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Prenatal Exposure to Nitrosatable Drugs, Vitamin C, and Risk of Selected Birth Defects

Mayura U. Shinde[1,*], **Ann M. Vuong**[1], **Jean D. Brender**[1], **Martha M. Werler**[2], **Katherine E. Kelley**[2], **John C. Huber Jr**[1], **Joseph R. Sharkey**[1], **Qi Zheng**[1], **Lucina Suarez**[3], **Peter H. Langlois**[4], **Mark A. Canfield**[4], **Paul A. Romitti**[5], **Sadia Malik**[6], and **The National Birth Defects Prevention Study**

[1]Texas A&M Health Science Center, School of Rural Public Health, College Station, Texas

[2]Slone Epidemiology Center at Boston University, Boston, Massachusetts

[3]Texas Department of State Health Services, Environmental Epidemiology and Disease Registries, Austin, Texas

[4]Texas Department of State Health Services, Birth Defects Epidemiology and Surveillance Branch, Austin, Texas

[5]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, Iowa

[6]Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Abstract

BACKGROUND—Nitrosatable drugs, such as secondary or tertiary amines and amides react with nitrite in an acidic environment to form *N*-nitroso compounds, teratogens in animal models. Vitamin C is a known nitrosation inhibitor.

METHODS—Using data from the National Birth Defects Prevention Study, we assessed nitrosatable drug exposure and vitamin C intake during the first trimester among 11,606 case-mothers of infants with oral clefts, limb deficiencies (LDs), or congenital heart defects and 6807 control-mothers of infants without major birth defects during 1997–2005. Daily intake of vitamin C was estimated from maternal interviews that elicited information about supplement use and dietary intake.

RESULTS—With no reported use of nitrosatable drugs as the referent group, a lower odds ratio (OR) was observed for transverse LDs among births to mothers exposed to secondary amine drugs and daily vitamin C supplementation (adjusted odds ratio [aOR] 1.2, 95% confidence interval [CI] 0.83–1.8) compared with women taking these drugs and no supplementation (aOR 2.7, 95% CI 1.5–4.6). The OR for longitudinal LDs associated with secondary amine exposure was lower with daily dietary vitamin C intake ≥85 mg (aOR 1.2, 95% CI 0.68–2.0) compared with <85 mg (aOR

[*]Correspondence to: Mayura Shinde, Texas A&M Health Science Center, School of Rural Public Health, 259, SRPH Administration Building, College Station, TX 77843-1266. shinde@srph.tamhsc.edu.

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1.9, 95% CI 1.2–3.1). Daily vitamin C supplementation in combination with higher dietary vitamin C intake reduced associations between nitrosatable drug exposures and limb deficiencies and atrial septal defects not otherwise specified.

CONCLUSION—Prenatal dietary and vitamin C supplement intake may diminish the association between nitrosatable drug exposure during pregnancy and selected birth defects.

Keywords

nitrosatable drug; vitamin C; dietary vitamin C; birth defects; oral clefts; limb deficiencies; heart defects

INTRODUCTION

N-nitroso compounds, including nitrosamines and nitrosamides can be formed in vivo when nitrosatable amines or amides react with nitrosating agents such as nitrite, in the acidic environment of the stomach (Preussmann, 1984). Experimental evidence has shown that drugs containing nitrosatable groups such as amines or amides can form *N*-nitroso compounds in the presence of nitrite under simulated gastric conditions (Gillatt et al., 1984). *N*-nitroso compounds have been observed to be teratogenic in animal models. In pregnant mice, exposure to acetoxymethyl-methylnitrosamine, a compound thought to have the same intermediate metabolite as dimethylnitrosamine, has been associated with birth defects such as exencephaly, cleft palate, and digital and long-bone deformities of the fore- and hindlimbs (Platzek et al., 1983). This association was observed when 10 mg/kg of *N*-nitroso compound was administered via intravenously, subcutaneously, and intraperitoneally. Inouye and Murakami (1978) observed similar abnormalities when pregnant mice were exposed to *N*-nitroso compound, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, with doses as low as 40 mg/kg injected intraperitoneally. In addition, frog embryos treated with *N*-nitrosodimethylamine (dimethylnitrosamine) has been linked with severe heart malformations (Fort et al., 1991).

Although some exposure to *N*-nitroso compounds occurs from exogenous sources, such as processed meat products, cured meats, smoked fish, alcohol, and tobacco (Spiegelhader, 1980; Tricker, 1997; Lijinsky, 1999), endogenous *N*-nitroso compound formation accounts for 45 to 75% of the total exposure to *N*-nitroso compounds (Tricker, 1997). *N*-nitroso compounds can be formed endogenously by the reduction of ingested nitrates to nitrites by oral bacteria, followed by a reduction of a portion of the nitrite to nitric oxide in the acidic environment of the stomach (Spiegelhader et al., 1976).

Certain medications that are classified as tertiary amines, secondary amines, or amides contribute to the formation of endogenous *N*-nitroso compounds. Nitrosatable drug use during the first trimester of pregnancy was observed among 24% of control participants of the National Birth Defects Prevention Study (NBDPS) (Brender et al., 2011a). In the NBDPS study population, prenatal exposure to nitrosatable drugs during the first trimester was associated with several birth defects, including limb deficiencies, cleft lip with cleft palate, cleft palate only, single ventricle heart defects, atrioventricular septal defects, and hypoplastic left heart syndrome (Brender et al., 2012). The associations were stronger among mothers who reported higher dietary nitrite intake.

Vitamin C has been shown to inhibit endogenous *N*-nitroso compound formation. Mirvish et al. (1972) first demonstrated that ascorbic acid inhibits *N*-nitroso compound formation by rapid reduction of nitrite to nitrous oxide, followed by the production of dehydroascorbic acid. A reduced risk was observed for peripheral nervous system tumors in offspring when pregnant hamsters were given 200 mg/kg, respectively, of ascorbic acid and ethylurea, and 100 mg/kg of nitrite (Rustia, 1975). In a clinical trial conducted with human volunteers, increased doses of ascorbic acid (1.76–1000 mg) with combined exposures to nitrate and proline (a nitrosatable precursor) significantly reduced the excretion of *N*-nitroso compounds by 44% compared with exposures to nitrate and proline without concomitant administration of vitamin C (Leaf et al., 1987). In a recent study, we found that women who took daily vitamin C supplementation along with tertiary or secondary amine drug exposure had lower odds of having anencephalic births than women who took these drugs without vitamin C supplementation (Brender et al., 2011b).

Given the previous animal and human data on the impact of vitamin C on ameliorating the effects of *N*-nitroso compounds, we expanded our work with the NBDPS data to examine the: (1) effects of vitamin C supplementation on the relation between drugs classified as nitrosatable such as secondary amines, tertiary amines, or amides and selected birth defects (oral clefts, limb deficiencies, and congenital heart defects); and the (2) effects of estimated dietary vitamin C intake on the association between nitrosatable drugs and these birth defects.

METHODS

Study Population

Data from the NBDPS, an ongoing population-based case–control study of major structural birth defects in the United States, were used to examine the effect of supplemental and dietary intake of vitamin C on prenatal exposure to nitrosatable drugs (secondary and tertiary amines, amides) and selected birth defects in offspring. Index cases are identified from birth defect surveillance programs at 10 sites: Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present). They include live births (all sites), stillbirths (all sites except New Jersey and New York before 2000), and elective terminations (all sites excluding Massachusetts, New Jersey, and New York before 2000) (Cogswell et al., 2009).

Case classifications have been standardized for the NBDPS (Rasmussen et al., 2003), and clinical information for an index case is evaluated by an on-site clinical geneticist. To reduce misclassification, clinical information is again reviewed independently by at least one other clinical geneticist. Index cases with single-gene conditions or chromosome abnormalities are excluded to narrow the focus to those of unknown etiology. Birth defects are further classified as either multiple or isolated. Index cases having a major birth defect accompanied by at least one other unrelated, major, and specified defect are considered as having multiple defects. Those having a single major defect with additional minor defects in the absence of a defined syndrome; having a major defect accompanied by other major defects in the same organ, organ system, or body part; or having a major defect accompanied by other

pathogenetically related defects are classified as having isolated defects (Rasmussen et al., 2003). Index cases with a congenital heart defect are also assigned a complexity category describing the heart phenotype as a simple (anatomically discrete or well-recognized single entities), an association (common, uncomplicated combinations of heart defects), or a complex form (phenotypes not described as either of the former) as described by Botto et al. (2007). For our study, we focused on index cases with estimated delivery dates (EDDs) between October 1, 1997 and December 31, 2005 and diagnosed with oral cleft defects (multiple/isolated), limb deficiencies (multiple/isolated), or congenital heart defects (simple and isolated, except for heterotaxia and single ventricle for which complex and isolated defects were studied).

Control-infants were live born without major birth defects delivered in the same time frame and study area as cases. They were randomly sampled from birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs before 2001; California; Georgia, for EDDs before 2001; New York; and Texas) (Cogswell et al., 2009). Control-infants were ineligible if they were not liveborn, had a major birth defect, or were born outside the study area. At the time of interview, prospective study participants were excluded if the infant was adopted or in foster care or if the mother has one or more of the following characteristics: did not speak English or Spanish, participated in the NBDPS previously, was incarcerated, was a donor or surrogate parent, was unable to answer questions, or was deceased. The institutional review boards in each state and the Centers for Disease Control and Prevention approved the NBDPS 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, vitamin C, and birth defects.

Data Collection

Following informed consent, interviews were conducted in either English or Spanish by trained female interviewers using a computer-assisted telephone interview (Yoon et al., 2001). Data collection procedures were the same for cases and controls, with interviews conducted 6 weeks to 24 months following the EDDs (or delivery of a full-term infant) and targeted for completion within 6 months of the EDD. The median times from the EDD to the time of interview collection were 10.3 and 7.6 months for cases and controls, respectively. The interview included detailed questions regarding maternal health during the index pregnancy (including medications taken), nutrition (food and beverage consumption, vitamin supplementation), infections, and behavioral factors.

