

Maternal smoking is associated with decreased 5-methyltetrahydrofolate in cord plasma¹⁻³

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ABSTRACT

Background: Maternal-fetal folate transport via the placenta has been shown to be concentrative. Exposure to cigarette smoke is associated with decreased maternal folate status through altered dietary intakes and possibly through nondietary mechanisms such as increased folate turnover. The effect of maternal smoking on fetal folate status has not been documented.

Objective: The objective was to determine the effect of maternal smoking on plasma 5-methyltetrahydrofolic acid (5-MTHFA) concentrations in umbilical cord blood.

Design: African American women were recruited from an antenatal clinic in Detroit, MI. Plasma 5-MTHFA concentrations were measured in maternal-umbilical cord pairings ($n = 58$). The participants completed a structured interview to determine demographic characteristics, including smoking.

Results: Concentrations of 5-MTHFA were significantly higher in venous cord plasma (16.8 ± 7.5 ng/mL) than in maternal plasma (13.0 ± 7.5 ng/mL) but remained associated ($r = 0.60$, $P < 0.001$) with each other. Cigarettes smoked by the mothers was negatively associated ($r = -0.31$, $P = 0.019$) with venous cord 5-MTHFA concentrations and remained so after control for maternal plasma 5-MTHFA and other variables. Venous cord plasma 5-MTHFA was significantly lower in smoking (15.1 ± 7.6 ng/mL; $n = 32$) than in nonsmoking (19.0 ± 7.0 ng/mL; $n = 26$) mothers.

Conclusions: Cord plasma 5-MTHFA concentrations were elevated relative to maternal blood, as expected, because the placenta is capable of concentrative folate transport to the fetus. The negative effect of maternal smoking on infant, but not on maternal, 5-MTHFA status indicates that maternal smoking may impair folate transport to the fetus. *Am J Clin Nutr* 2007;85:796–802.

KEY WORDS 5-Methyltetrahydrofolic acid, folate, folic acid, African American women, pregnancy, smoking, electrospray mass spectrometry, infants, umbilical cord, placenta

INTRODUCTION

Pregnant, inner-city African American women are at risk of consuming less than the recommended dietary allowance (RDA) of dietary folate and engage in lifestyle habits, such as smoking, that can further reduce maternal folate status (1). In the United States, population-wide serum and erythrocyte folate concentrations have increased with folic acid fortification, but concentrations remain lower in non-Hispanic black women than in other ethnic groups (2). Also, the incidence of infants born with neural tube defects has decreased less in non-Hispanic black populations than in Hispanic and non-Hispanic white populations (3). In

Wayne County, MI, or in the state of Michigan, the incidence of congenital abnormalities of the central nervous system has not decreased after folate fortification (4). The adequacy of current levels of folic acid fortification is debatable, but clearly other factors must be considered (5).

The effect of cigarette smoking on pregnancy outcomes is complex because it is associated with other behaviors, including reduced micronutrient and increased alcohol intakes (6). Maternal smoking during pregnancy has been identified as the most important determinant of birth weight in developed countries (7) and may increase the risk of neural tube defects (8, 9), orofacial clefts (10, 11), and congenital heart defects (12). Adequate folate status is associated with a reduced risk of each of these outcomes (13–16). Folate intake, which is reduced with smoking (17), is the major determinant of blood folate concentrations; however, the negative association between smoke exposure and blood folate status persists after adjustment for folate intakes in studies before and after folic acid fortification (1, 18).

Smoking is associated with reduced circulating maternal folate concentrations (1, 6, 19). To our knowledge, there have only been 2 reports of the effects of maternal smoking on umbilical cord or infant folate concentrations, with both showing no effect (14, 20). No data exist on the effects of maternal smoking on umbilical cord folate concentrations in African American women. Maternal-to-fetal folate transfer is concentrative, as shown in perfused human placenta studies (21–23) and in maternal-umbilical cord comparisons (24, 25). The mechanism of placental folate transport is yet to be fully elucidated, but it

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appears that there is a high affinity folate receptor at the syncytiotrophoblast that mediates endocytosis at the microvillous membrane followed by efflux into fetal blood at the basolateral side by a reduced folate carrier (21). Maternal smoking can also alter placental development, induce placental hypoxia, and impair placental nutrient transport (6, 26).

