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Original Contribution

Nitrosatable Drug Exposure During Early Pregnancy and Neural Tube Defects in Offspring

National Birth Defects Prevention Study

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Nitrosatable drugs, such as secondary or tertiary amines and amides, form *N*-nitroso compounds in the presence of nitrite. Various *N*-nitroso compounds have been associated with neural tube defects in animal models. Using data from the National Birth Defects Prevention Study, the authors examined nitrosatable drug exposure 1 month before and 1 month after conception in 1,223 case mothers with neural tube defect-affected pregnancies and 6,807 control mothers who delivered babies without major congenital anomalies from 1997 to 2005. Nitrite intakes were estimated from mothers' responses to a food frequency questionnaire. After adjustment for maternal race/ethnicity, educational level, and folic acid supplementation, case women were more likely than were control women to have taken tertiary amines (odds ratio = 1.60, 95% confidence interval (CI): 1.31, 1.95). This association was strongest with anencephalic births (odds ratio = 1.96, 95% CI: 1.40, 2.73); odds ratios associated with tertiary amines from the lowest tertile of nitrite intake to the highest tertile were 1.16 (95% CI: 0.59, 2.29), 2.19 (95% CI: 1.25, 3.86), and 2.51 (95% CI: 1.45, 4.37), respectively. Odds ratios for anencephaly with nitrosatable drug exposure were reduced among women who also took daily vitamin supplements that contained vitamin C. Prenatal exposure to nitrosatable drugs may increase the risk of neural tube defects, especially in conjunction with a mother's higher dietary intake of nitrites, but vitamin C might modulate this association.

anencephaly; ascorbic acid; neural tube defects; nitrites; nitrosation; pharmaceutical preparations; spinal dysraphism

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; EDD, estimated delivery date; NBDPS, National Birth Defects Prevention Study; NTD, neural tube defect; OR, odds ratio.

Various *N*-nitroso compounds have been associated with neural tube defects (NTDs) in animal models (1, 2), and DNA alkylation of embryonic cells has been suggested as one of the mechanisms for teratogenicity (1). Extensive experimental evidence indicates that *N*-nitroso compounds can be formed in vivo by nitrosatable amines or amides reacting with nitrosating agents, such as nitrite, in an acidic environment like that found in the stomach (3). Endogenous *N*-nitroso compound formation contributes 40%–75% of exposure to such compounds in humans (4), and a variety of drugs contribute nitrosatable amines or amides in the endogenous formation of *N*-nitrosamines and *N*-nitrosamides. In experiments with simulated gastric conditions (5–7), the combination of drugs containing secondary or tertiary amines or amides with nitrite have yielded a range of *N*-nitroso compounds, depending on the chemical structure of the drug.

Few epidemiologic studies have been conducted on the relation between nitrosatable drugs and NTDs. Olshan and Faustman (8) noted an association between exposure to nitrosatable drugs during the first 4 months of pregnancy and spina

bifida, whereas Croen et al. (9) did not detect this association with NTDs in their study population. In a study that also took into account dietary nitrate and nitrite intakes, Brender et al. (10) noted a significant positive association between prenatal use of drugs with the potential for nitrosation in Mexican-American women and NTDs in their offspring, especially among offspring of women who also had higher prenatal dietary intakes of nitrites. This finding was consistent with results from an experimental study with mice in which the percentage of NTDs and other defects increased in conjunction with a nitrosatable compound as the dose of nitrite increased (11), indicating that the combined teratogenicity of these compounds might be due to the nitrosation products formed within the stomach.

Previous studies of maternal exposure to nitrosatable drugs and birth defects were based on an incomplete assessment of drugs with respect to their nitrosatability. In their recent review, Brambilla and Martelli (12) identified 182 drugs that had been tested, of which 173 (95%) were found to form *N*-nitroso compounds or other reactive species. Furthermore, none of the previous studies examined the effects of nitrosatable drugs by their molecular structure (secondary or tertiary amines vs. amides) or examined the potential modulating effect of vitamin C, a well-documented inhibitor of nitrosation (13). Many drugs that are tertiary amines form *N*-nitrosodimethylamine in the presence of nitrite (12). In animal studies, prenatal administration of acetoxymethylmethylnitrosamine, which has the same active intermediate metabolite as *N*-nitrosodimethylamine, was associated with exencephaly and other defects at doses not associated with maternal toxicity (1). In the present study, we examined 1) the relation between prenatal exposure to nitrosatable drugs by their molecular structure (secondary amines, tertiary amines, and amides) in conjunction with dietary intake of nitrites and NTDs in offspring and 2) the effect of supplemental and dietary intake of vitamin C on these associations.