Classification of Nitrosatable

Drugs Information about prescription and non-prescription drug usage (medication name and frequency of use) and corresponding dates of usage were obtained during the interview from 3 months before the estimated date of conception to the end of pregnancy. The Slone Epidemiology Center Drug Dictionary system was used to link the reported medications to their active ingredient (Kelley et al., 2003). Detailed methods used to classify drugs with regards to their nitrosatability, functional groups, and indications were described in previous publications (Brender et al., 2011a,b). Briefly, the methodology for classification included:

(1) identification of active ingredients for all medications; (2) cross-referencing the drugs with a comprehensive nitrosatable medicinal compounds list (Brambilla and Martelli, 2007; McKean-Cowdin et al., 2003); (3) drug categorization based on the presence of functional groups (e.g., secondary amine, tertiary amine, or amide); and (4) further drug classification by its primary indication or therapeutic use and pharmacologic class. The Supporting Information Appendix contains a list of these drugs with their respective functional groups and primary indications. This study focuses on drugs reported to have been taken during the first trimester (the first, second, or third month of pregnancy). Complete data on nitrosatable drug use and covariates were available for 2523 (91.0%), 669 (90.3%), 7274 (89.9%), and 6167 (90.6%) mothers of infants with oral cleft defects, limb deficiencies, congenital heart defects, and unaffected infants, respectively.

Assessment of Vitamin C Intake

Information on average food consumption throughout the year before conception was gathered during the NBDPS interview using the 58-item food frequency questionnaire (FFQ), which was adapted from the short Willett FFQ. This Willett FFQ has been validated and reproduced in other studies and provides useful information about nutrient intake in women during pregnancy (Willett et al., 1987; Suitor et al., 1989). Information was also obtained for cereal intake 3 months preconception through the date of delivery. Nutrient calculations, such as dietary intake of vitamin C, were based on the USDA National Nutrient Database for Standard Reference 19 (USDA, 2006). Complete data for any nitrosatable drug use stratified by dietary vitamin C intake were available for mothers of 669 (90.3%) infants with limb deficiencies, 2523 (91.0%) infants with oral cleft defects, 7273 (89.9%) infants with congenital heart defects, and 6167 (90.6%) controls.

Vitamin C supplementation was assessed using the NBDPS questionnaire, which contained questions regarding start and stop dates, duration of use, and frequency of vitamin use (single, prenatal, and multivitamins) from 3 months before conception to the end of pregnancy. Depending on the frequency of intake, vitamin C supplementation was categorized into none, less than daily, and daily. Women who reported using a daily vitamin C supplement during the study period (second or second and third month of pregnancy) were classified as “daily” while those who reported no vitamin C supplementation during the same period were classified as “none.” Women were classified as “less than daily” if they took a vitamin C supplement less than 30 days in a given month or less than every day in a given period. Complete data for any nitrosatable drug use stratified by vitamin C supplementation were available for mothers of 680 (91.8%) infants with limb deficiencies, 2449 (88.2%) infants with oral cleft defects, 7400 (91.5%) infants with congenital heart defects, and 6278 (92.2%) controls.

Data Analysis

Logistic regression was used to estimate odds ratios and 95% confidence intervals for limb deficiencies, oral cleft defects, and congenital heart defects in relation to any nitrosatable drug use and use of secondary amines, tertiary amines, and amides by supplemental and dietary intake of vitamin C. Women who reported no nitrosatable drug use during the first trimester served as the reference group in all analyses. For analyses involving oral cleft

defects, 137 control women with EDDs during 2003 in Utah were excluded since case-mothers of infants with oral cleft defects were not obtained from Utah during that time.

Nitrosatable drug exposure was stratified by vitamin C supplementation (none, less than daily, daily) during the second month of pregnancy for limb deficiencies and congenital heart defects. For oral cleft defects, the second month of pregnancy was used for cleft lip only since the formation of lip and primary palate occurs during this time (Itikala et al., 2001). For cleft palate alone and cleft lip with cleft palate, the second and third months were used since the critical period for formation of the secondary palate is during those months of pregnancy. Covariates included in the logistic models for each defect group studied (limb deficiencies, oral cleft defects, and heart defects) were selected based on their association with the selected defect group and with maternal factors associated with nitrosatable drug usage based on previous literature. Nonsignificant covariates as well as those that did not change the odds ratio by 10% or more were eliminated from the final model using backward selection. Covariates were consistently included in regression analyses for subcategories of each major defect group. For limb deficiencies, the final model included maternal age (<18, 18– 19, 20–24, 25–29, 30–34, ≥35), maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), maternal education (<12 years, 12 years, 13–15 years, >15 years), study site, alcohol use 1 month preconception through the first trimester (yes, no), and body mass index categorized according to NIH guidelines (underweight, normal, overweight, and obese). In addition to the covariates mentioned, analyses of oral cleft defects also included any reported maternal smoking 1 month preconception through the first trimester (yes/no) since previous studies reported maternal smoking to be significantly associated with oral clefts (Little et al., 2004). For heart defect analyses, all above covariates were included in the final model except maternal age.

Nitrosatable drug exposure was also stratified by dietary vitamin C intake (<85 mg/day or ≥85 mg/day). Cut points were based on the recommended dietary allowance for pregnant women over 18 years of age (NIH, 2011) which corresponded to the 41st percentile for control participants. The median cut-point for dietary vitamin C among the controls was 101.31 mg/day. Dietary vitamin C analyses were restricted to women who had estimated daily caloric intakes between 500 and 5000 kcal. Models examining the effect of dietary intake of vitamin C in relation to nitrosatable drug usage included covariates previously described for each major birth defect group in addition to daily caloric intake, folic acid supplementation for oral cleft defects and multivitamin supplementation for limb deficiencies and heart defects during the first trimester.

Additive and multiplicative interaction was assessed for the associations of birth defects with nitrosatable drugs by supplemental and dietary vitamin C. To determine whether significant additive interaction was present, a statistical program estimating measures of relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP) was utilized (Andersson et al., 2005). If the 95% confidence intervals of both RERI and AP excluded 0, additive interaction was considered present which implies that the risk of birth defects attributable to the two risk factors in combination is greater than the sum of risks associated with each risk factor separately. Multiplicative interaction was assessed with the inclusion of product terms of nitrosatable drug groups with supplemental and dietary

vitamin C in the logistic models and was considered significant if the *p* value was less than 0.05.

RESULTS

Mothers of 741 infants with limb deficiencies, 2774 with oral clefts defects, 8091 with congenital heart defects and those of 6807 control-infants with an EDD from 1997 to 2005 participated in the NBDPS. Maternal participation rates for limb deficiency, oral cleft defect, congenital heart defect, and controls were 69%, 74%, 69%, and 66%, respectively. Twelve percent of the case-mothers and 17% of the control-mothers declined to participate in the study. The median time between EDD and interview was slightly longer for case-mothers (9 months for oral cleft defects; 10 months for either limb deficiencies or congenital heart defects) compared with control-mothers (8 months). Case-mothers were more likely than control-mothers to be Hispanic, less educated, smoke, and have a BMI greater than 29.9 kg/m^2, and slightly less likely than control mothers to use multivitamin or folic acid containing supplements during the first trimester. The proportion of supplemental vitamin C usage varied slightly between case and control-mothers (Table 1).

Among women exposed to secondary amine drugs, a lower odds ratio (OR) was observed with daily vitamin C supplementation for offspring with limb deficiencies (all types) (aOR 1.33, 95% CI 1.00–1.77) compared with those taking these drugs and no vitamin C supplementation (aOR 1.99, 95% CI 1.26–3.15) (Table 2). A similar pattern was also noted for transverse limb deficiencies for mothers with secondary amine drug exposure with daily (aOR 1.23, 95% CI 0.83–1.81) compared with no vitamin C supplementation (aOR 2.65, 95% CI 1.54–4.55); significant multiplicative interaction (*p* value 0.026) was noted between exposure to secondary amines and supplemental vitamin C with respect to risk for this type of limb deficiency. For cleft lip with cleft palate defects, the OR was closer to the null with daily (aOR 1.04, 95% CI 0.76–1.43) compared with no vitamin C supplementation (aOR 1.78, 95% CI 0.95–3.34) in relation to amide drug exposure. Similar findings were seen when analyses were restricted to isolated cleft defects (data not shown). No significant additive and multiplicative interactions were observed for oral cleft defects by vitamin C supplement in relation to nitrosatable drug exposures.

For atrial septal defect not otherwise specified (ASDNOS), a lower OR was observed among women with daily (aOR 1.12, 95% CI 0.59–2.11) compared with no vitamin C supplementation (aOR 2.90, 95% CI 1.22–6.93) in relation to amide drug exposure, although fewer cases were observed to be exposed to amides than other types of nitrosatable drugs. Significant additive (AP 0.46, 95% CI 0.11–0.82) and multiplicative interactions (*p* value 0.03) were noted between amide exposure and supplemental vitamin C in relation to these heart defects. Compared with no daily supplementation, lower ORs were noted for conotruncal and perimembranous ventricular septal heart defects among women with daily intake of vitamin C supplement (aORs, respectively, of 0.86, 95% CI 0.65–1.13; and 0.91, 95% CI 0.68–1.22) in conjunction with tertiary amine drug exposure (Table 3). Significant additive and multiplicative interactions were observed between exposure to tertiary amines and supplemental vitamin C for conotruncal (respectively, AP 0.30, 95% CI 0.04–0.56; *p*

value 0.03) and perimembranous ventricular septal heart defects (respectively, AP 0.26, 95% CI 0.002–0.52; *p* value 0.04).