The purpose of the present study was to examine the effects of maternal smoking at the time of the first prenatal visit on 5-methyltetrahydrofolic acid (5-MTHFA) concentrations in maternal plasma and umbilical cord plasma in an inner-city African American population at-risk for behaviors detrimental to health. Accurate and specific measurements of 5-MTHFA were made in maternal plasma, cord venous plasma, and cord arterial plasma by stable-isotope HPLC-mass spectroscopy-electrospray ionization (HPLC-MS-ESI) (27) and combined with detailed demographic, clinical, and behavioral data, including smoking, alcohol, and dietary intakes.

SUBJECTS AND METHODS

Subjects and sample collection

Pregnant African American women attending the Antenatal Clinic at Wayne State University (Detroit, MI) between February 1999 and January 2001 were recruited into the present study as described previously (1). All procedures and protocols received prior approval by the Wayne State University Human Investigations Committee, and informed consent was obtained during the initial clinical visit. Women with high-risk pregnancies, with known fatty acid metabolism disorders, with diabetes, and who developed gestational diabetes were excluded from the study. All of the infants in the present study were singleton and free of malformations as determined by dysmorphology and neurobehavioral testing procedures common in fetal alcohol syndrome screening (28).

A structured interview at the first antenatal visit determined eligibility and assessed demographic characteristics, alcohol intake, and smoking exposure (29, 30). In this interview, quantitative smoking (cigarettes smoked/d) was determined at the time of the interview and before pregnancy by maternal recall. Socioeconomic status was measured by using a modified Hollingshead index (31). Demographic and clinical characteristics were collected for 282 mother-singleton infant pairs at delivery. Nutrient intakes were assessed at the time of delivery as previously published (1, 30, 32) with a food-frequency questionnaire validated for nutrient and folate intakes in low-income pregnant women (33, 34). The US Department of Agriculture National Nutrient Database for Standard Reference, release 14, was used for quantification (35). Individual nutrient intakes were adjusted for total energy intake with the nutrient residual method (36) to reduce measurement error (37). Dietary folate equivalents (DFEs) were calculated as recommended by the Food and Nutrition Board in the Dietary Reference Intakes for folate (38): for natural food sources of folate, $1 \mu\text{g folate} = 1 \mu\text{g DFE}$; for synthetic vitamin preparations, $1 \mu\text{g folic acid} = 2 \mu\text{g DFE}$; and for mixed food and supplement products, $\mu\text{g DFE}$ is calculated as $\text{food folate (in } \mu\text{g)} \times 1 + \text{folic acid (in } \mu\text{g)} \times 1.7$. All participants were advised about nutrient supplementation during pregnancy and received a prescription to obtain prenatal vitamins, but compliance with daily multivitamin use in urban African Americans is very low (39).

Maternal blood (15 mL) was collected shortly before infant delivery by venipuncture of the antecubital vein. At delivery, infant demographics were recorded and as much separate arterial and venous umbilical cord blood was collected as possible. Both the maternal and umbilical cord blood specimens were collected into heparinized tubes and kept cold (4°C) until centrifugation (5 min, $2000 \times g$) to separate plasma and erythrocytes, and the plasma was frozen at -75°C until analyzed.

Laboratory analyses

Plasma 5-MTHFA concentrations in maternal and umbilical venous and arterial samples were measured by HPLC-MS-ESI with an intraassay CV of 5.3% and an interassay CV of 7.6% as described previously (27). Before extraction, $[^{13}\text{C}_5]$ 5-MTHFA (10 ng) was added to 0.5 mL plasma as an internal standard. The analyte was isolated by using solid-phase extraction (Strata phenyl column 100 mg/mL; Phenomenex, Torrance, CA), washed with 0.03 mol $\text{K}_2\text{HPO}_4/\text{L}$, and eluted with 0.5 mL of the HPLC mobile phase (acetonitrile:methanol:water, 26:14:60). Forty microliters of extract was injected onto a C_{18} HPLC column (150×4.6 mm; Phenomenex) with the use of a binary pumped Agilent 1100 HPLC (Palo Alto, CA) interfaced to an ion-trap MS (Finnigan LCQ San Jose, CA), and samples were analyzed by ESI in the positive ion mode.

