Published in final edited form as:

Ann Epidemiol. 2014 July ; 24(7): 498–503.e1. doi:10.1016/j.annepidem.2014.04.004.

Serum Cotinine and Whole Blood Folate Concentrations in Pregnancy

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Abstract

Background—Prenatal tobacco smoke exposure may be associated with low maternal folate levels that increase the risk of adverse infant and child health outcomes by reducing folate availability during fetal development.

Methods—Using data from the HOME Study, we examined the relationship between secondhand or active tobacco smoke exposure and whole blood folate concentrations in pregnant women from Cincinnati, OH (n=362) at approximately 16 weeks gestation. We used multivariable linear regression to examine the association between continuous or categorical serum cotinine levels and whole blood folate levels, adjusting for sociodemographic, dietary, and perinatal variables.

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Disclaimer Statement:

The findings and conclusions in this report are those of the authors and do not necessarily represent the official views or positions of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry or the Department of Health and Human Services.

Disclosures:

Dr. Lanphear has served as an expert witness and as a consultant to the California Attorney General's Office, but he has not personally received any compensation for these services. Dr. Lanphear has also served as a consultant on a US Environmental Protection Agency research study which he does receive compensation. Dr. Braun was financially compensated for conducting a re-analysis of the international pooled study of lead exposure for the plaintiffs in a public nuisance case.

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Results—After adjustment for potential confounders, an interquartile range increase in serum cotinine concentration (0.012 to 0.224 ng/mL) was suggestively associated with decreased whole blood folate levels (β : -23 nmol/L; 95% CI: -49, 3, p-value=0.08). Compared to unexposed women, reductions in mean whole blood folate were observed among active smokers (β : -94, 95% CI: -195, 6 nmol/L, p-value=0.40); smaller reductions were observed among women with secondhand exposure (β : -26; CI: -84, 32 nmol/L, p-value=0.07).

Conclusions—Consistent with prior studies, active smoking was associated with reduced whole blood folate levels among these pregnant women. Secondhand tobacco smoke exposures were associated with small and imprecise reductions in whole blood folate levels.

MeSH Keywords

Epidemiology; folic acid; pregnancy; tobacco smoke pollution; smoking

Introduction

Tobacco smoke exposure remains a prevalent and preventable cause of disease, disability, and death in the United States (US).^{1–3} Smoking and secondhand tobacco smoke (SHS) exposure can cause adverse health effects across the lifespan, but the developing fetus may be particularly sensitive due to reduced nicotine detoxification capacity compared to adults.^{4,5} Despite the well-known adverse health effects of tobacco smoke, approximately 14% of US women smoke during their pregnancy and at least 30% of US women experience SHS exposures before or during pregnancy.^{6–8} Active and SHS exposures during pregnancy may increase the risk for numerous adverse maternal, fetal, infant, and child health outcomes. These include, but are not limited to placental abruption, intrauterine growth restriction, preterm birth, low birth weight, sudden infant death syndrome, neurodevelopmental disorders, and possibly neural tube defects (NTDs).^{2,9–14}

Prenatal tobacco smoke exposures may increase infant or child disease risk by reducing folate bioavailability during the sensitive prenatal period of development.^{15–23} Folate is an essential B-vitamin that functions as a coenzyme in single-carbon transfer reactions and is essential for DNA synthesis, methylation, and repair in cells.²⁴ Folate deficiency during pregnancy is associated with NTDs, such as spina bifida and anencephaly.²⁵ While lower serum and red blood cell folate concentrations have been observed in pregnant smokers compared to nonsmokers, we are not aware of prior studies examining the association between SHS exposure and folate status using validated biomarkers of secondhand tobacco smoke exposure (e.g., cotinine) while considering potential sociodemographic or nutritional confounders. Biomarkers of tobacco smoke exposure have several advantages over self-reported exposures: 1) pregnant women may not report active smoking because of social desirability, 2) questionnaires may not be sensitive enough to identify all relevant sources of SHS exposure, and 3) biomarkers of cumulative dose, like cotinine, can be used to examine dose-response relationships across the entire range of secondhand and active tobacco smoke exposures.^{26–28}

Since tobacco smoke exposures and low folate levels during pregnancy may increase the risk of subsequent disease, we examined the relationship between maternal serum cotinine

and whole blood folate concentrations in pregnant women, while adjusting for sociodemographic and dietary factors.