Estimated daily dietary vitamin C intake above 85 mg in conjunction with nitrosatable drug exposure during the first trimester showed lower ORs for limb deficiencies, cleft lip without cleft palate, hypoplastic left heart syndrome, aortic stenosis, and atrial septal secundum births. Among women who reported taking secondary amine drugs, a lower point estimate was observed for limb deficiencies among those who had daily vitamin C intake of more than 85 mg (aOR 1.21, 95% CI 0.87–1.69) in comparison to those with lower dietary vitamin C intake (aOR 1.71, 95% CI 1.25–2.35). This difference was most notable for longitudinal limb deficiencies in which a smaller effect was observed for women with more than 85 mg of daily dietary vitamin C intake (aOR 1.15, 95% CI 0.68–1.95) compared with less than 85 mg (aOR 1.93, 95% CI 1.21–3.07); significant additive interaction (AP 0.41, 95% CI 0.04–0.77) was observed between secondary amine exposure and dietary vitamin C with respect to risk for this type of limb deficiency (Table 4). A similar pattern was found between cleft lip only and secondary amine drug exposure with a lower OR among women with daily dietary vitamin C intake above 85 mg (aOR 0.96, 95% CI 0.67–1.38) compared with less than 85 mg (aOR 1.39, 95% CI 0.99–1.97). In contrast, higher ORs were noted between exposure to amides and several types of limb deficiencies and oral clefts among women with higher dietary vitamin C intake (≥85 mg/day) compared with those with lower dietary vitamin C intake. Because differences were small between ORs for nitrosatable drugs with high and low dietary vitamin C, no significant additive or multiplicative interaction were observed for oral cleft defects.

Higher dietary intake of vitamin C appeared to confer less beneficial effects against congenital heart defects in offspring than vitamin C supplementation in conjunction with nitrosatable drug exposures (Table 5). In fact, the ORs for ASDNOS were notably higher among offspring of women who had higher dietary intake of vitamin C in conjunction with tertiary amine drug exposures (aOR 2.09, 95% CI 1.28–3.41) than for women with this drug exposure and lower dietary intake of vitamin C (aOR 1.06, 95% CI 0.59–1.92). We also analyzed the effects of dietary vitamin C (using the median cut-point) on the relation between nitrosatable drugs and birth defects and found no change in the overall conclusion.

When examining total vitamin C intake, a smaller OR was observed for any limb deficiencies in relation to secondary amine drug exposure among women with ≥85 mg of dietary vitamin C and daily vitamin C supplementation (aOR 1.06, 95% CI 0.70–1.62) compared with those with lower dietary vitamin C and no vitamin C supplementation (aOR 3.03, 95% CI 1.57–5.84) (Table 6). Significant additive (AP 0.63, 95% CI 0.34–0.91) and multiplicative (*p* value 0.01) interactions were observed between secondary amine exposure and total daily vitamin C intake with respect to risk for any limb deficiency. For transverse limb deficiency, in relation to secondary amine exposure, the OR was notably lower among women with high dietary vitamin C and daily vitamin C supplementation (aOR 1.23, 95% CI 0.72–2.10) compared with those with <85 mg of dietary vitamin C and no vitamin C supplementation (aOR 4.16, 95% CI 1.89–9.17). A significant additive interaction (AP 0.70, 95% CI 0.42–0.97) was found between secondary amine exposure and total daily vitamin C intake for transverse limb deficiency. A similar finding was also observed for longitudinal

limb deficiency, although the difference in associations was not as pronounced. Although the confidence intervals were wide, a lower OR was noted with higher dietary vitamin C intake and daily vitamin C supplementation for ASDNOS with amide exposure (aOR 0.79, 95% CI 0.30–2.04 vs. aOR 4.43, 95% CI 1.34–14.63); significant additive (AP 0.68, 95% CI 0.32–1.04) interaction was noted between total daily vitamin C intake and nitrosatable drug exposure in relation to these types of heart defects. Associations were also diminished between secondary and tertiary amine exposures and ASDNOS among births to women with higher dietary vitamin C intake coupled with daily vitamin C supplementation.

DISCUSSION

In this large, population-based case–control study, we found that daily vitamin C supplementation reduced associations between nitrosatable drug exposure during the first trimester and several types of birth defects in offspring. Women who reported taking a daily vitamin C supplement appeared to have lower odds of having infants with several birth defects associated with nitrosatable drug exposure compared with those who reported no vitamin C supplement use. With daily use of supplements containing vitamin C, ORs were lower for transverse limb deficiency in conjunction with secondary amine drug exposures, cleft lip without cleft palate with tertiary amine exposures, and several congenital heart defects in conjunction with tertiary amine and amide drug exposures. Conversely, higher ORs were observed for longitudinal limb deficiency and right ventricular outflow tract obstruction in relation to secondary amine drug exposure, and cleft palate alone, and atrial septal defect secundum with amide drug exposure.

Associations between nitrosatable drug use and birth defects were diminished less with higher dietary intake of vitamin C than those noted with daily vitamin C supplementation. However, higher ORs were observed for several birth defects across nitrosatable drug groups with higher dietary vitamin C intake. For ASDNOS, higher ORs were noted among women with higher dietary vitamin C intake compared with those with <85 mg/day in relation to tertiary amine drug exposure. The ORs were notably higher with several birth defects among women with higher intake of dietary vitamin C in relation to amide drug exposure. Total vitamin C modified the association between nitrosatable drug exposure and several birth defects, with lower ORs observed for limb deficiencies and atrial septal defects (not otherwise specified) among births to women with higher dietary vitamin C intake and daily supplementation.

Although maternal multivitamin use has been reported to reduce the risks for limb deficiencies, congenital heart defects, most notably conotruncal heart defects, and oral cleft defects (cleft with or without cleft palate, cleft palate only) (Shaw et al., 1995; Yang et al., 1997; Werler et al., 1999; Botto et al., 2000; Shaw et al., 2000), the effects of supplementation with an overall multivitamin or vitamin C in particular on reducing risk of birth defects in relation to nitrosatable drug exposure during pregnancy have been rarely studied. Only one published study examined the effects of vitamin C supplementation on risk of neural tube births among women exposed to nitrosatable drugs during the periconceptional period (Brender et al., 2011b). Women exposed to tertiary amine drugs had lower risk for anencephalic births if they took a daily vitamin C supplement (OR 1.5, 95%

CI 0.86–2.7) compared with less than daily (OR 2.8, 95% CI 1.5–5.2) or no vitamin C supplements (OR 2.1, 95% CI 1.3–3.6). In the present study, we found lower associations for transverse limb deficiencies, cleft lip without cleft palate, and several heart defects with daily compared with no vitamin C supplementation in relation to nitrosatable drug exposure during the first trimester of pregnancy.

The present study has several strengths. Data were used from the NBDPS, one of the largest collaborative population-based studies of birth defects in the United States. Case classifications were standardized (Rasmussen et al., 2003), and each was reviewed by clinical geneticists; those with congenital heart defects were also reviewed by clinicians with expertise in pediatric cardiology and further classified depending on their complexity (Botto et al., 2007). Lastly, the median time of interview from EDD was comparable between case and control mothers.

The findings in our study are subject to several limitations. The proportion of non-response varied between cases and controls with approximately 24% to 31% of eligible case mothers and one third of the eligible control mothers not participating in the study. Cogswell et al. (2009) evaluated the representativeness of control participants in the NBDPS and found them to be representative of their base populations with respect to age, previous live births, and smoking. Slight differences were noted for maternal race/ethnicity, education, and infant characteristics especially if controls were selected from hospitals compared with birth certificates. Although, the absolute difference in the distribution of maternal race/ethnicity between the control participants and base population exceeded five percentage points, the differences seemed attributable to participation rather than selection of participants.

Another limitation in our study pertains to the potential maternal recall bias of drug exposures. Mothers of infants with birth defects may more likely recall drug exposures during the first trimester in comparison with mothers of infants without malformations. Some studies have found little evidence for differential recall of drugs classified as nitrosatable in the present study (Feldman et al., 1989; Werler et al., 1989; Delgado-Rodriguez et al., 1995). No difference in overall recall was observed for several drugs containing nitrosatable compounds including analgesics, antibiotics, and benzodiazepines between women with normal or adverse pregnancy outcomes (Feldman et al., 1989). The sensitivity and specificity of recall for antibiotic and antinauseant drug use was observed to be similar for cases and controls (Delgado-Rodriguez et al., 1995). In contrast, Werler et al. (1989) reported sensitivity of exposure for antibiotic drug use 20% higher in case mothers compared with control mothers. To reduce recall bias, the NBDPS utilized a two-level approach to assess drug use by asking participants about drug use by indication and medication name. Use of a standardized questionnaire on drug use by indication and medication has shown to be more accurate than an open-ended questionnaire (Mitchell et al., 1986; Werler et al., 2011). In the NBDPS, women were asked about medication use during pregnancy and the drugs were later classified into secondary amines, tertiary amines, and amides depending on their nitrosatability. Because women were not questioned specifically about nitrosatable drug use, recall bias would be unlikely overall with this exposure; however, it is possible that some types of drugs within the various categories of nitrosatable drugs might have been recalled differentially between case- and control-women.

Because participants were interviewed about the frequency of foods consumed a year before conception, possible recall inaccuracy could lead to misclassification of foods consumed during the first trimester of pregnancy. But such misclassification might be non-differential with respect to the outcomes as the same period of dietary assessment was used for all NBDPS participants. Studies have shown that consumption patterns of meats and vegetables do not significantly differ before and during pregnancy (Cuco et al., 2006), and strong correlations have been reported between healthy dietary patterns during pregnancy and nutrient intake such as vitamin C (Northstone et al., 2008).

In the NBDPS, women were interviewed about their medications and supplement use 3 months preconception and during pregnancy. Questions concerning diet were collected a year before pregnancy. However, they were not asked about the specific timing of dietary or supplemental vitamin C intake in relation to nitrosatable drug use. Vitamin C is known to inhibit *N*-nitroso compound formation when administered concurrently with a nitrosatable precursor. Since the timing of vitamin C intake could not be ascertained, the reduced risk observed for selected birth defects may not be due to the effect of vitamin C itself, but could be due to other factors or healthy behaviors correlated with vitamin C supplementation or higher dietary intake of vitamin C.

Furthermore, in addition to single vitamin C supplements, prenatal vitamins and multivitamins both contain vitamin C. This would cause an overlap between vitamin C contributions from different sources of supplementation. In this study, women who reported taking a single vitamin C preparation, prenatal vitamins, or multivitamins were considered to have taken a vitamin C supplement. During the second month of pregnancy, approximately 1.36% of women took a daily single vitamin C supplement and 0.8% less than daily. The remaining obtained their vitamin C supplementation from either a multivitamin or prenatal vitamin. In addition, any effect modification by supplemental vitamin C could be due to other nutrients, such as vitamin E, that are commonly part of prenatal or multivitamins. For the defects selected in this study, we defined exposure to nitrosatable drugs as taking any such drugs during the first trimester. Future studies might examine nitrosatable drug use by month as well as the first trimester since several birth defects have critical windows of development which occur within a certain month.