Statistical analyses

Data were normally distributed as determined by the Kolmogorov-Smirnov procedure. Comparisons between maternal plasma 5-MTHFA, venous cord plasma 5-MTHFA, and arterial cord plasma 5-MTHFA were made by repeated-measures ANOVA; individual mean comparisons were made by using Tukey's honestly significantly different (HSD) test. Maternal plasma 5-MTHFA and venous plasma 5-MTHFA were compared by paired *t* test. Bivariate associations of cigarettes smoked by the mothers before pregnancy and at the first prenatal visit, cigarettes smoked by the fathers, maternal plasma 5-MTHFA, maternal DFE intake, and venous cord plasma 5-MTHFA were determined by Pearson's correlations (two-tailed). Plasma 5-MTHFA in smokers and nonsmokers at delivery were compared by independent *t* test for maternal and venous cord samples and by general linear models with various covariates. Associations with venous cord plasma 5-MTHFA were also evaluated by using multiple linear regression analyses. A parsimonious model with independent variables having *P* values <0.10 were included and a controlled model were generated. Variables were included based on information from the literature, influence on the model *r* value, and degree of collinearity with other variables. The consistency of the statistical results were examined after the exclusion of 2 participants who consumed a prenatal multivitamin that included folic acid regularly throughout pregnancy, as determined by dietary records and maternal 5-MTHFA. All statistical analyses were completed with SPSS for WINDOWS statistical software (release 11.5.1; SPSS Inc, Chicago, IL) with statistical significance inferred when *P* values were <0.05 .

RESULTS

Maternal characteristics

Sample size in the present study was limited primarily by the difficulties in collecting adequate amounts of separate and unhemolyzed venous and arterial cord blood combined with sample

TABLE 1
Characteristics and dietary intakes of pregnant African American women¹

	All subjects (<i>n</i> = 282)	Maternal-cord folate subset		
		Smokers (<i>n</i> = 32)	Nonsmokers (<i>n</i> = 26)	All subjects (<i>n</i> = 58)
Maternal age (y)	24.8 ± 5.4	26.1 ± 5.9	23.8 ± 5.1	25.1 ± 5.6
Gestational age at 1st prenatal visit (wk)	16.3 ± 6.4	16.9 ± 6.9	17.2 ± 6.9	17.0 ± 6.8
Height (m)	1.65 ± 0.07	1.66 ± 0.06	1.66 ± 0.09	1.66 ± 0.07
Prepregnancy weight (kg)	74.3 ± 20.8	78.4 ± 21.7	75.8 ± 18.1	77.3 ± 20.1
Education (highest grade)	11.8 ± 1.4	11.7 ± 1.3	12.0 ± 1.2	11.8 ± 1.3
Socioeconomic status (Hollingshead class)	3.9 ± 1.0	4.0 ± 1.2	3.7 ± 1.1	3.9 ± 1.1
Total pregnancies	3.7 ± 2.4	3.9 ± 2.5	3.2 ± 1.6	3.6 ± 2.2
Smoking (cigarettes/d)	5.7 ± 8.5	9.9 ± 8.5	NA	5.5 ± 8.0
Absolute alcohol intake				
(mL/d)	30.6 ± 43.7	44.0 ± 56.6	19.1 ± 29.0 ²	32.8 ± 47.6
(oz/d)	1.0 ± 1.5	1.5 ± 1.9	0.6 ± 1.0 ²	1.1 ± 1.6
Selected dietary intakes ³				
Protein (g/d)	72 ± 14	67 ± 15	71 ± 14	69 ± 14
Carbohydrates (g/d)	259 ± 46	256 ± 46	243 ± 40	250 ± 44
Fat (g/d)	94 ± 16	89 ± 41	94 ± 43	91 ± 42
Vitamin B-6 (mg/d)	1.9 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	1.8 ± 0.4
Vitamin B-12 (μg/d)	5.5 ± 2.2	5.6 ± 2.1	5.2 ± 1.6	5.4 ± 1.9
Methionine (g/d)	1.7 ± 0.4	1.5 ± 0.4	1.6 ± 0.4	1.6 ± 0.4
Dietary folate equivalents (μg/d)	466 ± 121	428 ± 129	442 ± 110	434 ± 120
Food folate (μg/d)	216 ± 83	208 ± 84	224 ± 79	215 ± 82
Fortified folate (μg/d)	147 ± 61	129 ± 66	128 ± 55	129 ± 61
Total energy (MJ/d)	9.2 ± 4.4	9.0 ± 3.6	9.0 ± 4.0	9.0 ± 3.7
Maternal plasma 5-MTHFA (ng/mL)	ND	12.5 ± 7.9	13.5 ± 7.0	13.0 ± 7.5