MATERIALS AND METHODS

Study population

The National Birth Defects Prevention Study (NBDPS), previously described by Yoon et al. (14), is an ongoing population-based case-control study of birth defects in the United States that began in 1997. Ten Centers for Birth Defects Research and Prevention (CBDRP) (in Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (1998 to present); New Jersey (1998 to 2002); and North Carolina and Utah (2003 to present)) have participated or are currently participating in this national study. Case infants are identified from livebirths (all centers), stillbirths (all centers except New Jersey and New York from 1997 to 1999), and elective pregnancy terminations (Arkansas, California, Georgia, Iowa, North Carolina, Texas, and Utah) (15). Case definitions are standardized across centers, and clinical information on potential cases is evaluated by a clinical geneticist at each center (16) and independently reviewed by 1 or more other clinical geneticists. Infants with single-gene or chromosome abnormalities are excluded from the NBDPS (16). For this study on nitrosatable drugs, we included cases with NTDs (anencephaly, craniorachischisis, spina bifida, and encephalocele) and controls who had estimated delivery dates (EDDs) from October 1, 1997, through December 31, 2005.

Controls (livebirths who had no major birth defects and whose mothers resided in the study area at delivery) with EDDs during the same period were randomly selected from live birth certificates (Iowa, Massachusetts, New Jersey, North Carolina, and Utah) or hospital records (California, New York, and Texas) (15). Centers in Arkansas and Georgia initially selected controls from hospital records but switched to live birth certificates exclusively in January of 2001. The institutional review boards in each state and the Centers for Disease Control and Prevention approved the study protocol, and the institutional review boards of Texas A&M University, the University of Iowa, and the Texas Department of State Health Services also approved this project on nitrosatable drugs and birth defects.

Data collection

In the NBDPS, women are interviewed via telephone by trained interviewers who administer a standard questionnaire after informed consent is obtained. The interview takes approximately 1 hour to complete and covers topics regarding maternal health (including medications being taken), diet (vitamin, food supplement, and food and beverage consumption), home/work (residence and occupation), demographic characteristics, and water use (14). Interviews are targeted for completion within 6 months of delivery, with a maximum time from delivery/termination to interview of no more than 24 months. Time of interview is determined by the EDD, and no women are interviewed until 6 weeks after the EDD (or delivery of a full-term infant).

Classification of nitrosatable drugs

As part of the interview, women in the NBDPS are questioned about their use of prescription and nonprescription drugs from 3 months before the estimated date of conception to the date of birth of the index pregnancy. In addition to the name of the medication, information is collected regarding the dates that the drug(s) were taken and the frequency of use. Reported drugs are linked to their active ingredients by using the Slone Epidemiology Center Drug Dictionary system (17), which identifies individual ingredients in multiple-component products (18). Methods used to classify drugs with respect to nitrosatability, functional groups, and indications have been published previously (19). Briefly, all orally administered and orally inhaled prescription and nonprescription medications and their active ingredients reported by case and control women were identified. These drugs were cross-referenced against previously compiled lists of nitrosatable medicinal compounds (12, 20) and categorized on the basis of the presence of amine (secondary or tertiary) and amide functional groups. Within its functional group, each component was also categorized by its primary indication or therapeutic use. Complete data on nitrosatable drug use and covariates were available for 1,168 (95.5%) participating case women and 6,553 (96.3%) participating control women. The Web

Appendix (available at http://aje.oxfordjournals.org/) contains a list of the drugs that were identified as nitrosatable and reported as taken by NBDPS subjects with EDDs during the period of 1997–2005. For the present study on NTDs, we focused on drugs that women reported taking from 1 month before conception to 1 month after conception.