Another limitation was the small sample sizes available for some of the selected birth defects after stratifying by supplemental or dietary vitamin C. Among women who reported taking no vitamin C supplements, we were able to detect ORs for secondary or tertiary amine drug exposure as low as 1.5 for conotruncal heart defects with 80% power, but for longitudinal limb deficiencies, the smallest detectable OR at the same power level was 2.3. Among women with low dietary intake of vitamin C, detectable ORs for secondary or tertiary amine drug exposures ranged from 1.4 for conotruncal heart defect to 1.9 for longitudinal limb deficiencies with 80% power.

Multiple analyses were performed to test the effect of supplement or dietary vitamin C on the relation between nitrosatable drugs and birth defects. Forty-five statistical tests were conducted to assess the interaction between nitrosatable drugs and vitamin C supplementation. Seven statistically significant interactions were observed while only two

would be expected by chance alone. For dietary vitamin C, two statistically significant interactions were noted; however, two tests are expected by chance alone.

In conclusion, findings from this study give support to the hypothesis that vitamin C supplementation may decrease associations between nitrosatable drug exposure during the first trimester of pregnancy and selected birth defects. To our knowledge this is one of the first studies to examine the effect of vitamin C on the relation between nitrosatable drugs and selected birth defects. Further research is needed to examine the role of vitamin C in reducing the potential risks of birth defects associated with nitrosatable compounds, especially with respect to timing of supplement use in conjunction with intake of nitrosatable drugs and the effects of dietary intake of vitamin C with more sensitive instruments of dietary assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Selected Maternal Characteristics of Limb, Oral Cleft, and Heart Defects Cases and Controls in the National Birth Defects Prevention Study, 1997–2005

	Controls (*n* = 6807)		Limb deficiency defects (*n* = 741)		Oral cleft defects (*n* = 2774)		Congenital heart defects (*n* = 8091)	
Characteristics of participants	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Race-ethnicity								
Non-Hispanic white	4026	59.1	416	56.1	1724	62.2	4698	58.1
Non-Hispanic black	771	11.3	74	10.0	183	6.6	930	11.5
Hispanic	1502	22.1	204	27.5	659	23.8	1868	23.1
Asian/Pacific Islander	200	2.9	14	1.9	82	3.0	216	2.7
Native American and Other	279	4.1	33	4.5	118	4.3	361	4.5
Missing	29	0.4	0	0	8	0.3	18	0.2
Education (yrs)								
<12	1130	16.6	126	17.0	525	18.9	1399	17.3
12	1652	24.3	200	27.0	778	28.1	2091	25.8
13–15	1806	26.5	208	28.1	731	26.4	2196	27.1
>15	2110	31.0	195	26.3	711	25.6	2270	28.1
Missing	109	1.6	12	1.6	29	1.1	135	1.7
Age at delivery (yrs)								
<18	255	3.8	31	4.2	90	3.2	271	3.4
18–19	478	7.0	59	8.0	200	7.2	489	6.0
20–24	1552	22.8	188	25.4	704	25.4	1842	22.8
25–29	1807	26.6	190	25.6	738	26.6	2130	26.3
30–34	1759	25.8	185	25.0	626	22.6	2086	25.8
>34	956	14.0	88	11.9	416	15.0	1272	15.7
Missing	0	0	0	0	0	0	1	0
Study center								
Arkansas	848	12.5	70	9.5	310	11.2	1208	14.9
California	858	12.6	120	16.2	450	16.2	864	10.7
Georgia	735	10.8	78	10.5	333	12.0	994	12.3
Iowa	759	11.2	80	10.8	306	11.0	769	9.5
Massachusetts	859	12.6	92	12.4	389	14.0	1102	13.6

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Characteristics of participants	Controls (*n* = 6807)		Limb deficiency defects (*n* = 741)		Oral cleft defects (*n* = 2774)		Congenital heart defects (*n* = 8091)	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
North Carolina	412	6.1	16	2.2	101	3.6	295	3.6
New Jersey	574	8.4	84	11.3	192	6.9	548	6.8
New York	601	8.8	54	7.3	249	9.0	580	7.2
Texas	792	11.6	97	13.1	348	12.6	1242	15.4
Utah	369	5.4	50	6.7	96	3.5	489	6.0
Smoking[a]								
No	5444	80.0	582	78.5	2074	74.8	6344	78.4
Yes	1274	18.7	150	20.2	675	24.3	1629	20.1
Missing	89	1.3	9	1.2	25	0.9	118	1.5
Body mass index (kg/m^2)								
<18.5	361	5.3	42	5.7	187	6.7	420	5.2
18.5–24.9	3640	53.5	358	48.3	1402	50.5	3911	48.3
25.0–29.9	1463	21.5	174	23.5	580	20.9	1809	22.4
>29.9	1055	15.5	130	17.5	482	17.4	1588	19.6
Missing	288	4.2	37	5.0	123	4.4	363	4.5
Folic acid-containing supplement use[b]								
No	860	12.6	97	13.1	416	15.0	1101	13.6
Yes	5782	84.9	621	83.8	2299	82.9	6766	83.6
Missing	165	2.4	23	3.1	59	2.1	224	2.8
Multivitamin use[b]								
No	785	11.5	92	12.4	371	13.4	999	12.3
Yes	5865	86.2	629	84.9	2350	84.7	6882	85.1
Missing	157	2.3	20	2.7	53	1.9	210	2.6
Vitamin C supplementation[c]								
None	1764	25.9	192	25.9	752	27.1	2117	26.2
<Daily	1174	17.3	114	15.4	490	17.7	1383	17.1
Daily	3834	56.3	432	58.3	1523	54.9	4546	56.2
Missing	35	0.5	3	0.4	9	0.3	45	0.6

[a]Any smoking 1 month before conception through the first trimester of pregnancy.

[b] Refers to use during the first trimester of pregnancy.

[c] Refers to use during the second month of pregnancy.

Table 2

Effects of Maternal Nitrosatable Drug Exposures During the First Trimester of Pregnancy on Limb Deficiencies and Oral Clefts Stratified by Vitamin C Supplementation, National Birth Defects Prevention Study, 1997–2005

			Cases		Controls					
Type of malformation	**Frequency of Vitamin C supplement[a]**	**Type of drug exposure**	**No.**	**%[b]**	**No.**	**%[b]**	**Unadjusted OR[c]**	**95% CI**	**Adjusted OR[c]**	**95% CI**
Any limb deficiency[d,e]	None	No nitrosatable drug exposure	123	74.1	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	29	19.1	155	11.1	1.88	1.22–2.92	1.99	1.26–3.15
		Tertiary amines	17	12.1	135	9.8	1.27	0.74–2.17	1.53	0.86–2.71
		Amides	10	7.5	88	6.6	1.14	0.58–2.26	1.32	0.65–2.70
	Less than daily	No nitrosatable drug exposure	74	71.2	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	14	15.9	145	15.3	1.05	0.58–1.91	1.09	0.59–2.03
		Tertiary amines	16	17.8	173	17.7	1.01	0.57–1.77	1.15	0.64–2.07
		Amides	11	12.9	95	10.6	1.26	0.65–2.46	1.28	0.63–2.57
	Daily	No nitrosatable drug exposure	295	72.0	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	71	19.4	513	16.0	1.26	0.96–1.67	1.33	1.00–1.77
		Tertiary amines	52	15.0	493	15.5	0.96	0.71–1.31	1.06	0.77–1.45
		Amides	36	10.9	320	10.6	1.03	0.71–1.48	1.07	0.74–1.55
Longitudinal limb deficiency[d,e]	None	No nitrosatable drug exposure	51	77.3	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	8	13.6	155	11.1	1.25	0.58–2.69	1.21	0.54–2.72
		Tertiary amines	8	13.6	135	9.8	1.44	0.67–3.10	1.66	0.72–3.79
		Amides	1	1.9	88	6.6	0.28	0.04–2.02	0.31	0.04–2.34
	Less than daily	No nitrosatable drug exposure	26	76.5	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	5	16.1	145	15.3	1.07	0.40–2.83	1.14	0.41–3.16
		Tertiary amines	3	10.3	173	17.7	0.54	0.16–1.79	0.66	0.19–2.32
		Amides	4	13.3	95	10.6	1.30	0.45–3.82	1.27	0.40–4.02
	Daily	No nitrosatable drug exposure	114	69.5	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	33	22.5	513	16.0	1.52	1.02–2.27	1.59	1.05–2.39
		Tertiary amines	20	14.9	493	15.5	0.96	0.59–1.56	0.99	0.60–1.64
		Amides	15	11.6	320	10.6	1.11	0.64–1.92	1.15	0.66–2.00
Transverse limb deficiency[d,e]	None	No nitrosatable drug exposure	68	70.8	1238	80.8	1.00	Referent	1.00	Referent