¹ All values are $\bar{x} \pm$ SD. NA, not applicable; ND, not determined; 5-MTHFA, 5-methyltetrahydrofolate.

² Significantly different from smokers, $P \leq 0.05$ (two-tailed independent *t* test).

³ Values adjusted for energy by the nutrient residual model.

requirements for other clinical and research analyses. In particular, instances of a complete set of unhemolyzed maternal plasma, venous, and arterial cord plasma were limited ($n = 15$). Matching only the maternal and the venous cord plasma resulted in a larger sample set ($n = 58$). The maternal age in the present analysis ranged from 17 to 38 y. Maternal characteristics and selected dietary intakes in the present analysis ($n = 58$) were similar to those in the entire sample ($n = 282$) (Table 1). The percentage of women smoking during pregnancy reported in the present analysis was 55% (95% CI: 42%, 68%), which was similar to the 48% of the entire sample who reported smoking (95% CI: 42%, 54%).

In the present sample, alcohol intake was higher in the smoking than in the nonsmoking mothers (Table 1). This was also true in the entire sample. In addition, the nonsignificant differences in education, socioeconomic status, and total pregnancies in the present analysis reached significance with the increase in power available in the entire sample. In summary, smokers had lower levels of education (grade completed: 11.5 ± 1.5 compared with 12.0 ± 1.2 ; $P = 0.003$), lower socioeconomic status (Hollingshead score: 4.1 ± 1.0 compared with 3.7 ± 1.0 ; $P < 0.001$), and a greater number of total pregnancies (4.1 ± 2.7 compared with 3.4 ± 1.0 ; $P = 0.015$).

Maternal DFE intakes were estimated at 434 ± 120 μg/d (median: 420 μg/d). This suggests that 79.3% of the subjects had DFE intakes below the Estimated Average Requirement (EAR) of 520 μg/d and 91.4% below the RDA of 600 μg/d for pregnant women. The intake of vitamin B-6 was 1.8 ± 0.4 mg/d (median: 1.8 mg/d) and was below the EAR of 1.6 mg/d in 29.3% of the

subjects and below the RDA of 1.9 mg/d in 65.5% of the subjects. In contrast, vitamin B-12 intakes were estimated at 5.4 ± 1.9 μg/d (median: 5.3 μg/d); 96.6% of the subjects met the EAR of 2.2 μg/d and the RDA of 2.6 μg/d.

Maternal and umbilical cord plasma folate

Plasma 5-MTHFA concentrations were higher in both the arterial and venous cord plasma (18.4 ± 7.4 and 18.7 ± 9.6 ng/mL, respectively) than in the maternal plasma (13.5 ± 7.8 ng/mL) (Figure 1). No significant difference between arterial and venous plasma concentrations of 5-MTHFA were observed. In the expanded sample ($n = 58$), venous cord plasma 5-MTHFA concentrations (16.8 ± 7.5 ng/mL) were again significantly higher than maternal plasma 5-MTHFA concentrations (13.0 ± 7.5 ng/mL) (Figure 1). Exclusion of the 2 subjects determined to be taking folic acid supplements regularly resulted in no changes in the statistical results, although slight reductions were found in the mean and SDs for both the maternal-arterial-venous analysis ($n = 14$) and the maternal-venous analysis ($n = 56$).