Methods

Study Sample

The data used in this study were collected from women participating in the Health Outcomes and Measures of the Environment (HOME) Study in Cincinnati, Ohio. The HOME Study has measured environmental chemical exposures and a variety of health outcomes in pregnant women and their children. Initial identification of participants was conducted at 9 prenatal clinics affiliated with 3 hospitals between March 2003 and January 2006. Eligibility criteria included: 18 years of age, living in a home built before 1978, 16±3 weeks gestation at enrollment; residing in 5 counties in Southwest Ohio and 1 county in Northern Kentucky; intention to continue prenatal care and deliver at collaborating obstetric practices and hospitals; HIV negative; and not receiving seizure, thyroid or chemotherapy/radiation medications. Recruitment letters were mailed to 5,512 women who were at least 18 years old and lived in a home built prior to 1978. Of 1,263 (23%) eligible women, 468 (37%) enrolled in the study.²⁶ The HOME Study was approved by the Institutional Review Board at the Cincinnati Children's Hospital Medical Center and all subjects provided written informed consent prior to participation.

Biomarkers of Tobacco Smoke Exposure

Serum and whole blood samples were obtained via venipuncture from women at approximately 16 weeks gestation. Samples were kept at -20°C prior to being shipped on dry ice to the Centers for Disease Control and Prevention (CDC) laboratory. Serum was analyzed for cotinine, a sensitive and specific biomarker of SHS and active tobacco smoke exposure using high-performance liquid chromatography-tandem mass spectroscopy (HPLC-MS/MS).^{29,30} We used the actual measured cotinine concentrations for samples with results below the limit of detection (Limit of Detection [LOD]=0.015 ng/mL) rather than imputing values since this is statistically more efficient.³¹

Using data from this cohort, we previously found that repeated serum cotinine concentrations collected during the latter two-thirds of pregnancy were highly correlated with each other (Pearson $R>0.7$) and associated with self-reported secondhand and active tobacco smoke exposures.²⁷ Furthermore, women with no self-reported tobacco smoke exposures had serum cotinine concentrations consistent with SHS exposure, indicating that self-report may misclassify women's actual exposure.²⁶

Biomarkers of Folate Status

Whole blood samples were analyzed for folate vitamers by HPLC-MS/MS.^{32,33} Whole blood (total) folate was calculated as the sum of five folate vitamers, with 5-methyltetrahydrofolate being the major folate form (~80% of total folate) (Supplemental Method 1). Whole blood folate is a specific measure of long-term folate status with a biological half-life of ~8 weeks.³⁴ Folate vitamer values <LOD were substituted by the LOD/ 2. To assess the between-run variability ($n = 14$), 3 known quality control (QC) pools

(127–357 nmol/L whole blood [total] folate and 100–248 nmol/L whole blood 5-methyltetrahydrofolate) were analyzed in duplicate during each analytic run and 2 unknown QC pools (447–665 nmol/L whole blood [total] folate and 359–408 nmol/L whole blood 5-methyltetrahydrofolate) were analyzed as part of each analytic run at a rate of 1 unknown pool for every 20 study samples. The between-run variability for whole blood (total) folate for the 3 known and 2 unknown QC materials was 3.9–6.7% and 1.5–1.9%, respectively. The between-run variability for whole blood 5-methyltetrahydrofolate was 2.1–3.6% and 2.0–2.2%, respectively.