Type of malformation	Frequency of Vitamin C supplement[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c]	95% CI
			No.	%[b]	No.	%[b]				
		Secondary amines	21	23.6	155	11.1	2.47	1.47–4.14	2.65[g]	1.54–4.55
		Tertiary amines	9	11.7	135	9.8	1.21	0.59–2.49	1.50	0.70–3.21
		Amides	9	11.7	88	6.6	1.86	0.90–3.86	2.21	1.02–4.80
	Less than daily	No nitrosatable drug exposure	46	69.7	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	8	14.8	145	15.3	0.97	0.45–2.09	0.96[g]	0.43–2.14
		Tertiary amines	11	19.3	173	17.7	1.11	0.56–2.19	1.24	0.61–2.53
		Amides	7	13.2	95	10.6	1.29	0.57–2.94	1.38	0.58–3.28
	Daily	No nitrosatable drug exposure	164	72.9	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	35	17.6	513	16.0	1.12	0.77–1.63	1.23[g]	0.83–1.81
		Tertiary amines	28	14.6	493	15.5	0.93	0.62–1.41	1.10	0.72–1.67
		Amides	20	10.9	320	10.6	1.03	0.64–1.66	1.10	0.67–1.78
Cleft lip alone[f]	None	No nitrosatable drug exposure	103	76.3	1221	80.5	1.00	Referent	1.00	Referent
		Secondary amines	19	15.6	155	11.3	1.45	0.87–2.44	1.23	0.72–2.10
		Tertiary amines	18	14.9	135	10.0	1.58	0.93–2.69	1.42	0.81–2.49
		Amides	8	7.2	88	6.7	1.08	0.51–2.28	0.99	0.45–2.16
	Less than daily	No nitrosatable drug exposure	73	70.9	789	73.9	1.00	Referent	1.00	Referent
		Secondary amines	17	18.9	137	14.8	1.34	0.77–2.34	1.37	0.76–2.47
		Tertiary amines	15	17.1	164	17.2	0.99	0.55–1.77	1.06	0.58–1.94
		Amides	10	12.1	94	10.7	1.15	0.57–2.30	1.13	0.55–2.33
	Daily	No nitrosatable drug exposure	254	72.6	2632	73.9	1.00	Referent	1.00	Referent
		Secondary amines	56	18.1	500	16.0	1.16	0.86–1.57	1.08	0.79–1.48
		Tertiary amines	45	15.1	484	15.5	0.96	0.69–1.34	0.98	0.70–1.38
		Amides	36	12.4	315	10.7	1.18	0.82–1.71	1.18	0.81–1.71
Cleft lip with cleft palate[f]	None	No nitrosatable drug exposure	147	78.6	706	81.1	1.00	Referent	1.00	Referent
		Secondary amines	17	10.4	80	10.2	1.02	0.59–1.77	1.01	0.56–1.79
		Tertiary amines	24	14.0	81	10.3	1.42	0.87–2.32	1.50	0.89–2.54
		Amides	17	10.4	46	6.1	1.77	0.99–3.18	1.78	0.95–3.34
	Less than daily	No nitrosatable drug exposure	212	78.5	1138	74.8	1.00	Referent	1.00	Referent
		Secondary amines	27	11.3	198	14.8	0.73	0.48–1.12	0.76	0.49–1.18

Type of malformation	Frequency of Vitamin C supplement[a]	Type of drug exposure	Cases No.	Cases %[b]	Controls No.	Controls %[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c]	95% CI
		Tertiary amines	30	12.4	219	16.1	0.74	0.49–1.11	0.81	0.53–1.23
		Amides	26	10.9	124	9.8	1.13	0.72–1.76	1.22	0.77–1.95
	Daily	No nitrosatable drug exposure	413	71.6	2586	74.0	1.00	Referent	1.00	Referent
		Secondary amines	84	16.9	490	15.9	1.07	0.83–1.38	1.05	0.80–1.36
		Tertiary amines	96	18.9	468	15.3	1.28	1.01–1.64	1.30	1.01–1.67
		Amides	53	11.4	309	10.7	1.07	0.79–1.46	1.04	0.76–1.43
Cleft palate alone[f]	None	No nitrosatable drug exposure	114	83.8	706	81.1	1.00	Referent	1.00	Referent
		Secondary amines	13	10.2	80	10.2	1.00	0.54–1.87	0.89	0.47–1.69
		Tertiary amines	15	11.6	81	10.3	1.15	0.64–2.06	1.14	0.61–2.13
		Amides	7	5.8	46	6.1	0.94	0.42–2.14	0.94	0.40–2.22
	Less than daily	No nitrosatable drug exposure	148	69.5	1138	74.8	1.00	Referent	1.00	Referent
		Secondary amines	37	20.0	198	14.8	1.44	0.97–2.12	1.40	0.93–2.10
		Tertiary amines	38	20.4	219	16.1	1.33	0.91–1.96	1.30	0.87–1.95
		Amides	23	13.5	124	9.8	1.43	0.89–2.30	1.41	0.85–2.32
	Daily	No nitrosatable drug exposure	358	72.6	2586	74.0	1.00	Referent	1.00	Referent
		Secondary amines	69	16.2	490	15.9	1.02	0.77–1.34	0.98	0.74–1.30
		Tertiary amines	58	13.9	468	15.3	0.90	0.67–1.20	0.92	0.68–1.25
		Amides	56	13.5	309	10.7	1.31	0.96–1.78	1.31	0.96–1.79

[a] Vitamin C supplementation during the second month post-conception was used for limb deficiencies and cleft lip without cleft palate, and during the second and third month post-conception for cleft lip with cleft palate and cleft palate.

[b] Percentages for no nitrosatable drug exposure are based on total participants with complete information, while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d] Any limb deficiency includes longitudinal, transverse, intercalary, and not elsewhere classified limb deficiencies.

[e] Adjusted for maternal age, race/ethnicity, education, BMI, alcohol use, and study center.

[f] Adjusted for maternal age, race/ethnicity, education, smoking status, BMI, alcohol use, and study center.

[g] Significant multiplicative interaction ($p < 0.05$).

OR, odds ratio; CI, confidence interval.

Table 3

Effects of Maternal Nitrosatable Drug Exposures During the First Trimester of Pregnancy on Isolated Congenital Heart Defects Stratified by Vitamin C Supplementation, National Birth Defects Prevention Study, 1997–2005

			Cases		Controls					
Type of malformation[a]	**Frequency of Vitamin C supplement**[b]	**Type of drug exposure**	**No.**	**%**[c]	**No.**	**%**[c]	**Unadjusted OR**[d]	**95% CI**	**Adjusted OR**[d,e]	**95% CI**
Conotruncal defects	None	No nitrosatable drug exposure	201	79.5	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	22	9.9	155	11.1	0.87	0.55–1.40	0.83	0.51–1.34
		Tertiary amines	28	12.2	135	9.8	1.28	0.83–1.97	1.26[f,g]	0.80–1.98
		Amides	17	7.8	88	6.6	1.19	0.69–2.04	1.14	0.65–1.99
	Less than daily	No nitrosatable drug exposure	114	75.0	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	13	10.2	145	15.3	0.63	0.35–1.15	0.63	0.34–1.16
		Tertiary amines	25	18.0	173	17.7	1.02	0.64–1.62	1.09[f,g]	0.68–1.77
		Amides	11	8.8	95	10.6	0.82	0.43–1.57	0.81	0.41–1.59
	Daily	No nitrosatable drug exposure	440	75.3	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	78	15.1	513	16.0	0.93	0.72–1.21	0.90	0.69–1.18
		Tertiary amines	69	13.6	493	15.5	0.86	0.65–1.12	0.86[f,g]	0.65–1.13
		Amides	52	10.6	320	10.6	1.00	0.73–1.36	0.99	0.72–1.35
Right ventricular outflow tract obstruction	None	No nitrosatable drug exposure	137	81.6	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	15	9.9	155	11.1	0.87	0.50–1.53	0.83	0.47–1.48
		Tertiary amines	21	13.3	135	9.8	1.41	0.86–2.30	1.19	0.71–2.00
		Amides	13	8.7	88	6.6	1.33	0.73–2.45	1.35[f,g]	0.72–2.55
	Less than daily	No nitrosatable drug exposure	104	75.4	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	18	14.8	145	15.3	0.96	0.57–1.63	0.88	0.51–1.52
		Tertiary amines	22	17.5	173	17.7	0.98	0.60–1.60	0.84	0.51–1.40
		Amides	14	11.9	95	10.6	1.14	0.63–2.07	1.08[f,g]	0.58–2.01
	Daily	No nitrosatable drug exposure	308	70.5	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	70	18.5	513	16.0	1.19	0.91–1.57	1.15	0.86–1.52
		Tertiary amines	69	18.3	493	15.5	1.22	0.93–1.62	1.21	0.91–1.61
		Amides	38	11.0	320	10.6	1.04	0.73–1.48	1.01[f,g]	0.70–1.45

Type of malformation[a]	Frequency of Vitamin C supplement[b]	Type of drug exposure	Cases		Controls		Unadjusted OR[d]	95% CI	Adjusted OR[d,e]	95% CI
			No.	%[c]	No.	%[c]				
Left ventricular outflow tract obstruction	None	No nitrosatable drug exposure	103	77.4	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	18	14.9	155	11.1	1.40	0.82–2.37	1.20	0.69–2.09
		Tertiary amines	14	12.0	135	9.8	1.25	0.69–2.24	1.17	0.63–2.17
		Amides	11	9.7	88	6.6	1.50	0.78–2.90	1.56	0.78–3.10
	Less than daily	No nitrosatable drug exposure	98	71.5	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	13	11.7	145	15.3	0.74	0.40–1.35	0.67	0.36–1.24
		Tertiary amines	29	22.8	173	17.7	1.38	0.88–2.15	1.26	0.79–2.01
		Amides	19	16.2	95	10.6	1.64	0.96–2.81	1.57	0.90–2.74
	Daily	No nitrosatable drug exposure	354	71.5	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	79	18.2	513	16.0	1.17	0.90–1.52	1.10	0.84–1.43
		Tertiary amines	82	18.8	493	15.5	1.27	0.98–1.64	1.19	0.91–1.56
		Amides	50	12.4	320	10.6	1.19	0.87–1.63	1.17	0.84–1.61
Hypoplastic left heart syndrome	None	No nitrosatable drug exposure	40	76.9	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	5	11.1	155	11.1	1.00	0.39–2.57	0.80	0.30–2.13
		Tertiary amines	7	14.9	135	9.8	1.60	0.71–3.65	1.45	0.61–3.46
		Amides	3	7.0	88	6.6	1.06	0.32–3.48	1.19	0.35–4.11
	Less than daily	No nitrosatable drug exposure	43	67.2	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	5	10.4	145	15.3	0.65	0.25–1.66	0.58	0.22–1.52
		Tertiary amines	16	27.1	173	17.7	1.73	0.95–3.15	1.66	0.89–3.12
		Amides	12	21.8	95	10.6	2.36	1.20–4.64	2.18	1.08–4.43
	Daily	No nitrosatable drug exposure	131	69.7	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	33	20.1	513	16.0	1.32	0.89–1.96	1.27	0.84–1.90
		Tertiary amines	34	20.6	493	15.5	1.42	0.96–2.09	1.34	0.90–2.00
		Amides	20	13.3	320	10.6	1.29	0.79–2.09	1.27	0.78–2.08
Aortic stenosis	None	No nitrosatable drug exposure	24	77.4	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	6	20.0	155	11.1	2.00	0.80–4.96	1.60	0.61–4.22
		Tertiary amines	3	11.1	135	9.8	1.15	0.34–3.86	0.94	0.26–3.40
		Amides	2	7.7	88	6.6	1.17	0.27–5.04	1.20	0.26–5.57
	Less than daily	No nitrosatable drug exposure	18	66.7	805	73.4	1.00	Referent	1.00	Referent

