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Original Contribution

Association of Folic Acid Supplementation During Pregnancy and Infant Bronchiolitis

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Viral bronchiolitis affects 20%–30% of infants; because there is no known effective treatment, it is important to identify risk factors that contribute to its pathogenesis. Although adequate folate intake during the periconceptional period prevents neural tube defects, animal data suggest that higher supplementation may be a risk factor for child respiratory diseases. Using a population-based retrospective cohort of 167,333 women and infants, born in 1995–2007 and enrolled in the Tennessee Medicaid program, we investigated the association between the filling of folic acid–containing prescriptions and infant bronchiolitis. We categorized women into the following 4 groups in relation to the first trimester: "none" (no prescription filled), "first trimester only," "after first trimester," and "both" (prescriptions filled both during and after the first trimester). Overall, 21% of infants had a bronchiolitis diagnosis, and 5% were hospitalized. Most women filled their first prescriptions after the fifth to sixth weeks of pregnancy, and most prescriptions contained 1,000 μg of folic acid. Compared with infants born to women in the "none" group, infants born to women in the "first trimester only" group had higher relative odds of bronchiolitis diagnosis (adjusted odds ratio = 1.17, 95% confidence interval: 1.11, 1.22) and greater severity (adjusted odds ratio = 1.16, 95% confidence interval: 1.11, 1.22). This study's findings contribute to an understanding of the implications of prenatal nutritional supplement recommendations for infant bronchiolitis.

bronchiolitis; folate; folic acid supplementation; Medicaid; pregnancy; prenatal vitamin

Abbreviations: CI, confidence interval; ICD-9, *International Classification of Diseases, Ninth Revision*; LMP, last menstrual period; OR, odds ratio; Th1, T helper type 1.

Viral lower respiratory tract infections, such as bronchiolitis, are associated with substantial morbidity among infants in the United States and with death worldwide (1–3). Viral bronchiolitis affects 20%–30% of all infants in the United States and is the most common cause of infant hospitalization (1–4). Approximately 3% of healthy infants are hospitalized yearly for bronchiolitis, resulting in an estimated 120,000 hospitalizations (1, 2, 5). Because of the associated morbidity of bronchiolitis and the lack of effective treatment or vaccines to prevent the most common viral causes, such as respiratory syncytial virus, it is important to identify potentially modifiable risk factors that contribute to its pathogenesis. Given that lung organogenesis and immune development occur during discrete periods of fetal development, and that folic acid supplementation during the periconceptional period prevents neural tube defects, it is important to investigate the association of maternal folic acid supplementation and the development of respiratory diseases in children during the first years of life.

Since 1992, the US Public Health Service has recommended that women of child-bearing age take folic acid–containing supplements to help prevent neural tube defects (6–8). Folate plays an important role in several biological mechanisms, including DNA methylation, which, in conjunction with other epigenetic mechanisms, affects gene expression; although data are limited, basic science studies have

demonstrated that DNA methylation is important in the expression of transcription factors that regulate T cell development, particularly T helper type 1 (Th1) cells, because demethylation may be important in immune cell development (9–12). In an animal model, a high versus low methyl donor diet during pregnancy was associated with an enhanced allergic phenotype in the offspring (13). Subsequent epidemiologic studies regarding the association of maternal folic acid exposure or folate levels and child respiratory outcomes have included early wheezing outcomes and/or lower respiratory tract infections (14, 15), and others have focused on asthma and atopic disease outcomes at later ages (16–23) with inconsistent findings. In a racially diverse retrospective cohort of 167,333 predominately low-income mother-infant dyads, we investigated the association of infant bronchiolitis and the filling of maternal folic acid–containing prescriptions during pregnancy.

METHODS

Study population

This population-based retrospective cohort study included 167,333 women and their biological infants, born during 1995–2007 and enrolled in the Tennessee Medicaid program (TennCare) (1, 24). Approximately 50% of the infants born in Tennessee are enrolled in TennCare (25). We linked TennCare administrative files with state vital records to obtain study data, including information on participant demographic characteristics, insurance enrollment, filled prescriptions, and health care encounters as previously described (1, 26–28). The study protocol was approved by the institutional review board of Vanderbilt University (Nashville, Tennessee) and by representatives of the Bureau of TennCare (Nashville, Tennessee).