Potential Confounders

Dietary folate intake, sociodemographic factors, and lifestyle are strong determinants of both serum cotinine and blood folate levels. Drawing on prior knowledge, we selected potential confounders based on whether they might be related to either serum cotinine or whole blood folate concentrations among pregnant women.^{3,8,35–37} Trained research assistants collected sociodemographic, dietary, and perinatal variables using standardized, computer-assisted interviews at 20-weeks gestation and medical chart reviews after delivery.

Sociodemographic covariates included maternal race, age, education, marital status, household income, and insurance status. Dietary variables included prenatal vitamin use frequency, food security (variety and quantity of food), and fresh fruit and vegetable consumption frequency. Perinatal variables included maternal depressive symptoms at 16 weeks gestation (Beck Depression Inventory-II),³⁸ maternal body mass index (BMI) at 16 weeks gestation, and parity.

Statistical analysis

We began our analysis by calculating the median and interquartile range of serum cotinine and whole blood folate concentrations according to sociodemographic, dietary, and perinatal characteristics of the study participants.

We characterized tobacco smoke exposure at 16 weeks gestation using both continuous and categorical measures of serum cotinine concentrations. For analysis of continuous cotinine levels, we applied a Box-Cox transformation with a gamma value of 0.1 to serum cotinine levels to account for the right-skew of the distribution.³⁹ For categorical analysis, prenatal serum cotinine concentrations were categorized into 3 levels: no exposure (<LOD), SHS exposure (LOD to 3 ng/mL), and active exposure (>3 ng/mL).²³ WBF concentrations were approximately normal and not mathematically transformed.

In our primary analysis using linear regression, we examined the unadjusted and adjusted change in whole blood folate concentrations for an interquartile range (IQR) increase in serum cotinine concentrations. We also estimated the unadjusted and adjusted mean whole blood folate concentrations according to categories of maternal serum cotinine concentrations. We examined Variance Inflation Factors and Condition Indices to determine whether there was multi-collinearity among the variables in our adjusted model. Variance Inflation Factors and Condition Indices did not have values that would suggest multi-collinearity.⁴⁰ All statistical analyses were conducted in SAS v9.2.

In sensitivity analyses we examined whether the week of gestation that samples were collected, gravidity, or maternal BMI - assessed among a subset of participants with complete height and weight data measured at 16 weeks gestation - confounded the association between cotinine and whole blood folate concentrations. We also examined whether very low whole blood folate levels, which might be indicative of sample degradation, biased our results by excluding samples with concentrations <100 nmol/L.³² Next, we examined whether the association between cotinine and whole blood folate levels different according to maternal race (Black vs. Caucasian), age (18–<25, 25–34, and 35+ years old), or prenatal vitamin intake (rarely/never, weekly, or daily). Finally, we modeled whole blood 5-methyltetrahydrofolate levels as the outcome instead of whole blood folate concentrations.

Results

Of the 468 women who enrolled in the HOME Study, 67 dropped out before delivery. We excluded nine women who delivered twins and 3 women who delivered stillborn infants. Of the remaining 389 (83%) women, we included 362 (77%) women with complete covariate, exposure, and outcome data in our primary analysis.

The women included in our analysis were predominantly non-Hispanic white (62%), married (66%), and between 25–35 years of age (60%). Higher socioeconomic status indicators were associated with higher whole blood folate concentrations and lower serum cotinine concentrations (Table 1). Non-Hispanic white women had higher whole blood folate (median: 569 nmol/L vs. 334 nmol/L) and lower serum cotinine concentrations (median: 0.017 ng/mL vs. 0.378 ng/mL) compared to non-Hispanic blacks. Whole blood folate concentrations were highest and serum cotinine concentrations lowest among women who were first-time mothers, had lower depression scores, or had normal BMI. Consistent with prior literature, women who reported taking prenatal vitamins weekly or every day had higher folate concentrations than women who reported rarely or never consuming prenatal vitamins.^{41,42} In addition, women who reported adequate food security and frequent consumption of fresh fruits and vegetables had higher whole blood folate and lower serum cotinine concentrations. Associations between covariates and whole blood 5-methyltetrahydrofolate levels were similar to those observed for whole blood folate levels (Supplemental Table 1).