Effect of smoking

Cigarettes smoked by the mothers at the first prenatal visit as compared with other quantitative smoking estimates had the strongest Pearson's correlation with plasma 5-MTHFA determinations. Venous cord plasma 5-MTHFA was positively associated with maternal plasma 5-MTHFA ($r = 0.60$, $P < 0.001$; Figure 2) and negatively associated with cigarettes smoked by the mothers at the first prenatal visit ($r = -0.31$, $P = 0.019$).

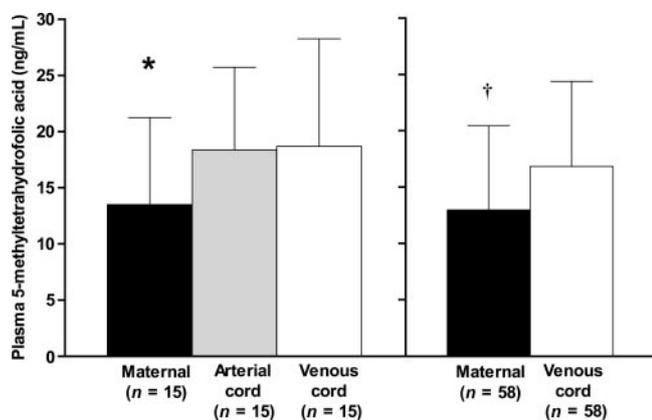


FIGURE 1. Mean (\pm SD) 5-methyltetrahydrofolic acid concentrations in maternal plasma and arterial and venous cord plasma samples from African American women. *Significantly different from arterial and venous cord plasma, by Tukey's honestly significantly different test ($P < 0.01$), after significance was determined by repeated-measures ANOVA ($P = 0.0023$). †Significantly different from venous cord plasma, $P < 0.0001$ (paired t test).

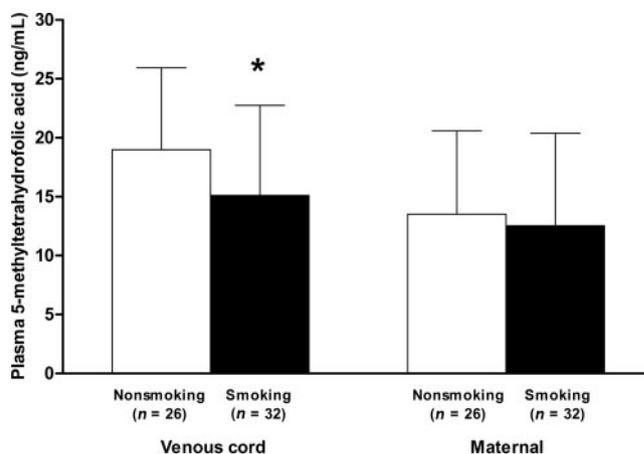


FIGURE 3. Mean (\pm SD) venous cord and maternal plasma 5-methyltetrahydrofolic acid concentrations in smoking African American women. *Significantly different from the nonsmoking group, $P = 0.0498$ (independent t test).

Cigarettes smoked by the mothers was not significantly associated with maternal plasma 5-MTHFA ($r = -0.05$, $P = 0.71$). Similarly, prepregnancy cigarettes smoked was negatively associated with venous cord 5-MTHFA ($r = -0.30$, $P = 0.023$) and not associated with maternal 5-MTHFA ($r = -0.04$, $P = 0.78$). Prepregnancy and first prenatal visit cigarettes smoked were strongly correlated ($r = 0.99$, $P < 0.001$). Cigarettes smoked by the fathers (data available for 53 maternal-cord pairings) was significantly correlated only with maternal first prenatal visit cigarettes smoked, although the correlation coefficients between cigarettes smoked per day by the fathers and plasma 5-MTHFA concentrations were similar to our previous finding at 24 wk of gestation ($r = -0.21$, $P = 0.043$; $n = 92$) (1). Exclusion of the 2 subjects who were taking folic acid supplements had no statistical effect on these bivariate associations.