The eligible study population included women aged 15–44 years who were continuously enrolled in TennCare and their singleton infants. Continuous enrollment for women was defined as enrollment 6 months prior to pregnancy through the date of delivery with no more than 60 days of nonenrollment. We included infants who were term (estimated gestational age of ≥37 weeks), non–low birth weight (≥2,500 g), and who had no more than 90 days of nonenrollment during the first year of life. All infants were followed through the first 365 days of life. We calculated infant gestational age using the date of the last menstrual period (LMP) listed on the birth certificate (85.5%) or the median gestational age for the infant's race, birth weight, and birth year (14.5%) if LMP information was not available (1, 29). To study risk factors for bronchiolitis in infants without preexisting pulmonary, airway, or cardiac disease, we excluded infants with *International Classification of Diseases, Ninth Revision* (ICD-9), diagnostic codes indicating congenital heart disease, chronic lung disease, or congenital upper airway anomalies (1, 30).

Outcome

The main outcome was bronchiolitis during the first year of life, as determined by ICD-9 diagnosis codes (1). We captured health care visits (clinic, emergency department, 23-hour observation, hospitalization) using ICD-9 codes for bronchiolitis (code 466.1) or respiratory syncytial virus pneumonia (code 480.1) (1, 26). We classified infants as having either at least 1 health care visit for bronchiolitis or having no such visits. To estimate severity, we categorized infants into the following 3 groups on the basis of their most advanced level of health care for bronchiolitis: no visit (no bronchiolitis visits), outpatient (only clinic or emergency department visit), and hospitalization (23-hour observation or hospitalization) (1).

Predictor

The main predictor was timing of the filling of folic acid–containing supplements during pregnancy. In assembling records for this study, we found that 99% of folic acid–containing prescriptions filled during pregnancy were prenatal vitamins. Therefore, we used prenatal vitamin prescriptions as a surrogate for folic acid supplementation. From pharmacy files, we obtained the national drug code to identify the specific medication, the date the prescription was filled, the dose of folic acid, and the total days' supply. Because of the TennCare policy of administering a 30-day supply for prescription medications and our findings from preparatory work for the research study, we assigned each prenatal vitamin prescription filling to a maximum of 30 days' supply (31). We conducted medication database and internet searches to determine the specific folic acid dose in each prenatal vitamin if the information was not available in TennCare files and obtained a specific dose for 97% of prescriptions in the cohort.

Using the date filled and the number of days' supply, we ascertained whether women were potentially exposed to folic acid through filled prescriptions. Women who did not fill any prenatal vitamin prescriptions during pregnancy were assigned to the "none" group. Women who filled prenatal vitamin prescriptions that included at least 1 day in the first trimester but none after the first trimester were assigned to the "first trimester only" group. Women who filled prenatal vitamin prescriptions that included at least 1 day after the first trimester but none in the first trimester were assigned to the "after first trimester" group. Women who filled prenatal vitamin prescriptions that included at least 1 day during and 1 day after the first trimester were assigned to the "both" group.

Covariates

Potential confounders and important covariates were identified a priori on the basis of known associations with our predictor and outcome (1, 26, 32). From infant birth certificates, we identified birth weight, sex, number of siblings (number of prior livebirths), and pregnancy year, as well as maternal characteristics including age at delivery, marital status, education, and smoking during pregnancy (yes/no). From the enrollment files, we identified maternal race and region of residence (urban, suburban, or rural), which was based on standard metropolitan statistical areas (33). We determined prenatal care adequacy using the Kotelchuck Index, which considers the date that a woman initiates prenatal care and the number of prenatal visits from LMP through delivery

Table 1. Maternal and Infant Characteristics by Maternal Folic Acid Supplementation During Pregnancy in a Cohort of 167,333 Term, Healthy Infants Born in 1995–2007 and Enrolled in TennCare