In unadjusted analyses, we observed a 55 nmol/L decrease in whole blood folate for each IQR (0.012 to 0.224 ng/mL) increase in serum cotinine concentration (95% confidence interval [CI]: –75, –36, p-value<0.001) (Table 2). After adjusting for maternal race, education, age, depressive symptoms, insurance status, marital status, prenatal vitamin intake, food security, income, fruit and vegetable intake, and parity, each IQR increase in serum cotinine was associated with a 23 nmol/L (CI: –49, 3, p-value=0.08) decrease in whole blood folate concentrations, although the CI for this point estimate included the null value. A similar association, with a negligible difference in the precision was observed when adjusting for only maternal race and insurance status (β : –21; CI: –45, 3, p-value=0.09). This confounding arose because of the higher whole blood folate *and* dramatically lower

serum cotinine levels among white or privately insured women compared with black or publicly/un-insured women.

In unadjusted analyses, serum cotinine concentrations indicative of SHS or active tobacco smoke exposures were associated with decreased whole blood folate concentrations (Table 3). Compared to unexposed women, unadjusted whole blood folate concentrations were 97 nmol/L (CI: -151, -42, p-value=0.0008) and 217 nmol/L (CI: -307, -134, p-value<0.0001) lower among women exposed to secondhand and active tobacco smoke, respectively. Adjusted mean differences in whole blood folate concentrations among women with SHS (β : -26; CI: -84, 32 nmol/L, p-value=0.38) or active (β : -94; CI: -195, 6 nmol/L, p-value=0.07) tobacco smoke exposure were markedly attenuated, less precise, and no longer statistically significant after we adjusted for all the above listed covariates or only race and insurance status.

Additional adjustment for the week of gestation that samples were collected (β per IQR: -24; CI: -50, 3 nmol/L, p-value=0.08), maternal BMI (β per IQR: -24; CI: -52, 4 nmol/L, p-value=0.10), or gravidity (β per IQR: -23; CI: -49, 4 nmol/L, p-value=0.09) did not change the magnitude or precision of our point estimates. The exclusion of 23 participants with whole blood folate levels <100 nmol/L did not appreciably alter our results (β per IQR: -26; CI: -52, 1 nmol/L, p-value=0.05). The association between serum cotinine and whole blood folate levels did not vary by maternal age, race, or prenatal vitamin intake (all p-value interaction terms >0.23). After adjustment for confounders, our results were similar when we used 5-methyltetrahydrofolate concentrations as the outcome instead of whole blood folate concentrations (β per IQR: -18; CI: -41, 6 nmol/L, p-value=0.14).

Discussion

There is growing interest in identifying prenatal environmental determinants of health and disease. Both tobacco smoke exposure and poor folate status during pregnancy are associated with similar adverse developmental outcomes during infancy and childhood.^{9,15-18,43,44} Given the importance of folic acid intake in preventing neural tube defects and the increasing recognition that folate deficiency may play a role in adverse fetal development, the identification of modifiable sources of low folate status, like tobacco smoke, may offer potential avenues to reduce subsequent disease risk.^{25,45}

We observed an inverse association between serum cotinine and whole blood folate concentrations among pregnant women at approximately 16 weeks gestation in unadjusted analyses that was markedly attenuated but not eliminated after adjustment for potential confounders. Reductions in whole blood folate levels were largest among smokers compared to women with no tobacco smoke exposure. SHS exposures were associated with a small, imprecise reduction in whole blood folate concentrations after adjustment. Race and insurance status, which were predictive of cotinine and whole blood folate levels, were primarily responsible for this attenuation.