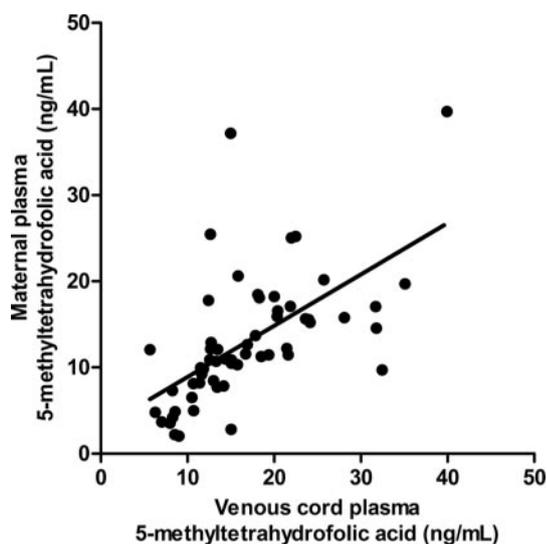


FIGURE 2. Scatterplot of the relation between maternal and venous cord plasma 5-methyltetrahydrofolic acid concentrations in African American women ($n = 58$). Each circle represents an individual data point. Bivariate associations were determined by Pearson's correlations: $r = 0.60$, $R^2 = 0.36$, $P < 0.001$. $y = 0.60x + 2.957$.

Venous cord plasma 5-MTHFA concentrations in smoking mothers (15.1 ± 7.6 ng/mL; $n = 32$) were significantly lower than venous cord concentrations in nonsmoking mothers (19.0 ± 7.0 ng/mL; $n = 26$) (Figure 3). These differences persisted after the inclusion of maternal plasma 5-MTHFA concentrations ($P = 0.039$), energy-adjusted DFE intakes ($P = 0.038$), and both maternal plasma 5-MTHFA and adjusted DFE ($P = 0.034$) in general linear models as covariates. Maternal plasma 5-MTHFA concentrations were slightly but not significantly lower in the smoking (12.5 ± 7.9 ng/mL; $n = 32$) than in the nonsmoking (13.5 ± 7.0 ng/mL; $n = 26$; $t_{(56)} = 0.50$, $P = 0.62$) mothers (Figure 3), and the inclusion of maternal energy-adjusted DFE intake as a covariate had no effect.

Maternal plasma 5-MTHFA was positively associated (standardized $\beta = 0.59$, $P < 0.001$) and cigarettes smoked by the mothers at the first prenatal visit was negatively associated (standardized $\beta = -0.28$, $P = 0.008$) with venous cord plasma 5-MTHFA concentrations and were the only independent variables remaining in the parsimonious multiple linear regression model (Table 2). These associations also remained significant in a controlled model that included energy intake, maternal alcohol consumption, education, maternal age, and BMI. Energy-adjusted DFE intakes and socioeconomic status were also considered but excluded from the model because of collinearity with other independent variables. In the present sample for which maternal and umbilical cord concentrations were measured ($n = 58$), infant birth weight did not differ significantly between smoking and nonsmoking mothers (3190 ± 107 and 3345 ± 119 , respectively; $P = 0.34$) in a simple univariate analysis; however, the effect of maternal smoking on birth weight was significant when gestational age and infant sex were entered as covariates ($P = 0.042$).