Characteristic	Pattern of Prenatal Vitamin Prescription Filling During Pregnancy								Total Cohort	
	None ($n = 30,983$)		During First Trimester Only ($n = 16,656$)		After First Trimester ($n = 44,042$)		Both[a] ($n = 75,652$)			
	No.	%	No.	%	No.	%	No.	%	No.	%
Maternal race[b]										
White	18,286	59	11,084	67	20,015	45	47,533	63	96,918	58
Black	11,840	38	5,212	31	22,988	52	26,256	35	66,296	40
Asian	201	1	90	1	297	1	569	1	1,157	1
Other	656	2	270	2	742	2	1,294	2	2,962	2
Maternal age, years[b,c]	22 (20–26)[d]		22 (20–25)[d]		22 (19–25)[d]		22 (19–26)[d]		22 (19–26)[d]	
Maternal education, years[b]										
<12	12,468	40	6,897	41	20,047	46	29,358	39	68,770	41
12	13,347	43	7,430	45	18,229	41	33,803	45	72,809	44
>12	5,102	17	2,300	14	5,656	13	12,349	16	25,407	15
Maternal smoking[b,e]										
Yes	9,468	31	5,411	33	11,295	26	22,246	29	48,420	29
No	21,447	69	11,216	67	32,689	74	53,297	71	118,649	71
Marital status[b]										
Single	19,909	64	10,113	61	32,998	75	47,558	63	110,578	66
Married	11,063	36	6,537	39	11,023	25	28,071	37	56,694	34
Adequacy of prenatal care[b,f]										
Adequate plus	6,768	22	5,359	32	7,739	18	22,098	29	41,964	25
Adequate	10,675	34	7,231	43	14,207	32	33,458	44	65,571	39
Intermediate	3,687	12	2,051	12	5,912	13	10,387	14	22,037	13
Inadequate	8,517	27	1,320	8	14,161	32	6,527	9	30,525	18
Unknown	1,336	4	695	4	2,023	5	3,182	4	7,236	4

Table continues

(34). In addition, we identified mothers with asthma using data on asthma-specific health care visits and medication (32).

Statistical analyses

We performed descriptive analyses using proportions for categorical variables and medians and interquartile ranges for continuous variables. We compared differences in maternal and infant characteristics across the 4 prescription filling groups using χ^2 contingency table statistics for categorical variables and the Kruskal-Wallis test for continuous variables. In addition, we calculated the week that each woman filled her first prenatal vitamin prescription after her LMP. We conducted logistic regression to determine the association between the timing of the filling of prenatal vitamin prescriptions and infant bronchiolitis diagnosis, while adjusting for infant sex, gestational age, birth weight, number of siblings, maternal race, region of residence, pregnancy year, marital status, maternal age, education, smoking, and prenatal care adequacy. We did not detect important correlations among covariates to warrant omission due to multicollinearity issues or redundancy. We conducted a proportional odds model to investigate the timing of prescription filling and the ordinal outcome of bronchiolitis severity (categorized as "no visit," "outpatient" (clinic or emergency department visit), or "hospitalization" (23-hour observation and hospitalization)). We performed graphical assessments to check for the proportional odds assumption using partial residual plots (35), and we did not find substantial departure from the assumption. We performed a sensitivity analysis with restriction to subjects with available LMP data and no missing data on covariates (complete case analysis, Appendix Table 1, case-wise deletion of missing estimated gestational age) and 1 that prevented case-wise deletion using multiple imputation methods (Appendix Table 1, all dyads). Multiple imputations were performed using the aregImpute() and the fit.mult.impute() function procedures in the Hmisc package in R statistical software (36). Maternal asthma was hypothesized to be a potential effect modifier; thus, an interaction term of maternal asthma history and folic acid supplementation was tested in the model (32). A 2-sided 5% significance level was used for all statistical inferences. SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina) was used for