Estimation of dietary nitrates and nitrites

As part of the NBDPS interview, women are questioned about their average consumption of foods and beverages during the year before becoming pregnant with the index pregnancy through the use of a 58-item food frequency questionnaire adapted from the short Willett food frequency questionnaire (21, 22). Separate, more detailed questions to assess consumption of breakfast cereals cover intake from 3 months before conception through the end of pregnancy. From these 2 sources of information, dietary intakes of nitrates and nitrites in milligrams per day were estimated using procedures described in detail elsewhere (23, 24). Briefly, 1) weighted means for nitrates and nitrites in milligrams per 100 grams were calculated for each food item based on the relevant literature; 2) the respective means were multiplied by the serving size (in grams) assigned to each food; 3) nitrates and nitrites in each serving size were multiplied by the number of servings per month; and 4) nitrate and nitrite levels across all food items were summed and then divided by 30 to obtain milligrams per day of dietary nitrate and nitrite intake. We calculated total dietary nitrite intake with the formula suggested by Choi (25) (total nitrite = dietary nitrite intake + (0.05 × dietary nitrate intake)). Dietary intakes of nitrites and total nitrites were categorized into tertiles based on the control women's distributions, as was done in a previous study (10). We excluded from the stratified analyses of nitrosatable drugs and dietary nitrites women whose calculated daily kilocalorie intake was less than 500 or greater than 5,000. These lower and upper limits are consistent with other dietary studies (26) and with what has previously been used with the NBDPS population (27, 28). Complete data for any nitrosatable drug use stratified by total nitrite intake were available for 1,132 (92.6%) participating cases and 6,376 (93.7%) participating controls.

Covariates

Covariate selection was based on factors associated with NTDs in other studies and maternal factors associated with nitrosatable drug exposure (19). Potential confounders that were assessed included maternal race/ethnicity, educational level, and age; study site; body mass index based on self-reported height and weight (in kg/m^2); folic acid supplementation around the time of conception; and dietary folate intake (expressed as dietary folate equivalents in quartiles based on the control women's distribution).

Statistical analysis

Logistic regression was used to estimate odds ratios and 95% confidence intervals for NTDs in relation to any nitrosatable drug use and for secondary amines, tertiary amines, and amides. Women who did not report taking any drugs classified as nitrosatable around conception served as the reference group in all analyses. Final models for the association between nitrosatable drugs and NTDs included maternal race/ethnicity, educational level, and folic acid use as covariates.

Nitrosatable drug exposure was stratified by tertiles of dietary nitrite and total nitrite intake, and odds ratios and 95% confidence intervals were estimated for NTDs for each stratum, with adjustment for maternal race/ethnicity, educational level, folic acid use, and total energy intake. Multiplicative interaction was assessed by including the product terms of secondary and tertiary amines with dietary nitrite and total nitrite in the logistic models. Additive interaction was examined using the methods discussed by Andersson et al. (29), in which the relative excess risk due to interaction and the attributable proportion due to interaction were calculated with their respective 95% confidence intervals. In the absence of additive interaction, both measures equaled zero.

Nitrosatable drug exposure was also stratified by vitamin C supplementation during the first month of pregnancy and by dietary vitamin C intake (categorized as <85 mg/day or ≥ 85 mg/day, cutpoints that were based on recommended dietary allowances for pregnant women >18 years of age (30) and that corresponded to the 41st percentile for study participants). The presence of multiplicative and additive interactions was assessed for secondary and tertiary amines with vitamin C supplementation and dietary intake with the same methods used for these drugs as with dietary nitrite/total nitrite.

RESULTS

A total of 1,223 eligible case women with NTD-affected pregnancies (352 with anencephaly or craniorachischisis, 730 with spina bifida, and 141 with encephalocele) and 6,807 control women with an EDD during 1997–2005 participated in the NBDPS. Participation rates for case women with NTD-affected pregnancies and control women were 68% and 66%, respectively, with median time from EDD to interview of 9 months for case women and 8 months for control women. Case women were more likely than were control women to be Hispanic, to be less educated, to have a body mass index of 30 or higher, and to live in California or Texas, but they were slightly less likely than controls to use a folic acid preparation around the time of conception (Table 1).

A higher proportion of case women (19.5%) than of control women (16.9%) reported taking drugs classified as nitrosatable (adjusted odds ratio (AOR) = 1.31, 95% confidence interval (CI): 1.12, 1.55), especially drugs classified as tertiary amines (AOR = 1.60, 95% CI: 1.31, 1.95) (Table 2). Women with anencephalic births were approximately twice as likely as were controls (AOR = 1.96, 95% CI: 1.40, 2.73) to report taking drugs classified as nitrosatable tertiary amines. Use of tertiary amines was less strongly associated with spina bifida (AOR = 1.48, 95% CI: 1.15, 1.91) and encephalocele (AOR = 1.48, 95% CI: 0.83, 2.63). Restriction of analyses to data from study centers that included terminations in case findings did not materially change the adjusted odds ratios for

Table 1. Selected Maternal Characteristics of Neural Tube Defect Cases and Controls in the National Birth Defects Prevention Study, 1997–2005