Several prior studies have observed inverse associations between tobacco smoke exposure and folate biomarker levels among pregnant women; however, few studies have examined

SHS among pregnant women and adjusted for socioeconomic or dietary confounders.^{20–23} Only one prior study of pregnant women used a biomarker of tobacco smoke exposure, but they did not differentiate between secondhand and active tobacco smoke exposure.²² Two studies by Ulvik et al. and Mannino et al. examined tobacco-folate relationship in large samples of men and non-pregnant women, respectively, using serum cotinine to differentiate between active and SHS exposure.^{46,47} Ulvik et al. reported lower adjusted serum folate concentrations among smokers with low (<14 ng/mL) and high (≥14 ng/mL) serum cotinine concentrations compared to self-reported nonsmokers. Using data from the National Health and Nutrition Examination Survey (1988–1994), Mannino et al. observed a 25–50 and 86 nmol/L decrease in red blood cell (RBC) folate among those with serum cotinine levels indicative of secondhand and active tobacco smoke exposure compared to those with the lowest cotinine levels, respectively. The magnitude of these reductions are consistent with those we observed in women exposed to SHS (β : -24; CI: -81, 33 nmol/L) and active smoke (-93; 95% CI: -193, 7 nmol/L), thus, suggesting that we may have had insufficient statistical power to detect statistically significant associations.

Most previous studies examining the relationship between tobacco smoke exposure and folate levels among pregnant women have relied on self-reports to distinguish smokers and nonsmokers, while also including women with SHS exposures among those characterized as nonsmokers. This may attenuate the association between active smoking and folate levels if there is an inverse association between SHS exposure and folate. One strength of our study is the use of a sensitive and specific tobacco smoke biomarker that can accurately differentiate secondhand and active exposures.^{26,29} This is an advantage when examining self-reported SHS exposures, since these may be misclassified because women may be unaware of secondhand tobacco smoke in their environment or questionnaires may not be sensitive enough to detect low-level exposures.⁴⁸ While we and others have previously reported that serum cotinine concentrations are valid and reliable measures of tobacco smoke exposure during pregnancy, additional non-differential exposure misclassification may attenuate the observed associations towards the null.^{26,27,29}

There are several limitations of this study that should be considered when interpreting these results. First, the cross-sectional analysis limits the ability to infer causality between tobacco smoke exposure and lower whole blood folate concentrations; however, serum cotinine levels are relatively stable during pregnancy.²⁷

While we considered numerous confounders in the present study, unmeasured or misclassified confounders have the potential to further attenuate our results and the observed associations may be due to these factors. We were unable to adjust for some medical conditions that might affect diet quality and possibly be associated with tobacco smoke exposures (e.g., hyperemesis). Our assessment of dietary quality was based upon self-reported prenatal vitamin use and fresh fruit and vegetable intake, which may not adequately capture the diet of these women. Although several studies have observed differences in dietary folic acid intake between smokers and nonsmokers, the nutritional variables we examined do not appear to be strong confounders of the cotinine-folate association after adjusting for race and insurance status.^{49,50} In addition, more refined measures of the

overarching constructs that race and insurance status represent might further explain the association between serum cotinine and folate concentrations.

We measured whole blood folate, but did not have hematocrit or serum folate levels for these women, which would have allowed us to calculate RBC folate and directly compare the folate status of these women to prior studies. In addition, folate may have degraded due to the length of sample storage prior to analysis (4–6 years). Fazili et al. have shown that ~10–20% of whole blood folate was lost over a 2-year period when whole blood was stored frozen at -70°C .⁵¹ However, our results were similar when we excluded samples with whole blood folate concentrations less than 100 nmol/L. Additional non-differential misclassification of folate status could have arisen since our WBF measurement reflects both the more variable serum component and the less variable RBC component.³⁴

Finally, the generalizability of our findings to other populations of pregnant women may be limited by our cohort's age. However, folate levels have remained relatively stable in the US since the 1998 fortification of the US food supply.⁵² Furthermore, the pattern of associations between sociodemographic factors and serum cotinine or WBF concentrations in our cohort is similar to those observed in the general US population. These two observations suggest that the results from our cohort may be generalizable to present day pregnant women in the US.^{36,52}

The results from this study of pregnant women suggest that active smoking may be associated with lower whole blood folate levels. We observed small, imprecise, and non-significant reductions in whole blood folate levels among women with SHS exposures that were consistent with other studies of men and non-pregnant women that used serum cotinine to characterize tobacco exposures. Because these results show that associations between tobacco smoke exposure and folate status may be confounded by sociodemographic factors, the interpretation and design of epidemiological studies will need to consider these potential confounders given their importance in determining diet and other lifestyle factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grants Supporting this Work: This work was supported by NIEHS grants R00 ES020346, PO1 ES11261, and R01 ES020349.