Table 1. Continued

Characteristic	Pattern of Prenatal Vitamin Prescription Filling During Pregnancy								Total Cohort	
	None ($n = 30,983$)		During First Trimester Only ($n = 16,656$)		After First Trimester ($n = 44,042$)		Both[a] ($n = 75,652$)			
	No.	%	No.	%	No.	%	No.	%	No.	%
Maternal asthma[b]										
Yes	919	3	710	4	1,597	4	4,184	6	7,410	4
No	30,064	97	15,946	96	42,445	96	71,468	94	159,923	96
No. of siblings[b,g]										
None	6,270	20	4,571	27	12,764	29	26,676	35	50,281	30
1	11,325	37	6,789	41	14,686	33	27,431	36	60,231	36
≥2	13,315	43	5,270	32	16,505	38	21,366	28	56,456	33
Region of residence[b]										
Urban	10,089	33	7,112	43	11,290	26	28,064	37	56,555	34
Suburban	8,170	26	4,093	25	8,652	20	17,660	23	38,575	23
Rural	12,680	41	5,441	33	24,068	55	29,860	40	72,049	43
Infant sex										
Male	15,973	52	8,434	51	22,734	52	38,795	51	85,936	51
Female	15,010	48	8,222	49	21,307	48	36,856	49	81,395	49
Birth weight, g[b]	3,260 (2,977–3,572)[d]		3,264 (2,992–3,572)[d]		3,260 (2,977–3,558)[d]		3,289 (3,005–3,600)[d]		3,275 (3,005–3,574)[d]	
Infant EGA, weeks[b]	39 (38–40)[d]		39 (38–40)[d]		39 (38–40)[d]		39 (38–40)[d]		39 (37–42)[d]	

Abbreviations: EGA, estimated gestational age; IQR, interquartile range; TennCare, Tennessee Medicaid program.
[a] Prenatal vitamin prescriptions filled both during and after the first trimester.
[b] $P < 0.05$ (χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables).
[c] Maternal age at delivery.
[d] Value expressed as median (interquartile range).
[e] Maternal smoking during pregnancy.
[f] Kotelchuk Index (34).
[g] Based on prior livebirths.

data management, and R, version 3.0.1, software was used for analysis (37).

RESULTS

A total of 167,333 mother-infant dyads were included in the study (Table 1). The median maternal age at delivery was 22 years. Among women in the cohort, 40% were black, 66% were single, and 41% had less than a high school education. Among infants, 51% were boys, and the median birth weight and gestational age were 3,275 g and 39 weeks, respectively. Table 1 provides maternal and infant characteristics by the 4 prescription filling groups with statistically significant differences detected for most characteristics studied ($P < 0.05$) in this large cohort. The "first trimester only" and "both" groups had the highest percentages of white women (67% and 63%, respectively) and women who received at least adequate prenatal care (75% and 73%, respectively), whereas 59% of women in the "none" group were white, and 56% received at least adequate prenatal care. The "first trimester only" and "none" groups had similar percentages of women who reported smoking (33% and 31%, respectively), were single (61% and 64%, respectively), and had less than a high school education (41% and 40%, respectively).

Overall, 19% of women did not fill any prenatal vitamin prescriptions during pregnancy. Ten percent of women filled prenatal vitamin prescriptions during the first trimester only with a median of 30 days (interquartile range, 30–30) of potential folic acid supplementation exposure, whereas 26% filled prescriptions after the first trimester with a median of 47 days (interquartile range, 30–88) of potential folic acid supplementation exposure, respectively. Lastly, 45% of women filled prescriptions both during and after the first trimester with a median of 92 days (interquartile range, 60–157) of potential folic acid supplementation exposure. Approximately 83% of women filled their first prescriptions after the fifth to sixth weeks of pregnancy. The median gestational ages at which women in the "first trimester only" and "both" groups filled their first prenatal vitamin prescriptions were between the sixth to seventh weeks and the eighth to ninth weeks, respectively. Overall, 98% of prescriptions contained 1,000 μg of folic acid.

Twenty-one percent of infants in the study had at least 1 health care visit for bronchiolitis, of which 9%, 6%, 1%, and 5% had a clinic, emergency department, 23-hour

observation, or hospitalization, respectively, as the most advanced level of care. The proportion of infants with a bronchiolitis diagnosis differed by prenatal vitamin prescription filling group. Twenty percent of infants born to women in the "none" group were diagnosed with bronchiolitis compared with 25%, 19%, and 22% of infants born to women in the "first trimester only," "after first trimester," and "both" groups, respectively.