Type of malformation[a]	Frequency of Vitamin C supplement[b]	Type of drug exposure	Cases No.	Cases %[c]	Controls No.	Controls %[c]	Unadjusted OR[d]	95% CI	Adjusted OR[d,e]	95% CI
		Secondary amines	4	18.2	145	15.3	1.23	0.41–3.70	1.22	0.38–3.92
		Tertiary amines	8	30.8	173	17.7	2.07	0.88–4.83	1.83	0.73–4.60
		Amides	3	14.3	95	10.6	1.41	0.41–4.88	1.60	0.41–6.16
	Daily	No nitrosatable drug exposure	79	73.2	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	15	16.0	513	16.0	1.00	0.57–1.75	0.86	0.49–1.52
		Tertiary amines	17	17.7	493	15.5	1.18	0.69–2.00	1.01	0.58–1.74
		Amides	8	9.2	320	10.6	0.85	0.41–1.78	0.79	0.37–1.67
Septal defects	None	No nitrosatable drug exposure	435	79.7	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	47	9.8	155	11.1	0.86	0.61–1.22	0.81	0.56–1.16
		Tertiary amines	65	13.0	135	9.8	1.37	1.00–1.88	1.20[g]	0.86–1.67
		Amides	44	9.2	88	6.6	1.42	0.98–2.08	1.34	0.90–1.99
	Less than daily	No nitrosatable drug exposure	241	69.1	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	60	19.9	145	15.3	1.38	0.99–1.93	1.42	1.00–2.02
		Tertiary amines	58	19.4	173	17.7	1.12	0.80–1.56	1.07[g]	0.76–1.53
		Amides	39	13.9	95	10.6	1.37	0.92–2.04	1.39	0.91–2.13
	Daily	No nitrosatable drug exposure	817	72.8	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	150	15.5	513	16.0	0.96	0.79–1.18	0.95	0.78–1.17
		Tertiary amines	165	16.8	493	15.5	1.10	0.91–1.34	1.01[g]	0.83–1.24
		Amides	119	12.7	320	10.6	1.23	0.98–1.54	1.18	0.94–1.49
Perimembranous ventricular septal defect	None	No nitrosatable drug exposure	173	76.6	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	21	10.8	155	11.1	0.97	0.60–1.57	0.87	0.53–1.44
		Tertiary amines	34	16.4	135	9.8	1.80	1.20–2.71	1.43[f,g]	0.93–2.21
		Amides	23	11.7	88	6.6	1.87	1.15–3.04	1.65	1.00–2.74
	Less than daily	No nitrosatable drug exposure	99	68.3	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	26	20.8	145	15.3	1.46	0.91–2.33	1.42	0.88–2.31
		Tertiary amines	27	21.4	173	17.7	1.27	0.80–2.00	1.17[f,g]	0.73–1.89
		Amides	15	13.2	95	10.6	1.28	0.72–2.30	1.25	0.68–2.30
	Daily	No nitrosatable drug exposure	349	74.3	2695	73.9	1.00	Referent	1.00	Referent

Type of malformation[a]	Frequency of Vitamin C supplement[b]	Type of drug exposure	Cases No.	Cases %[c]	Controls No.	Controls %[c]	Unadjusted OR[d]	95% CI	Adjusted OR[d,e]	95% CI
		Secondary amines	59	14.5	513	16.0	0.89	0.66–1.19	0.89	0.66–1.20
		Tertiary amines	62	15.1	493	15.5	0.97	0.73–1.29	0.91[f,g]	0.68–1.22
		Amides	47	11.9	320	10.6	1.13	0.82–1.57	1.10	0.79–1.54
Atrial septal defect secundum	None	No nitrosatable drug exposure	168	84.0	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	11	6.2	155	11.1	0.52	0.28–0.98	0.53	0.27–1.01
		Tertiary amines	16	8.7	135	9.8	0.87	0.51–1.50	0.84	0.48–1.50
		Amides	13	7.2	88	6.6	1.09	0.59–1.99	1.19	0.63–2.26
	Less than daily	No nitrosatable drug exposure	82	70.1	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	22	21.2	145	15.3	1.49	0.90–2.46	1.43	0.83–2.45
		Tertiary amines	18	18.0	173	17.7	1.02	0.60–1.75	0.88	0.50–1.56
		Amides	13	13.7	95	10.6	1.34	0.72–2.50	1.25	0.65–2.43
	Daily	No nitrosatable drug exposure	305	70.6	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	67	18.0	513	16.0	1.16	0.87–1.53	1.09	0.81–1.46
		Tertiary amines	69	18.5	493	15.5	1.24	0.94–1.63	1.06	0.79–1.42
		Amides	50	14.1	320	10.6	1.38	1.00–1.90	1.34	0.96–1.88
Atrial septal defect, not otherwise specified	None	No nitrosatable drug exposure	54	69.2	1238	80.8	1.00	Referent	1.00	Referent
		Secondary amines	14	20.6	155	11.1	2.07	1.12–3.82	2.68	1.34–5.37
		Tertiary amines	14	20.6	135	9.8	2.38	1.29–4.39	2.70[g]	1.33–5.49
		Amides	8	12.9	88	6.6	2.08	0.96–4.52	2.90[f,g]	1.22–6.93
	Less than daily	No nitrosatable drug exposure	41	71.9	805	73.4	1.00	Referent	1.00	Referent
		Secondary amines	6	12.8	145	15.3	0.81	0.34–1.95	1.19	0.45–3.13
		Tertiary amines	7	14.6	173	17.7	0.79	0.35–1.80	1.03[g]	0.41–2.60
		Amides	8	16.3	95	10.6	1.65	0.75–3.63	2.48[f,g]	0.97–6.35
	Daily	No nitrosatable drug exposure	95	75.4	2695	73.9	1.00	Referent	1.00	Referent
		Secondary amines	12	11.2	513	16.0	0.66	0.36–1.22	0.74	0.40–1.40
		Tertiary amines	22	18.8	493	15.5	1.27	0.79–2.03	1.35[g]	0.81–2.24
		Amides	12	11.2	320	10.6	1.06	0.58–1.96	1.12[f,g]	0.59–2.11

[a]Defects are simple heart defects without major extracardiac malformations.

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[b] Vitamin supplement intake during the second month post-conception.

[c] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[d] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[e] Adjusted for maternal race/ethnicity, education, smoking status, BMI, alcohol use, and study center.

[f] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

[g] Significant multiplicative interaction (p <0.05).

OR, odds ratio; CI, confidence interval.

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Table 4

Effect of Maternal Nitrosatable Drug Exposures During the First Trimester of Pregnancy on Limb Deficiencies and Oral Clefts Stratified by Dietary Vitamin C, National Birth Defects Prevention Study, 1997–2005

			Cases		Controls					
Type of malformation	**Dietary Vitamin C (mg/d)**	**Type of drug exposure**	**No.**	**%[a]**	**No.**	**%[a]**	**Unadjusted OR[b]**	**95% CI**	**Adjusted OR[b]**	**95% CI**
Any limb deficiency[c,d]	<85	No nitrosatable drug exposure	197	66.6	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	63	24.2	372	16.9	1.57	1.16–2.13	1.71	1.25–2.35
		Tertiary amines	51	20.6	391	17.6	1.21	0.87–1.68	1.32	0.94–1.85
		Amides	27	12.1	231	11.2	1.09	0.71–1.66	1.11	0.72–1.72
	≥85	No nitrosatable drug exposure	275	76.4	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	49	15.1	426	13.4	1.15	0.84–1.59	1.21	0.87–1.69
		Tertiary amines	33	10.7	393	12.5	0.84	0.58–1.23	0.93	0.63–1.36
		Amides	29	9.5	261	8.6	1.12	0.75–1.67	1.22	0.81–1.84
Longitudinal limb deficiency[c,d]	<85	No nitrosatable drug exposure	77	65.3	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	28	26.7	372	16.9	1.79	1.14–2.79	1.93[f]	1.21–3.07
		Tertiary amines	19	19.8	391	17.6	1.15	0.69–1.93	1.19	0.70–2.04
		Amides	9	10.5	231	11.2	0.93	0.46–1.87	0.95	0.46–1.95
	≥85	No nitrosatable drug exposure	104	76.5	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	18	14.8	426	13.4	1.12	0.67–1.87	1.15[f]	0.68–1.95
		Tertiary amines	12	10.3	393	12.5	0.81	0.44–1.49	0.86	0.46–1.60
		Amides	11	9.6	261	8.6	1.12	0.59–2.11	1.22	0.64–2.34
Transverse limb deficiency[c,d]	<85	No nitrosatable drug exposure	113	67.7	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	33	22.6	372	16.9	1.44	0.96–2.15	1.60	1.05–2.42
		Tertiary amines	30	21.0	391	17.6	1.24	0.82–1.88	1.43	0.93–2.21
		Amides	15	11.7	231	11.2	1.05	0.60–1.83	1.09	0.62–1.92
	≥85	No nitrosatable drug exposure	155	74.9	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	29	15.8	426	13.4	1.21	0.80–1.83	1.36	0.89–2.08
		Tertiary amines	18	10.4	393	12.5	0.82	0.49–1.34	0.97	0.58–1.63
		Amides	20	11.4	261	8.6	1.36	0.84–2.21	1.55	0.95–2.55
Cleft lip alone[e]	<85	No nitrosatable drug exposure	176	69.6	1791	72.3	1.00	Referent	1.00	Referent