DISCUSSION

Smoking by inner-city African American women during pregnancy significantly decreases 5-MTHFA concentrations in cord plasma. This effect is independent of maternal folate intake and independent of the effect of maternal smoking on maternal plasma 5-MTHFA concentrations. Venous cord plasma 5-MTHFA was

TABLE 2

Results of multiple linear regression with venous cord plasma 5-methyltetrahydrofolate (5-MTHFA) concentrations as the dependent variable and dietary folate, smoking, drinking, and maternal plasma 5-MTHFA as independent variables¹

Group and model variables	Model R^2	Standardized β	P
Parsimonious model	0.44	—	< 0.001
Maternal plasma 5-MTHFA (ng/mL)	—	0.59	<0.001
Cigarettes smoked per day	—	-0.28	0.008
Controlled model	0.47	—	< 0.001
Maternal plasma 5-MTHFA (ng/mL)	—	0.59	<0.001
Cigarettes smoked per day	—	-0.31	0.009
Dietary vitamin B-12 (μg)	—	-0.07	0.53
Drinking (absolute oz of alcohol)	—	0.04	0.75
Education (y)	—	0.06	0.60
Mother's age (y)	—	0.11	0.31
BMI (kg/m^2)	—	-0.10	0.37

¹ Independent variables in the multiple linear regression models were included on the basis of biological plausibility as reported previously in the literature, the influence on the model R^2 value, and the degree of collinearity with other independent variables ($n = 58$). A backward stepwise parsimonious model with all included independent variables with P values < 0.10 and a controlled model with potential confounders included as independent variables were generated.

20.5% lower in the infants from smoking mothers compared with infants from nonsmoking mothers.

This is the first study to report an effect of maternal smoking on infant folate status. Previously in the United Kingdom, Relton et al (14) showed that neonatal erythrocyte folate was associated with maternal erythrocyte folate and maternal vitamin B-12 and that smoking decreased maternal erythrocyte folate ($n = 319$) but not neonatal erythrocyte folate ($n = 271$). However, smoking was assessed categorically (never, ever, or current), and mother-infant paired data were used only for maternal-neonatal vitamin correlations and not for the effects of smoking on neonates. Bjørke Mønsen et al (20) reported no differences in serum folate and whole-blood folate between Norwegian neonates from smoking ($n = 30$) and nonsmoking mothers ($n = 143$) at birth and at 6 wk. A slight increase in serum folate and a significant decrease in serum vitamin B-12 concentrations were observed in neonates of "heavy" smoking mothers defined in that study as smoking >7 cigarettes/d.

The present study had several limitations. Erythrocyte folate, total folate, and forms of folate other than 5-MTHFA were not assessed in the present study. Vitamin B-12 measurements were also not completed because of limited volumes of cord blood samples and other analytic priorities. In addition, accurate determinations of maternal smoking and folate intake are difficult and subject to error, as discussed previously (1). However, accurate and specific determinations of plasma 5-MTHFA were made by HPLC-MS-ESI for maternal-infant pairings, and prepregnancy smoking, smoking at the time of the first prenatal visit, and the father's smoking habits were assessed both quantitatively (cigarettes smoked/d) and categorically (smokers compared with nonsmokers).

The present HPLC-MS-ESI method can be used to determine other forms of folate, including 5-MTHFA, tetrahydrofolate,

5-formyltetrahydrofolate, and folic acid if appropriate internal standards are available, as done recently in erythrocytes (40) but not in the present study. An effect of smoking on 5-MTHFA would likely persist for total plasma folate because 5-MTHFA is estimated to consist of >90% of total plasma folate in persons with low folate, such as the present population sample, and remain >80% in persons with higher folate concentrations (41). Little data on the concentrations of specific forms of folate in cord and infant plasma and erythrocytes are available, and the placental transport of the individual forms of folate has not been fully elucidated. Folic acid transport is inhibited in the presence of 5-MTHFA in BeWo monolayers (42).