The results for the relative odds of bronchiolitis diagnosis and greater severity associated with prenatal vitamin prescription filling are presented in Table 2. In adjusted models, infants born to women in the "first trimester only" group had higher relative odds of bronchiolitis diagnosis (adjusted odds ratio (OR) = 1.17, 95% confidence interval (CI): 1.11, 1.22) and greater severity (adjusted OR = 1.16, 95% CI: 1.11, 1.22) compared with infants born to women in the "none" group. Similarly, infants born to women in the "both" group had significantly higher relative odds of bronchiolitis diagnosis (adjusted OR = 1.06, 95% CI: 1.02, 1.09) and greater severity (adjusted OR = 1.06, 95% CI: 1.02, 1.09). Overall, 0.83% of dyads had missing information on covariates in our primary adjusted analysis using gestational age based on LMP or estimated by algorithm. Sensitivity analyses that 1) involved complete case analysis including deletion of missing estimated gestational age and 2) used the multiple imputation method for missing values including for gestational age had results consistent with our primary analysis (Appendix Table 1).

Four percent of women in the cohort had a history of asthma ($n = 7{,}410$), and the P value for an interaction effect of maternal asthma and folic acid supplementation exposure on bronchiolitis was 0.053. We conducted analyses stratified by maternal asthma history. In the subset of dyads without maternal asthma, infants born to women in the "first trimester only" group had 18% higher relative odds of bronchiolitis diagnosis (OR = 1.18, 95% CI: 1.12, 1.23) compared with the "none" group, whereas there was no significant association in the similar comparison in the subset of infants born to women with an asthma history (OR = 0.96, 95% CI: 0.80, 1.20).

DISCUSSION

Viral bronchiolitis is a lower respiratory tract infection that is associated with acute and chronic morbidity in children during the first years of life (1–3, 26). Overall, 20%–30% of infants develop bronchiolitis, and 3%–5% are hospitalized (1, 2, 4, 5). Because of the substantial burden and the higher risk of subsequent wheezing and asthma (1, 38), research efforts have focused on identifying risk factors for bronchiolitis (1, 14, 21–23, 32, 39–41). Because of the potential effect of folic acid on the developing fetus, we examined the association of the timing of the filling of maternal prenatal vitamin prescriptions, the primary source of folic acid supplementation in our cohort, and infant health care visits for bronchiolitis. In our cohort, 83% of women who filled a prescription for a folic acid–containing supplement did so after the fifth to sixth weeks of pregnancy. We found that infants born to women who filled folic acid–containing prescriptions during the first trimester had 17% and 16% higher relative odds of having a health care visit for bronchiolitis or greater severity of bronchiolitis, respectively, compared with infants born to women who did not fill folic acid–containing prescriptions during pregnancy.

Folate has several biological functions and reduces the incidence of neural tube defects (8, 42, 43). The importance of folate during the periconceptional period led to recommendations that women of child-bearing age take folic acid–containing supplements, followed by implementation of a folic acid food fortification program in the United States (8, 43–45). Although folic acid supplementation is considered safe during pregnancy, there have been concerns about potential adverse effects, including effects on the developing fetal immune system that could result in a predisposition to respiratory diseases (46). Recent studies have demonstrated that DNA methylation is important in the expression of transcription factors that regulate T cell development (9, 10, 12). In animal models, it has been demonstrated that intake of methyl micronutrients during pregnancy alters DNA methylation in the offspring and influences gene expression and disease phenotypes (13, 47). Experiments by Hollingsworth et al. (13) demonstrated that offspring born to mice fed a diet high in methyl donors, including folic acid, during pregnancy had an enhanced allergic phenotype compared with the offspring of mice fed a diet low in methyl donors. Although not demonstrated in humans, it is plausible that maternal folic acid supplementation could potentially influence immune phenotypes via epigenetic mechanisms and subsequently lead to greater susceptibility to respiratory diseases in infants.

Overall, 21% of infants in our cohort had at least 1 health care visit for bronchiolitis, which is consistent with population estimates (1, 4, 26). To control for factors that might influence a woman's health care–seeking behavior for herself or her child and that are also plausibly associated with bronchiolitis, we conducted regression analyses that adjusted for factors including maternal age at delivery, maternal smoking, educational level, and prenatal care adequacy (1). We found that infants born to women who filled prescriptions for folic acid–containing supplements during the first trimester had higher relative odds of a bronchiolitis diagnosis and greater severity compared with infants born to women who did not fill such prescriptions. Haberg et al. (14) reported 6% and 9% higher relative odds of wheezing and lower respiratory tract infections, respectively, in children up to 18 months of age with maternal folic acid supplement intake in the first trimester; in a subsequent nested case-control study, they found that maternal folate levels in the second trimester were associated with child asthma at 3 years of age (16). Although the authors did not specifically study bronchiolitis, Bekkers et al. (22) and Magdelijns et al. (23) found that self-reported maternal folic acid supplement use after the first trimester was associated with higher risk of wheezing in children at 1 year of age; however, a significant association was not detected at later ages. In contrast, Kiefte-de Jong et al. (21) did not find an association between higher maternal plasma folate levels during the first trimester and child wheeze. Unlike our study, in which most prescriptions contained 1,000 μg of folic acid, the estimated folic acid content of the supplements used by women in these studies was lower. We also found that infants born to women in the "both" group had significantly higher relative odds of bronchiolitis diagnosis,