Characteristic	Cases (n = 1,223)		Controls (n = 6,807)	
	No.	%	No.	%
Race/ethnicity*				
Non-Hispanic white	621	50.8	4,026	59.1
Non-Hispanic black	114	9.3	771	11.3
Hispanic	395	32.3	1,502	22.1
Asian/Pacific Islander	25	2.0	200	2.9
Other	64	5.2	279	4.1
Missing	4	0.3	29	0.4
Educational level, years*				
<9	98	8.0	330	4.8
9–11	159	13.0	800	11.8
12	347	28.4	1,652	24.3
13–15	338	27.6	1,806	26.5
>15	265	21.7	2,110	31.0
Missing	16	1.3	109	1.6
Age at delivery, years*				
<18	49	4.0	255	3.7
18–19	99	8.1	478	7.0
20–24	270	22.1	1,552	22.8
25–29	390	31.9	1,807	26.5
30–34	257	21.0	1,759	25.8
35–39	115	9.4	816	12.0
40–44	40	3.3	128	1.9
>44	3	0.2	12	0.2
Study center location*				
Arkansas	153	12.5	848	12.5
California	240	19.6	858	12.6
Georgia	148	12.1	735	10.8
Iowa	146	11.9	759	11.2
Massachusetts	74	6.1	859	12.6
North Carolina	70	5.7	412	6.1
New Jersey	70	5.7	574	8.4
New York	72	5.9	601	8.8
Texas	171	14.0	792	11.6
Utah	79	6.5	369	5.4
Body mass index*,[a]				
<18.5	49	4.0	361	5.3
18.5–24.9	576	47.1	3,640	53.5
25.0–29.9	254	20.8	1,463	21.5
>29.9	265	21.7	1,055	15.5
Missing	79	6.5	288	4.2
Folic acid supplement use				
No	574	46.9	3,121	45.9
Yes[b]	605	49.5	3,521	51.7
Missing	44	3.6	165	2.4
Dietary folate equivalents, μg[c]				
<331.73	320	26.7	1,636	24.6
331.73–491.13	306	25.6	1,682	25.3
491.14–722.40	278	23.2	1,688	25.4
>722.40	293	24.5	1,639	24.7

* $P < 0.05$ (cases vs. controls; subjects with complete data only).

[a] Weight (kg)/height (m)^2.

[b] Refers to any use in the period 1 month before conception through 1 month after conception.

[c] Quartiles are based on control women's distributions in dietary folate equivalents, and data are restricted to women with daily energy intakes of 500–5,000 kcal. Dietary folate equivalents were calculated as food folate intake + (1.7 × synthetic folic acid intake).

NTDs (AOR = 1.59), anencephaly (AOR = 1.93), or spina bifida (AOR = 1.44) associated with tertiary amine drug exposure.

Overall, NTDs were associated with drugs classified as tertiary amines across a broad range of indications, such as analgesic opioids (odds ratio (OR) = 1.25), antidiabetic drugs (OR = 2.04), antiemetic phenothiazines (OR = 1.34), antiepileptics (OR = 3.48), antihistamines (OR = 1.46), antiinfective macrolides (OR = 2.90), cough medications (OR = 1.24), gastrointestinal histamine 2 blockers (OR = 1.66), and stimulants (OR = 1.17) (data not shown; associations reported are restricted to drugs with at least 5 exposed cases and 5 exposed controls). Exclusion of women who took antidiabetic, antiepileptic, and antibiotic drugs slightly reduced the adjusted odds ratios for NTDs (AOR = 1.45), anencephaly (AOR = 1.83), and spina bifida (AOR = 1.36), but the 95% confidence intervals for these odds ratios excluded 1.0. Antihistamines were the most commonly taken tertiary amine drugs by both case women (5.5%) and control women (3.9%) and were most strongly associated with anencephalic births (AOR = 2.39, 95% CI: 1.56, 3.67).

The strongest associations between anencephaly and nitrosatable secondary and tertiary amines were among women with nitrite and total nitrite levels (sum of dietary nitrite and 5% dietary nitrate intake) in the upper 2 tertiles of estimated intake (Table 3). Notably, the odds ratios were near or below 1.0 for nitrosatable drug use in the lowest tertiles of nitrite and total nitrite intake. Adjusted odds ratios for anencephaly associated with tertiary amines for the lowest tertile to the highest tertile of total nitrite were 1.16 (95% CI: 0.59, 2.29), 2.19 (95% CI: 1.25, 3.86), and 2.51 (95% CI: 1.45, 4.37), respectively ($P = 0.053$ for multiplicative interaction; relative excess risk due to interaction = 1.095 (95% CI: −0.163, 2.35); and attributable proportion due to interaction = 0.552 (95% CI: 0.105, 0.998)). Exposures to nitrosatable secondary and tertiary amines were also most strongly associated with encephalocele in the highest tertiles of dietary nitrite and total nitrite intake (data not shown). The pattern of increasing odds