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			Cases		Controls					
Type of malformation	**Dietary Vitamin C (mg/d)**	**Type of drug exposure**	**No.**	**%[a]**	**No.**	**%[a]**	**Unadjusted OR[b]**	**95% CI**	**Adjusted OR[b]**	**95% CI**
		Secondary amines	49	21.8	363	16.9	1.37	0.98–1.92	1.39	0.99–1.97
		Tertiary amines	37	17.4	379	17.5	0.99	0.69–1.44	1.04	0.71–1.52
		Amides	21	10.7	226	11.2	0.95	0.59–1.52	0.99	0.61–1.60
	≥85	No nitrosatable drug exposure	244	75.8	2703	77.6	1.00	Referent	1.00	Referent
		Secondary amines	41	14.4	412	13.2	1.10	0.78–1.56	0.96	0.67–1.38
		Tertiary amines	40	14.1	386	12.5	1.15	0.81–1.63	1.08	0.75–1.55
		Amides	33	11.9	257	8.7	1.42	0.97–2.09	1.35	0.91–2.01
Cleft lip with cleft palate[e]	<85	No nitrosatable drug exposure	338	70.6	1791	72.3	1.00	Referent	1.00	Referent
		Secondary amines	72	17.6	363	16.9	1.05	0.80–1.39	1.05	0.79–1.40
		Tertiary amines	79	18.9	379	17.5	1.10	0.84–1.45	1.12	0.85–1.49
		Amides	46	12.0	226	11.2	1.08	0.77–1.51	1.09	0.77–1.54
	≥85	No nitrosatable drug exposure	448	77.6	2703	77.6	1.00	Referent	1.00	Referent
		Secondary amines	60	11.9	412	13.2	0.88	0.66–1.17	0.89	0.66–1.20
		Tertiary amines	74	14.2	386	12.5	1.16	0.88–1.51	1.27	0.96–1.68
		Amides	52	10.4	257	8.7	1.22	0.89–1.67	1.30	0.94–1.81
Cleft palate alone[e]	<85	No nitrosatable drug exposure	278	72.0	1791	72.3	1.00	Referent	1.00	Referent
		Secondary amines	62	18.2	363	16.9	1.10	0.82–1.48	1.10	0.81–1.50
		Tertiary amines	61	18.0	379	17.5	1.04	0.77–1.40	1.08	0.79–1.47
		Amides	39	12.3	226	11.2	1.11	0.77–1.60	1.17	0.81–1.71
	≥85	No nitrosatable drug exposure	353	75.4	2703	77.6	1.00	Referent	1.00	Referent
		Secondary amines	56	13.7	412	13.2	1.04	0.77–1.41	0.97	0.71–1.32
		Tertiary amines	51	12.6	386	12.5	1.01	0.74–1.38	0.99	0.72–1.37
		Amides	48	12.0	257	8.7	1.43	1.03–1.98	1.34	0.96–1.87

[a] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b] Crude and adjusted odds ratio include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[c] Any limb deficiency includes longitudinal, transverse, intercalary, and not elsewhere classified limb deficiencies.

[d] Adjusted for maternal age, race/ethnicity, education, caloric intake, study center, BMI, alcohol use, and multivitamin use during the first trimester of pregnancy.

[e] Adjusted for maternal age, race/ethnicity, education, smoking status, study center, caloric intake, BMI, alcohol use, and folic acid supplementation during first trimester of pregnancy.

[f] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

OR, odds ratio; CI, confidence interval.

Table 5

Effect of Maternal Nitrosatable Drug Exposures During the First Trimester of Pregnancy on Congenital Heart Defects Stratified by Dietary Vitamin C, National Birth Defects Prevention Study, 1997–2005

			Cases		Controls					
Type of malformation[a]	**Dietary Vitamin C (mg/d)**	**Type of drug exposure**	**No.**	**%[b]**	**No.**	**%[b]**	**Unadjusted OR[c]**	**95% CI**	**Adjusted OR[c,d]**	**95% CI**
Conotruncal defects	<85	No nitrosatable drug exposure	303	75.4	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	54	15.1	372	16.9	0.88	0.64–1.19	0.87	0.63–1.19
		Tertiary amines	50	14.2	391	17.6	0.77	0.56–1.06	0.79	0.57–1.10
		Amides	30	9.0	231	11.2	0.78	0.53–1.17	0.80	0.53–1.20
	≥85	No nitrosatable drug exposure	423	77.3	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	54	11.3	426	13.4	0.83	0.61–1.12	0.77	0.57–1.05
		Tertiary amines	66	13.5	393	12.5	1.10	0.83–1.45	1.06	0.80–1.42
		Amides	47	10.0	261	8.6	1.17	0.85–1.63	1.17	0.84–1.63
Right ventricular outflow tract obstruction	<85	No nitrosatable drug exposure	250	74.6	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	50	16.7	372	16.9	0.98	0.71–1.36	0.98	0.70–1.37
		Tertiary amines	45	15.3	391	17.6	0.84	0.60–1.18	0.81	0.57–1.14
		Amides	29	10.4	231	11.2	0.92	0.61–1.38	0.89	0.58–1.34
	≥85	No nitrosatable drug exposure	283	73.1	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	51	15.3	426	13.4	1.17	0.85–1.60	1.10	0.79–1.51
		Tertiary amines	63	18.2	393	12.5	1.56	1.17–2.10	1.46	1.07–1.97
		Amides	32	10.2	261	8.6	1.20	0.81–1.76	1.18	0.79–1.74
Left ventricular outflow tract obstruction	<85	No nitrosatable drug exposure	242	69.3	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	59	19.6	372	16.9	1.20	0.88–1.63	1.13	0.83–1.55
		Tertiary amines	67	21.7	391	17.6	1.30	0.97–1.73	1.25	0.92–1.68
		Amides	38	13.6	231	11.2	1.24	0.86–1.80	1.21	0.83–1.76
	≥85	No nitrosatable drug exposure	297	75.6	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	48	13.9	426	13.4	1.05	0.76–1.44	0.94	0.67–1.31
		Tertiary amines	57	16.1	393	12.5	1.35	1.00–1.82	1.23	0.90–1.68
		Amides	37	11.1	261	8.6	1.32	0.91–1.90	1.31	0.90–1.90
Hypoplastic left heart syndrome	<85	No nitrosatable drug exposure	92	67.2	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	19	17.1	372	16.9	1.02	0.61–1.68	0.98	0.59–1.65

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Type of malformation[a]	Dietary Vitamin C (mg/d)	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
		Tertiary amines	32	25.8	391	17.6	1.63	1.07–2.47	1.57	1.02–2.41
		Amides	16	14.8	231	11.2	1.38	0.80–2.38	1.34	0.76–2.34
	≥85	No nitrosatable drug exposure	116	73.4	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	23	16.6	426	13.4	1.28	0.81–2.03	1.14	0.71–1.83
		Tertiary amines	25	17.7	393	12.5	1.51	0.97–2.36	1.38	0.87–2.19
		Amides	16	12.1	261	8.6	1.46	0.85–2.50	1.46	0.84–2.53
Aortic stenosis	<85	No nitrosatable drug exposure	59	67.8	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	16	21.3	372	16.9	1.33	0.76–2.34	1.21	0.68–2.17
		Tertiary amines	19	24.4	391	17.6	1.51	0.89–2.56	1.32	0.76–2.28
		Amides	9	13.2	231	11.2	1.21	0.59–2.47	1.08[e]	0.52–2.24
	≥85	No nitrosatable drug exposure	62	79.5	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	9	12.7	426	13.4	0.94	0.46–1.91	0.77	0.37–1.59
		Tertiary amines	9	12.7	393	12.5	1.02	0.50–2.07	0.92	0.44–1.89
		Amides	3	4.6	261	8.6	0.51	0.16–1.64	0.48[e]	0.15–1.55
Septal defects	<85	No nitrosatable drug exposure	620	72.1	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	121	16.3	372	16.9	0.96	0.77–1.20	0.97	0.77–1.23
		Tertiary amines	132	17.6	391	17.6	1.00	0.80–1.24	0.96	0.76–1.20
		Amides	98	13.7	231	11.2	1.25	0.97–1.61	1.24	0.95–1.61
	≥85	No nitrosatable drug exposure	830	75.5	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	129	13.5	426	13.4	1.01	0.81–1.24	1.00	0.80–1.25
		Tertiary amines	150	15.3	393	12.5	1.27	1.04–1.56	1.19	0.96–1.48
		Amides	99	10.7	261	8.6	1.26	0.99–1.61	1.26	0.98–1.62
Perimembranous ventricular septal defect	<85	No nitrosatable drug exposure	255	72.7	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	48	15.8	372	16.9	0.93	0.67–1.28	0.92	0.66–1.29
		Tertiary amines	54	17.5	391	17.6	0.99	0.72–1.35	0.93	0.67–1.28
		Amides	39	13.3	231	11.2	1.21	0.84–1.74	1.18	0.81–1.72
	≥85	No nitrosatable drug exposure	347	75.1	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	55	13.7	426	13.4	1.03	0.76–1.39	1.03	0.75–1.40
		Tertiary amines	65	15.8	393	12.5	1.32	0.99–1.75	1.23	0.92–1.66

Type of malformation[a]	Dietary Vitamin C (mg/d)	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
		Amides	41	10.6	261	8.6	1.25	0.88–1.77	1.23	0.86–1.76
Secundum atrial septal defect	<85	No nitrosatable drug exposure	231	71.1	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	51	18.1	372	16.9	1.09	0.79–1.50	1.08	0.77–1.52
		Tertiary amines	55	19.2	391	17.6	1.11	0.81–1.52	1.04	0.75–1.46
		Amides	35	13.2	231	11.2	1.20	0.82–1.76	1.22	0.81–1.82
	≥85	No nitrosatable drug exposure	315	76.5	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	47	13.0	426	13.4	0.97	0.70–1.34	0.94	0.66–1.32
		Tertiary amines	47	13.0	393	12.5	1.05	0.76–1.45	0.95	0.68–1.34
		Amides	41	11.5	261	8.6	1.38	0.97–1.95	1.39	0.96–2.02
Atrial septal defect, not otherwise specified	<85	No nitrosatable drug exposure	80	71.4	1829	72.1	1.00	Referent	1.00	Referent
		Secondary amines	14	14.9	372	16.9	0.86	0.48–1.53	1.04	0.56–1.93
		Tertiary amines	16	16.7	391	17.6	0.94	0.54–1.62	1.06	0.59–1.92
		Amides	16	16.7	231	11.2	1.58	0.91–2.76	1.95	1.06–3.56
	≥85	No nitrosatable drug exposure	103	73.6	2760	77.5	1.00	Referent	1.00	Referent
		Secondary amines	16	13.5	426	13.4	1.01	0.59–1.72	1.24	0.70–2.20
		Tertiary amines	26	20.2	393	12.5	1.77	1.14–2.76	2.09	1.28–3.41
		Amides	12	10.4	261	8.6	1.23	0.67–2.27	1.51	0.79–2.90

[a] Defects are simple heart defects without major extracardiac malformations.