Abbreviations

BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CI	95% Confidence Interval
HOME	Health Outcomes and Measures of the Environment

HPLC-MS/MS	High-performance Liquid Chromatography-Tandem Mass Spectroscopy
LOD	Limit of Detection
NTDs	Neural Tube Defects
QC	Quality Control
SHS	Secondhand Tobacco Smoke Exposure
US	United States

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Table 1

Descriptive characteristics of the study sample and whole blood folate and serum cotinine concentrations according to sociodemographic, dietary, and perinatal factors (n=362).

	N(%)	Median Whole Blood Folate nmol/L (25 th , 75 th Percentile)	Median Serum Cotinine ng/mL (25 th , 75 th Percentile)
Overall	362	494 (307, 642)	0.030 (0.012, 0.224)
Maternal Race			
Non-Hispanic White	226 (62)	569 (386, 698)	0.017 (0.008, 0.035)
Non-Hispanic Black	111 (31)	334 (255, 454)	0.378 (0.08, 2.53)
Other	25 (7)	469 (387, 650)	0.038 (0.012, 0.492)
Maternal Age (years)			
18–25	83 (23)	382 (290, 486)	0.276 (0.103, 1.41)
>25–35	218 (60)	528 (327, 659)	0.02 (0.01, 0.076)
>35	61 (17)	568 (332, 705)	0.019 (0.007, 0.062)
Maternal Education			
Less than grade 12	36 (10)	326 (251, 436)	2.2 (0.529, 60.1)
Grade 12 completed	50 (14)	402 (280, 587)	0.216 (0.094, 2.6)
Some college	89 (24)	437 (296, 602)	0.066 (0.02, 0.255)
College completed	107 (30)	563 (387, 698)	0.016 (0.008, 0.028)
Graduate school	80 (22)	578 (410, 697)	0.014 (0.006, 0.025)
Marital Status			
Married	239 (66)	563 (387, 687)	0.017 (0.008, 0.032)
Unmarried, living with someone	51 (14)	362 (257, 586)	0.565 (0.157, 6.89)
Unmarried, living alone	72 (20)	335 (262, 470)	0.458 (0.1, 5.415)
Household Income (dollars per year)			
5000–15000	76 (21)	335 (266, 463)	1.03 (0.213, 9.785)
25000–35000	63 (17)	422 (280, 601)	0.094 (0.025, 0.585)
45000–75000	122 (34)	563 (386, 674)	0.02 (0.01, 0.046)
85000–155000	101 (28)	563 (395, 698)	0.012 (0.006, 0.022)
Insurance Status			
Private	262 (72)	559 (387, 687)	0.017 (0.009, 0.038)
Public or uninsured	100 (28)	322 (254, 448)	0.677 (0.172, 7.41)
Maternal Depressive Symptoms			
Minimal depression (0–13)	282 (78)	525 (327, 668)	0.022 (0.01, 0.091)
Mild depression (14–19)	50 (14)	368 (280, 547)	0.172 (0.036, 2.61)
Moderate depression (20–28)	21 (6)	385 (280, 517)	0.276 (0.08, 25.4)
Severe depression (>28)	9 (2)	421 (322, 469)	2.13 (0.93, 62.1)
Body Mass Index (BMI)^a			0.027 (0.012, 0.185)
<25	143 (42)	466 (292, 640)	0.023 (0.009, 0.097)
25–30	116 (34)	544 (379, 661)	0.02 (0.01, 0.06)