Table continues

Table 2. Association Between Exposure to Nitrosatable Drugs Around the Time of Conception[a] and Neural Tube Defects, National Birth Defects Prevention Study, 1997–2005

Defect Group and Type of Drug Exposure	Cases		Controls		Unadjusted OR[b]	95% CI	Adjusted OR[c]	95% CI
	No.	%	No.	%				
Any neural tube defect								
No nitrosatable drug exposure	940	80.5	5,445	83.1	1	Referent	1	Referent
Secondary amines	125	11.7	646	10.6	1.12	0.91, 1.37	1.22	0.99, 1.50
Tertiary amines	141	13.0	570	9.5	1.43	1.18, 1.75	1.60	1.31, 1.95
Amides	64	6.4	340	5.9	1.09	0.83, 1.44	1.21	0.92, 1.61
Anencephaly[d]								
No nitrosatable drug exposure	263	79.0	5,445	83.1	1	Referent	1	Referent
Secondary amines	39	12.9	646	10.6	1.25	0.88, 1.77	1.41	0.99, 2.01
Tertiary amines	46	14.9	570	9.5	1.67	1.21, 2.31	1.96	1.40, 2.73
Amides	14	5.1	340	5.9	0.85	0.49, 1.48	0.98	0.56, 1.71
Spina bifida								
No nitrosatable drug exposure	569	81.4	5,445	83.1	1	Referent	1	Referent
Secondary amines	69	10.8	646	10.6	1.02	0.79, 1.33	1.08	0.83, 1.42
Tertiary amines	81	12.5	570	9.5	1.36	1.06, 1.74	1.48	1.15, 1.91
Amides	40	6.6	340	5.9	1.13	0.80, 1.58	1.23	0.87, 1.73
Encephalocele								
No nitrosatable drug exposure	108	79.4	5,445	83.1	1	Referent	1	Referent
Secondary amines	17	13.6	646	10.6	1.33	0.79, 2.23	1.54	0.90, 2.62
Tertiary amines	14	11.5	570	9.5	1.24	0.71, 2.18	1.48	0.83, 2.63
Amides	10	8.5	340	5.9	1.48	0.77, 2.86	1.76	0.90, 3.44

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] The period from 1 month before conception to 1 month after conception.
[b] Crude and adjusted odds ratios included only cases and controls for whom we had complete information on drug exposures and covariates.
[c] Adjusted for maternal race/ethnicity, educational level, and folic acid supplementation around the time of conception.
[d] This category included craniorachischisis.

ratios associated with nitrosatable drug exposure across increasing levels of dietary nitrites was less apparent for spina bifida (Table 4), and no significant multiplicative or additive interaction was noted. Exposure to tertiary amines was most strongly associated with spina bifida among women with an estimated dietary nitrite intake greater than 1.91 mg/day (AOR = 1.72, 95% CI: 1.12, 2.66).

Daily supplementation with preparations containing vitamin C diminished the association between nitrosatable drugs and anencephaly but not between nitrosatable drugs and spina bifida (Table 5). Among women who took a daily supplement with vitamin C, the odds ratio for having an anencephalic birth in association with nitrosatable tertiary amine use was lower (AOR = 1.52, 95% CI: 0.86, 2.71) than the corresponding estimates among women who did not take these supplements on a daily basis (AOR = 2.77, 95% CI: 1.48, 5.17) and among those who did not take the supplements at all (AOR = 2.11, 95% CI: 1.25, 3.57) during the first month of pregnancy ($P = 0.510$ for multiplicative interaction; relative excess risk due to interaction = 0.750 (95% CI: −0.377, 1.876); and attributable proportion due to interaction = 0.340 (95% CI: −0.106, 0.785)).

Among women whose dietary intake of vitamin C was estimated to be 85 mg/day or more, the adjusted odds ratio for spina bifida in conjunction with tertiary amine drug exposure was 1.15 (95% CI: 0.78, 1.69) compared with 1.81 (95% CI: 1.28, 2.56) among women with a lower dietary intake of this vitamin (data not shown) ($P = 0.130$ for multiplicative interaction; relative excess risk due to interaction = 0.659 (95% CI: −0.112, 1.430); and attributable proportion due to interaction = 0.337 (95% CI: 0.017, 0.656)). In contrast, tertiary amine drug exposure was more strongly associated with anencephaly in women with dietary vitamin C intakes estimated to be 85 mg/day or more (AOR = 2.69, 95% CI: 1.76, 4.12) than in women with intakes less than 85 mg/day (AOR = 1.15, 95% CI: 0.66, 2.02).