[b] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratio include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d] Adjusted for maternal race/ethnicity, education, smoking status, study center, caloric intake, BMI, alcohol use, and multivitamin use during the first trimester.

[e] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

OR, odds ratio; CI, confidence interval.

Table 6

Effect of Maternal Nitrosatable Drug Exposures during the First Trimester of Pregnancy and Selected Birth Defects Stratified by Total Vitamin C (Supplement and Diet), National Birth Defects Prevention Study, 1997–2005

			Cases		Controls					
Type of malformation[a]	**Total Vitamin C[a]**	**Type of drug exposure**	**No.**	**%[b]**	**No.**	**%[b]**	**Unadjusted OR[c]**	**95% CI**	**Adjusted OR[c]**	**95% CI**
Any limb deficiency[d]	Low/none	No nitrosatable drug exposure	51	66.2	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	18	26.1	67	12.4	2.49	1.37–4.51	3.03[g,h]	1.57–5.84
		Tertiary amines	12	19.1	65	12.1	1.71	0.87–3.37	1.99[g]	0.94–4.22
		Amides	5	8.9	36	7.1	1.29	0.48–3.42	1.36	0.47–3.88
	High/daily	No nitrosatable drug exposure	173	75.9	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	30	14.8	274	14.2	1.04	0.69–1.57	1.06[g,h]	0.70–1.62
		Tertiary amines	22	11.3	242	12.8	0.87	0.55–1.38	0.98[g]	0.61–1.57
		Amides	20	10.4	166	9.1	1.15	0.70–1.88	1.23	0.75–2.03
Longitudinal limb deficiency[d]	Low/none	No nitrosatable drug exposure	23	69.7	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	5	17.9	67	12.4	1.53	0.56–4.16	1.65	0.56–4.87
		Tertiary amines	5	17.9	65	12.1	1.58	0.58–4.30	1.90[g]	0.64–5.68
		Amides	1	4.2	36	7.1	0.57	0.07–4.34	0.56	0.07–4.53
	High/daily	No nitrosatable drug exposure	68	77.3	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	11	13.9	274	14.2	0.97	0.51–1.87	1.04	0.53–2.03
		Tertiary amines	6	8.1	242	12.8	0.60	0.26–1.40	0.68[g]	0.29–1.60
		Amides	8	10.5	166	9.1	1.17	0.55–2.47	1.29	0.60–2.77
Transverse limb deficiency[d]	Low/none	No nitrosatable drug exposure	27	62.8	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	13	32.5	67	12.4	3.39	1.67–6.90	4.16[g]	1.89–9.17
		Tertiary amines	7	20.6	65	12.1	1.88	0.79–4.50	2.29	0.88–5.98
		Amides	4	12.9	36	7.1	1.94	0.64–5.85	2.16	0.65–7.20
	High/daily	No nitrosatable drug exposure	93	72.7	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	18	16.2	274	14.2	1.17	0.69–1.96	1.23[g]	0.72–2.10
		Tertiary amines	14	13.1	242	12.8	1.03	0.58–1.83	1.22	0.67–2.22
		Amides	14	13.1	166	9.1	1.50	0.83–2.68	1.62	0.89–2.95

			Cases		Controls					
Type of malformation[a]	**Total Vitamin C**[a]	**Type of drug exposure**	**No.**	**%**[b]	**No.**	**%**[b]	**Unadjusted OR**[c]	**95% CI**	**Adjusted OR**[c]	**95% CI**
Cleft lip alone[e]	Low/none	No nitrosatable drug exposure	49	75.4	464	78.8	1.00	Referent	1.00	Referent
		Secondary amines	10	17.0	67	12.6	1.41	0.68–2.92	1.19	0.55–2.57
		Tertiary amines	9	15.5	65	12.3	1.31	0.62–2.79	1.12	0.50–2.49
		Amides	4	7.6	36	7.2	1.05	0.36–3.08	1.01	0.33–3.11
	High/daily	No nitrosatable drug exposure	157	78.1	1615	76.7	1.00	Referent	1.00	Referent
		Secondary amines	23	12.8	266	14.1	0.89	0.56–1.40	0.77	0.48–1.22
		Tertiary amines	21	11.8	240	12.9	0.90	0.56–1.45	0.85	0.52–1.38
		Amides	21	11.8	165	9.3	1.31	0.81–2.12	1.23	0.75–2.01
Cleft lip with cleft palate[e]	Low/none	No nitrosatable drug exposure	48	69.6	274	79.9	1.00	Referent	1.00	Referent
		Secondary amines	10	17.2	37	11.9	1.54	0.72–3.31	1.28	0.57–2.89
		Tertiary amines	10	17.2	40	12.7	1.43	0.67–3.04	1.15	0.51–2.58
		Amides	8	14.3	18	6.2	2.54	1.04–6.16	2.46[g]	0.90–6.75
	High/daily	No nitrosatable drug exposure	240	74.5	1586	76.9	1.00	Referent	1.00	Referent
		Secondary amines	38	13.7	261	14.1	0.96	0.67–1.39	0.90	0.61–1.32
		Tertiary amines	50	17.2	228	12.6	1.45	1.04–2.03	1.50	1.06–2.13
		Amides	25	9.4	159	9.1	1.04	0.67–1.62	1.08[g]	0.69–1.71
Conotruncal defects[f]	Low/none	No nitrosatable drug exposure	74	75.5	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	10	11.9	67	12.4	0.95	0.47–1.93	0.88	0.42–1.82
		Tertiary amines	11	12.9	65	12.1	1.08	0.54–2.14	1.07	0.52–2.18
		Amides	9	10.8	36	7.1	1.59	0.74–3.45	1.43	0.64–3.23
	High/daily	No nitrosatable drug exposure	246	75.9	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	37	13.1	274	14.2	0.91	0.63–1.31	0.82	0.56–1.20
		Tertiary amines	39	13.7	242	12.8	1.08	0.75–1.56	1.02	0.71–1.48
		Amides	30	10.9	166	9.1	1.21	0.80–1.83	1.14	0.75–1.73
Left ventricular outflow tract obstruction[f]	Low/none	No nitrosatable drug exposure	42	75.0	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	9	17.7	67	12.4	1.51	0.70–3.24	1.31	0.58–2.94
		Tertiary amines	7	14.3	65	12.1	1.21	0.52–2.81	1.23	0.50–2.99
		Amides	5	10.6	36	7.1	1.56	0.58–4.19	1.62	0.57–4.64
	High/daily	No nitrosatable drug exposure	194	73.2	1650	76.7	1.00	Referent	1.00	Referent

			Cases		Controls					
Type of malformation[a]	**Total Vitamin C[a]**	**Type of drug exposure**	**No.**	**%[b]**	**No.**	**%[b]**	**Unadjusted OR[c]**	**95% CI**	**Adjusted OR[c]**	**95% CI**
		Secondary amines	36	15.7	274	14.2	1.12	0.77–1.63	0.99	0.67–1.46
		Tertiary amines	40	17.1	242	12.8	1.41	0.97–2.03	1.29	0.89–1.89
		Amides	27	12.2	166	9.1	1.38	0.90–2.13	1.40	0.90–2.19
Septal defects[f]	Low/none	No nitrosatable drug exposure	183	81.3	472	79.1	1.00	Referent	1.00	Referent
		Secondary amines	19	9.4	67	12.4	0.73	0.43–1.25	0.69	0.39–1.21
		Tertiary amines	23	11.2	65	12.1	0.91	0.55–1.51	0.86	0.51–1.47
		Amides	18	9.0	36	7.1	1.29	0.71–2.33	1.24	0.67–2.30
	High/daily	No nitrosatable drug exposure	488	75.8	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	78	13.8	274	14.2	0.96	0.73–1.26	0.99	0.75–1.32
		Tertiary amines	85	14.8	242	12.8	1.19	0.91–1.55	1.14	0.86–1.50
		Amides	58	10.6	166	9.1	1.18	0.86–1.62	1.17	0.85–1.62
Atrial septal defect, not otherwise specified[f]	Low/none	No nitrosatable drug exposure	24	68.6	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	6	20.0	67	12.4	1.76	0.69–4.47	2.31	0.76–7.07
		Tertiary amines	6	20.0	65	12.1	1.82	0.72–4.61	2.44[g]	0.82–7.28
		Amides	5	17.2	36	7.1	2.73	0.98–7.59	4.43[g]	1.34–14.63
	High/daily	No nitrosatable drug exposure	60	79.0	1650	76.7	1.00	Referent	1.00	Referent
		Secondary amines	6	9.1	274	14.2	0.60	0.26–1.41	0.71	0.29–1.71
		Tertiary amines	14	18.9	242	12.8	1.59	0.88–2.89	1.65[g]	0.86–3.15
		Amides	5	7.7	166	9.1	0.83	0.33–2.09	0.79[g]	0.30–2.04

[a] Low/none refers to <85 mg of dietary vitamin C intake and no vitamin C supplementation; high/daily refers to ≥85 mg of dietary vitamin C intake and daily vitamin C supplementation.

[b] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratio include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d] Adjusted for maternal age, race/ethnicity, education, study center, BMI, alcohol, and caloric intake.

[e] Adjusted for maternal age, race/ethnicity, education, smoking status, study center, BMI, alcohol, and caloric intake.

[f] Adjusted for maternal race/ethnicity, education, smoking status, study center, BMI, alcohol, and caloric intake.

[g] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

[h] Significant multiplicative interaction ($p < 0.05$).

OR, odds ratio; CI, confidence interval.