Table 2. Maternal Folic Acid Supplementation During Pregnancy and Bronchiolitis Diagnosis and Severity Among Term, Otherwise Healthy Infants Enrolled in TennCare, 1995–2007

Folic Acid Supplementation During Pregnancy[a]	No.	%	Bronchiolitis Diagnosis[b]				Severity of Bronchiolitis[c]			
			Unadjusted OR	95% CI	Adjusted[d] OR	95% CI	Unadjusted OR	95% CI	Adjusted[d] OR	95% CI
None filled	30,983	19	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
During first trimester only	16,656	10	1.28	1.22, 1.33	1.17	1.11, 1.22	1.27	1.21, 1.33	1.16	1.11, 1.22
After first trimester	44,042	26	0.90	0.87, 0.93	0.98	0.95, 1.02	0.90	0.87, 0.93	0.98	0.94, 1.02
Both[e]	75,652	45	1.08	1.04, 1.11	1.06	1.02, 1.09	1.07	1.04, 1.11	1.06	1.02, 1.09

Abbreviations: CI, confidence interval; OR, odds ratio; TennCare, Tennessee Medicaid program.

[a] Indicates maternal prenatal vitamin prescriptions filled.

[b] At least 1 health care visit for bronchiolitis during the first year of life (logistic regression model).

[c] Proportional odds ordinal logistic regression was used for the severity of bronchiolitis as defined by ordinal outcome of "no visit" (no bronchiolitis visit), "outpatient" (only clinic or emergency department visits, representing the intermediate severity health care encounter outcome), and "hospitalization" (at least one 23-hour observation or hospitalization, representing the most severe health care encounter outcome).

[d] Odds ratio adjusted for infant sex, estimated gestational age (in weeks), infant birth weight, number of living siblings, maternal race, region of residence, year of pregnancy, maternal marital status, maternal age at delivery, maternal educational level, maternal asthma, maternal smoking during pregnancy, and adequacy of prenatal care.

[e] Prenatal vitamin prescriptions filled both during and after the first trimester.

although the point estimate is lower compared with infants of women in the "first trimester only" group, and more work is needed to further delineate this association. We studied potential effect modification of maternal asthma; however, future work is warranted in this area. With objective measures, such as prescriptions filled during pregnancy for folic acid supplementation and ICD-9 diagnostic codes for bronchiolitis health care visits, our work provides additional insight into the relationship between maternal folic acid supplementation and infant bronchiolitis. In addition, our study extends this work into a large and racially diverse population of women and children.

Adequate folate is necessary for the fusion of the neural tube during the third to fourth weeks of pregnancy and for spinal cord development during the fifth to sixth weeks of pregnancy (48, 49). We therefore focused on the timing of folic acid supplementation in relation to the first trimester and observed that the majority of women filled their first prenatal vitamin prescriptions after the fifth to sixth weeks of pregnancy. Hence, if follow-up work supports these findings, defining a time period during pregnancy when folic acid supplementation prevents neural tube defects but is not associated with infant bronchiolitis appears feasible. This may not be clinically desirable because of the benefits of folic acid supplementation in preventing neural tube defects and other congenital anomalies (44, 50); however, it is important to determine the magnitude of risks and benefits to understand the public health impact of interventions that may prevent 1 disease, but increase the risk of others.