DISCUSSION

In the present study, a large, population-based case-control study of nitrosatable drugs and birth defects, women who had NTD-affected pregnancies were more likely than were control women to have taken drugs classified as nitrosatable during the period 1 month before conception to 1 month after conception. To our knowledge, this birth defects study is the first in which nitrosatable drugs have been examined specifically by molecular structure. We found that tertiary amines

Table 3. Association Between Exposure to Nitrosatable Drugs Around the time of Conception[a] and Anencephaly[b], by Estimated Dietary Intake of Nitrites and Total Nitrites,[c] National Birth Defects Prevention Study, 1997–2005

Dietary Intake (mg/day) and Type of Drug Exposure	Cases		Controls		Unadjusted OR[d]	95% CI	Adjusted OR[e]	95% CI
	No.	%	No.	%				
Nitrites								
<1.27								
No nitrosatable drug exposure	78	86.7	1,743	82.3	1	Referent	1	Referent
Secondary amines	7	8.2	222	11.3	0.71	0.32, 1.55	0.77	0.35, 1.70
Tertiary amines	6	7.1	193	10.0	0.70	0.30, 1.62	0.77	0.33, 1.80
1.27–1.91								
No nitrosatable drug exposure	76	70.4	1,754	82.3	1	Referent	1	Referent
Secondary amines	16	17.4	220	11.1	1.68	0.96, 2.93	1.68	0.96, 2.97
Tertiary amines	23	23.2	177	9.2	3.00	1.84, 4.90	3.18	1.92, 5.27
>1.91								
No nitrosatable drug exposure	105	80.8	1,797	84.3	1	Referent	1	Referent
Secondary amines	15	12.5	193	9.7	1.33	0.76, 2.33	1.67	0.93, 2.99
Tertiary amines	16	13.2	190	9.6	1.44	0.83, 2.49	1.98	1.11, 3.51
Total nitrites								
<3.02								
No nitrosatable drug exposure	92	82.9	1,753	82.7	1	Referent	1	Referent
Secondary amines	10	9.8	220	11.2	0.87	0.44, 1.69	0.93	0.47, 1.83
Tertiary amines	10	9.8	183	9.5	1.04	0.53, 2.04	1.16	0.59, 2.29
3.02–4.56								
No nitrosatable drug exposure	77	76.2	1,747	81.8	1	Referent	1	Referent
Secondary amines	13	14.4	233	11.8	1.27	0.69, 2.31	1.38	0.74, 2.57
Tertiary amines	17	18.1	206	10.6	1.87	1.09, 3.23	2.19	1.25, 3.86
>4.56								
No nitrosatable drug exposure	90	77.6	1,789	84.4	1	Referent	1	Referent
Secondary amines	15	14.3	182	9.2	1.64	0.93, 2.89	1.86	1.03, 3.36
Tertiary amines	18	16.7	171	8.7	2.09	1.23, 3.55	2.51	1.45, 4.37

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] The period from 1 month before conception to 1 month after conception.

[b] This category included craniorachischisis.

[c] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

[d] Crude and adjusted odds ratios included only cases and controls for whom we had complete information on drug exposures and covariates.

[e] Adjusted for maternal race/ethnicity, educational level, folic acid supplementation, and total energy intake.

were more strongly associated with NTDs, particularly anencephaly, than were secondary amines or amides. Furthermore, the strongest associations between nitrosatable drug exposure and NTDs were noted among women with higher estimated intakes of dietary nitrites. In contrast, nitrosatable drug exposures showed little to no association with NTDs among women in the lowest tertile of dietary nitrite intake. The association between tertiary amine drug exposure and anencephalic births was reduced among women who took a daily supplement with vitamin C compared with women who took these supplements less than daily or not at all.

The findings of the present study show a pattern similar to that seen in a study of nitrosatable drug use among Mexican-American women in Texas and NTDs in their offspring (10). In the Texas study, odds ratios for NTDs associated with nitrosatable drug use for the lowest tertile to the highest tertile of total dietary nitrite intake were 0.9 (95% CI: 0.3, 3.4), 2.7 (95% CI: 0.8, 11), and 7.5 (95% CI: 1.8, 45), respectively. However, the overall odds ratio for any NTD in relation to nitrosatable drug use was higher (AOR = 2.7, 95% CI: 1.4, 5.3) than the odds ratio noted in the present study, including that found for Hispanic women in NBDPS (AOR = 1.26, 95% CI: 0.84, 1.89). Anencephaly and spina bifida were not examined separately by nitrite intake in the Texas study because of insufficient numbers by phenotype.