Maternal plasma 5-MTHFA concentrations in the present study (13.0 ± 7.5 ng/mL) were lower than those previously published in the same population of women at 24 wk of gestation (18.0 ± 7.1 ng/mL) (1). A decrease was expected because of the normal physiologic blood and plasma volume expansion that occurs during pregnancy (43). However, the increase in maternal plasma volume from 24 wk gestation to the time of infant delivery in this population was previously estimated at 10% (44), which would account for a difference of ≈ 1.8 ng/mL. The lower maternal plasma 5-MTHFA concentrations at delivery may also have been a consequence of reduced DFEs as pregnancy progressed, because the mean, median, and SD for DFE intakes were all lower in the present sample (mean, 434; median, 420; SD, 120 $\mu\text{g}/\text{d}$) than in previous samples at 24 wk gestation (mean: 605 $\mu\text{g}/\text{d}$; median: 590 $\mu\text{g}/\text{d}$; SD: 181 $\mu\text{g}/\text{d}$). Fortified folate intake was also estimated to be 147 ± 61 $\mu\text{g}/\text{d}$ in the entire sample at delivery ($n = 282$) and 129 ± 61 in the present sample ($n = 58$), as compared with 198 ± 98 $\mu\text{g}/\text{d}$ determined previously at 24 wk gestation (1). Gustatory sensitivity decreases during pregnancy (44), and, in the present population, the mass intakes of protein, carbohydrates, and fat are lower at delivery than at 24 wk gestation (32). In addition, folate catabolism progressively increases during pregnancy (45), and folate absorption and utilization may increase significantly after 27 wk gestation (46).

Folate fortification appears to have resulted in dietary folate increases greater than the anticipated 70–130 $\mu\text{g}/\text{d}$ (2). Folate values in the US Department of Agriculture National Nutrient Database for Standard Reference, release 14, are based on enrichment specifications (35). Therefore, the estimates of fortified folate intake in this population suggest that fortified food products were eaten in greater quantities than those estimated at the time of fortification. In addition, the folate intakes in the present study may be underestimates of actual intakes because folate fortification of the food supply in excess of enrichment targets has been reported (47).

Cord plasma 5-MTHFA concentrations in the present study (16.8 ± 7.5 ng/mL) were significantly higher than maternal plasma 5-MTHFA concentrations (13.0 ± 7.5 ng/mL) but were similar to plasma folate concentrations measured in an Irish population by Molloy et al (24): 20.7 ng/mL in cord plasma and 11.3 ng/mL in maternal plasma. Thus, the present study supports concentrative maternal-fetal folate transport via the placenta. Cigarettes smoked by the mothers and fathers was not associated with altered maternal plasma 5-MTHFA, and maternal plasma 5-MTHFA was not significantly different between smokers and nonsmokers. This was likely due to restricted statistical power (≈ 10 –20%) because of the small number of subjects. Smoking and smoke exposure have been negatively associated with serum



and erythrocyte folate (14, 18) and plasma 5-MTHFA (1) in larger sample sizes. There was sufficient power ($\approx 50\%$) in the present study to determine an effect of maternal smoking on venous cord 5-MTHFA.

Maternal smoking at the first prenatal visit (17.0 ± 6.8 wk gestation) appeared to affect cord plasma 5-MTHFA concentrations to a greater extent than maternal smoking affected maternal folate status at the time of delivery. However, maternal folate status is the major determinant of infant status, because maternal 5-MTHFA concentrations accounted for 35% of the variation and cigarette smoking accounted for 10% of the variation in venous cord 5-MTHFA. The effect of decreased cord folate concentrations at the time of delivery on neural tube defects is difficult to assess given the importance of adequate folate status at the time of conception and neural tube formation. It is possible that folate transport may be affected by smoking during early embryogenesis, because it has been speculated that smoking may impair folate receptor activity and the expression in squamous cell cancers of the lung (48). Further research on the effect of smoking on folate transport across the placenta and in other cell systems is required.

Extrapolation of the present findings to other populations requires caution given the low prevalence of methylenetetrahydrofolate reductase mutations, low DFE intakes, and adequate vitamin B-12 intakes in African Americans. The present study supports the hypothesis that lifestyle choices, such as smoking during pregnancy, may be partly responsible for the smaller reductions in neural tube defects than those expected after folic acid fortification in selected populations, such as inner-city African Americans. 

All authors contributed to the study design and concept and to the revision of the manuscript. JHH and NS were coprincipal investigators. JHH and RJS coordinated and supervised participant recruitment and sample collection. KDS and RJP performed the 5-methyltetrahydrofolate measurements. KDS conducted the statistical analyses and wrote the first draft of the manuscript. None of the authors had a conflict of interest.

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