This study has several limitations. We used filled prescriptions to determine the exposure; however, it is possible that women did not take vitamins as prescribed. In addition, it is possible that women who filled a prescription only once were less likely to take the prenatal vitamins than women who filled more than 1 prescription. It is also possible that some women obtained prenatal vitamins from over-the-counter or other sources, leading to misclassification. However, the TennCare pharmacy benefit covered prenatal vitamins throughout the study period, so we estimate that exposure misclassification in our low-income population is lower than might be seen in higher-income women covered by commercial health insurance. We captured data on folic acid intake in supplemental form and did not include fortified food sources. However, previous studies have found that women who reported taking folic acid supplements had the highest folic acid intake, were more likely to have folic acid intake that exceeded the tolerable upper limit, and had a higher prevalence of blood folate levels of 20 ng/mL or above (51, 52). It is likely that women who did not fill prescriptions consumed folic acid from fortified food sources, and this could possibly lead to misclassification (53, 54). However, it is unlikely that folic acid intake from fortified foods only would result in intake equivalent to the 1,000 μg of folic acid contained in the majority of prenatal vitamin prescriptions filled by women in this study (53). Because the majority of prenatal vitamin prescriptions contained 1,000 μg of folic acid, we were not able to investigate the association between daily dose of folic acid and infant bronchiolitis. In addition, prenatal vitamins contain additional micronutrients, which typically include other B vitamins, vitamin D, vitamin E, and zinc; previous studies have identified potential protective effects of these micronutrients on early childhood respiratory illnesses (15, 55). Approximately three-fourths of women in the "first trimester only" and "both" groups had at least adequate prenatal care, and it is possible that they were more likely to seek care for their infants when the infants were ill; however, we adjusted for prenatal care adequacy in our models. This study was conducted to investigate the association of folic acid supplementation and infant bronchiolitis without preexisting lung disease. For this reason, premature infants were not included. It is possible that maternal folate status is related to both gestational age and bronchiolitis (56, 57), and this could have induced a collider stratification bias into our estimates (58, 59). Future research on the association of prenatal folic acid supplementation with gestational age and bronchiolitis is

warranted. The study is retrospective in nature, and its findings could be influenced by unmeasured confounders, including environmental allergens and irritants (60, 61). Lastly, we identified bronchiolitis diagnoses using ICD-9 codes; this method represents an objective measure of provider-characterized outcomes at the time of illness and would serve to minimize recall bias.

Conclusion

In a population-based cohort of 167,333 mother-infant dyads, the timing of the filling of folic acid–containing prescriptions during the first trimester was associated with higher relative odds of bronchiolitis diagnosis and greater severity during the first year of life. These findings enhance what is currently known and support future investigations to help delineate the association between the dose and timing of prenatal nutritional supplements and the risk of childhood respiratory disease.

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Appendix Table 1. Multivariable Logistic Regression Analyses for Outcome of at Least 1 Bronchiolitis Visit During the First Year of Life by Maternal Folic Acid Supplementation During Pregnancy

Folic Acid Supplementation During Pregnancy[a]	At Least 1 Health Care Visit for Bronchiolitis Versus No Visit					
	Primary Adjusted Analysis[b] (*n* = 165,992)		Case-Wise Deletion of Missing Estimated Gestational Age[c] (*n* = 140,347)		All Dyads (Multiple Imputation)[d] (*n* = 167,333)	
	OR	95% CI	OR	95% CI	OR	95% CI
None filled	1.00	Referent	1.00	Referent	1.00	Referent
During first trimester only	1.17	1.11, 1.22	1.17	1.11, 1.23	1.17	1.11, 1.23
After first trimester	0.98	0.95, 1.02	0.98	0.94, 1.03	0.97	0.94, 1.01
Both[e]	1.06	1.02, 1.09	1.07	1.03, 1.11	1.05	1.02, 1.09

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

[a] Indicates maternal prenatal vitamin prescriptions filled.

[b] Primary adjusted analysis that included dyads with estimated gestational age (calculated when last menstrual period data were available or estimated when last menstrual period data were missing) and excluded dyads with missing covariates (complete case analysis).

[c] Sensitivity analysis that included only dyads in which estimated gestational age was calculated using available data on last menstrual period.

[d] Sensitivity analysis that included all dyads in the cohort (dyads in primary adjusted analysis and dyads with missing covariates for which multiple imputations was used).

[e] Prenatal vitamin prescriptions filled during and after the first trimester of pregnancy.