In 2 other studies, the relation between nitrosatable drug use and NTDs was examined. Utilizing data from the Collaborative Perinatal Project, Olshan and Faustman (8) found that nitrosatable drug use during the first 4 months of pregnancy was associated with spina bifida (OR = 4.55, 95% CI: 0.66, 31.62)

Table 4. Association Between Exposure to Nitrosatable Drugs Around the Time of Conception[a] and Spina Bifida, by Estimated Dietary Intake of Nitrites and Total Nitrites,[b] National Birth Defects Prevention Study, 1997–2005

Dietary Intake (mg/day) and Type of Drug Exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
	No.	%	No.	%				
Nitrites								
<1.27								
No nitrosatable drug exposure	168	82.8	1,743	82.3	1	Referent	1	Referent
Secondary amines	16	8.7	222	11.3	0.75	0.44, 1.27	0.80	0.47, 1.37
Tertiary amines	20	10.6	193	10.0	1.08	0.66, 1.75	1.14	0.69, 1.86
1.27–1.91								
No nitrosatable drug exposure	175	79.2	1,754	82.3	1	Referent	1	Referent
Secondary amines	26	12.9	220	11.1	1.19	0.77, 1.83	1.15	0.74, 1.80
Tertiary amines	28	13.8	177	9.2	1.59	1.03, 2.43	1.62	1.05, 2.50
>1.91								
No nitrosatable drug exposure	209	82.3	1,797	84.3	1	Referent	1	Referent
Secondary amines	26	11.1	193	9.7	1.16	0.75, 1.79	1.38	0.88, 2.16
Tertiary amines	30	12.6	190	9.6	1.36	0.90, 2.05	1.72	1.12, 2.66
Total nitrites[b]								
<3.02								
No nitrosatable drug exposure	177	80.5	1,753	82.7	1	Referent	1	Referent
Secondary amines	18	9.2	220	11.2	0.81	0.49, 1.34	0.82	0.49, 1.36
Tertiary amines	28	13.7	183	9.5	1.52	0.99, 2.32	1.57	1.02, 2.42
3.02–4.56								
No nitrosatable drug exposure	184	79.3	1,747	81.8	1	Referent	1	Referent
Secondary amines	28	13.2	233	11.8	1.14	0.75, 1.74	1.21	0.79, 1.87
Tertiary amines	31	14.4	206	10.6	1.43	0.95, 2.15	1.56	1.02, 2.37
>4.56								
No nitrosatable drug exposure	187	84.2	1,789	84.4	1	Referent	1	Referent
Secondary amines	22	10.5	182	9.2	1.16	0.73, 1.85	1.31	0.81, 2.11
Tertiary amines	19	9.2	171	8.7	1.06	0.65, 1.75	1.20	0.72, 2.00

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] The period from 1 month before conception to 1 month after conception.

[b] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

[c] Crude and adjusted odds ratios included only cases and controls for whom we had complete information on drug exposures and covariates.

[d] Adjusted for maternal race/ethnicity, educational level, folic acid supplementation, and total energy intake.

but not with anencephaly (0 of 6 case mothers exposed). They identified 13 nitrosatable drugs taken by the cohort. Using the list of nitrosatable drugs developed by Olshan and Faustman (8), Croen et al. (9) noted minimal association between nitrosatable drug use and NTDs among births to California residents (OR = 1.2, 95% CI: 0.77, 1.8); odds ratios for specific phenotypes were not reported.

A number of compounds have been found to inhibit nitrosation, with vitamin C being the most studied (13). In experimental studies in human volunteers, concomitant administration of ascorbic acid with nitrate and nitrosatable precursors such as proline (31) or a fish meal rich in amines (32) significantly reduced the excretion of *N*-nitroso compounds in comparison with these combined exposures without ascorbic acid. It is noteworthy that Brambilla and Martelli (12) concluded in their extensive review of nitrosatable drugs that the nitrosatable drug-nitrite interaction could be substantially reduced by the administration of vitamin C. In the present study, the associations between drugs classified as secondary or tertiary amines and anencephaly were reduced in women who took a daily vitamin supplement with vitamin C during the periconceptional period. The associations between these drugs and spina bifida were reduced among women who had an estimated dietary intake of vitamin C of 85 mg/day or more. However, we have no explanation for why we did not observe reduced associations between anencephaly and higher dietary vitamin C intake or reduced associations between spina bifida and vitamin C supplementation.