	N(%)	Median Whole Blood Folate nmol/L (25 th , 75 th Percentile)	Median Serum Cotinine ng/mL (25 th , 75 th Percentile)
>30	83 (24)	463 (287, 642)	0.167 (0.031, 1.14)
Parity			
0	163 (45)	527 (344, 670)	0.025 (0.012, 0.13)
1	115 (32)	476 (287, 612)	0.031 (0.01, 0.323)
2+	84 (23)	429 (277, 599)	0.059 (0.014, 0.525)
Prenatal Vitamin Intake			
Never and a few times a month	52 (14)	330 (254, 459)	0.251 (0.038, 2.605)
Weekly	45 (13)	422 (333, 597)	0.032 (0.015, 0.122)
Every day	265 (73)	545 (332, 672)	0.022 (0.01, 0.106)
Food Security			
Not enough	16 (4)	321 (256, 582)	0.233 (0.127, 4.25)
Enough but not always the kinds of food we want	67 (19)	386 (280, 583)	0.185 (0.032, 1.41)
Enough and the kinds of food we want	279 (77)	532 (333, 664)	0.021 (0.01, 0.094)
Fresh Fruit and Vegetable Consumption Frequency			
Monthly	43 (12)	395 (252, 602)	0.167 (0.032, 0.85)
Weekly	178 (49)	463 (309, 624)	0.039 (0.014, 0.239)
About once a day	70 (19)	537 (290, 626)	0.02 (0.01, 0.217)
More than once a day	71 (20)	568 (418, 705)	0.017 (0.008, 0.059)

^a n=342 because a total of 20 women missing height or weight data.

Table 2

Unadjusted and adjusted change in pregnant women's whole blood folate concentrations with an interquartile range increase in serum cotinine concentrations (n=362).

Covariate Adjustment	Change in Whole Blood Folate (nmol/L) Concentration β (95% CI)	p-value
Unadjusted	-56 (-75, -36)	<0.0001
Adjusted ^a	-23 (-49, 3)	0.08

^a Adjusted for maternal race (non-Hispanic Whites; non-Hispanic Blacks; and other), education (<12; 12; some college; college completed; and graduate school), depressive symptoms (continuous), insurance status (private insurance and public or uninsured), marital status (married; unmarried, living with someone; and unmarried, living alone), prenatal vitamin intake (never and few times a month; weekly; and every day), food security (not enough; enough but not always the kinds of foods we want; and enough and the kinds of food we want), maternal age (continuous, years), household income (continuous, dollars per year), fruit and vegetable intake (monthly, weekly, ~ once a day, and daily), and parity (0, 1, and 2+).

Table 3

Unadjusted and adjusted associations between categories of prenatal tobacco smoke exposure and maternal whole blood folate concentrations at 16 weeks gestation (n=362)

Serum Cotinine Concentration Category	N (%)	Unadjusted Change in Folate (nmol/L) (95% CI)	Adjusted Change in Folate ^a (nmol/L) (95% CI)	Adjusted p-value
Unexposed (<LOD) ^c	106 (29)	Ref	Ref	Ref
SHS exposure (LOD to 3 ng/mL)	217 (60)	-97 (-151, -42)	-26 (-84, 32)	0.38
Active exposure (>3ng/mL)	39 (11)	-221 (-307, -134)	-94 (-195, 6)	0.07

^a Adjusted for maternal race (non-Hispanic Whites; non-Hispanic Blacks; and other), education (<12; 12; some college; college completed; and graduate school), depressive symptoms (continuous), insurance status (private insurance and public or uninsured), marital status (married; unmarried, living with someone; and unmarried, living alone), prenatal vitamin intake (never and few times a month; weekly; and every day), food security (not enough; enough but not always the kinds of foods we want; and enough and the kinds of food we want), maternal age (continuous, years), household income (continuous, dollars per year), fruit and vegetable intake (monthly, weekly, ~ once a day, and daily), and parity (0, 1, and 2+).

^b LOD, limit of detection (0.015 ng/mL)

^c SHS, secondhand tobacco smoke.