The present study had several other limitations. Approximately one-third of the eligible case and control women did not participate in the study, although participation rates were similar between cases and controls. Cogswell et al. (15)

Table 5. Association Between Exposure to Nitrosatable Drugs Around the Time of Conception[a] and Neural Tube Defects, by Vitamin C Supplementation Level, National Birth Defects Prevention Study, 1997–2005

Type of Neural Tube Defect, Vitamin C Supplementation Level [c], and Type of Drug Exposure	Cases		Controls		Unadjusted OR[d]	95% CI	Adjusted OR[e]	95% CI
	No.	%	No.	%				
Anencephaly[b]								
None								
No nitrosatable drug exposure	147	86.0	2,804	86.7	1	Referent	1	Referent
Secondary amines	16	9.8	255	8.3	1.20	0.70, 2.04	1.44	0.84, 2.49
Tertiary amines	18	10.9	211	7.0	1.63	0.98, 2.71	2.11	1.25, 3.57
Less than daily								
No nitrosatable drug exposure	48	65.8	958	81.4	1	Referent	1	Referent
Secondary amines	13	21.3	123	11.4	2.11	1.11, 4.00	2.21	1.15, 4.23
Tertiary amines	15	23.8	117	10.9	2.56	1.39, 4.71	2.77	1.48, 5.17
Daily								
No nitrosatable drug exposure	77	77.0	1,785	79.1	1	Referent	1	Referent
Secondary amines	10	11.5	275	13.4	0.84	0.43, 1.65	0.91	0.46, 1.78
Tertiary amines	15	16.3	248	12.2	1.40	0.79, 2.48	1.52	0.86, 2.71
Spina bifida								
None								
No nitrosatable drug exposure	308	85.1	2,804	86.7	1	Referent	1	Referent
Secondary amines	31	9.1	255	8.3	1.11	0.75, 1.64	1.19	0.80, 1.77
Tertiary amines	35	10.2	211	7.0	1.51	1.04, 2.20	1.69	1.15, 2.48
Less than daily								
No nitrosatable drug exposure	108	81.8	958	81.4	1	Referent	1	Referent
Secondary amines	9	7.7	123	11.4	0.65	0.32, 1.31	0.68	0.34, 1.40
Tertiary amines	15	12.2	117	10.9	1.14	0.64, 2.02	1.25	0.70, 2.25
Daily								
No nitrosatable drug exposure	169	74.8	1,785	79.1	1	Referent	1	Referent
Secondary amines	31	15.5	275	13.4	1.19	0.80, 1.78	1.17	0.78, 1.76
Tertiary amines	34	16.8	248	12.2	1.45	0.98, 2.14	1.44	0.97, 2.13

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] The period from 1 month before conception to 1 month after conception.

[b] This category included craniorachischisis.

[c] Supplement taken the first month after conception (including prenatal multivitamin supplements, nonprenatal multivitamin supplements, and other vitamin C-containing supplements).

[d] Crude and adjusted odds ratios included only cases and controls for whom we had complete information on drug exposures and covariates.

[e] Adjusted for maternal race/ethnicity and educational level.

examined how well the NBDPS control women with EDDs from 1997 to 2003 represented the base population from which the case women were also identified. Control participants were similar to their base populations with respect to maternal age, smoking, and prevalence of diabetes mellitus but differed slightly by maternal race/ethnicity and educational level. Assessment of dietary nitrate and nitrite intakes was restricted to a food frequency questionnaire that might have not captured all dietary sources of these food contaminants or accurately reflected the actual amounts consumed (24). The potential for differential recall between case and control women regarding medications taken during early pregnancy is also of concern. However, women were not specifically asked about nitrosatable drug exposure, and it is unlikely that recall of medications taken would differ between cases and controls by type of nitrosatable drugs (e.g., secondary or tertiary amines) or by dietary intake of nitrites. Finally, although the present study utilized extensive reviews (12, 20) of nitrosatable drugs that were not available when previous studies of nitrosatable drugs and birth defects were conducted, exposures to some nitrosatable drugs might have been missed because the components have not been tested for nitrosatability or because results of such tests have not been published.

In conclusion, results of the present study suggest that early prenatal exposure to nitrosatable drugs, especially tertiary amines, in conjunction with nitrite intake might increase risk of NTDs. To our knowledge, this is the first study indicating that vitamin C might modulate the association

between prenatal exposure to nitrosatable drugs and NTDs in offspring.

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