ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Maternal and Pediatric Nutrition

Folic Acid Intake, Fetal Brain Growth, and Maternal Smoking in Pregnancy: A Randomized Controlled Trial

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ABSTRACT

Background: Folic acid supplementation during pregnancy plays an important role in fetal growth and development. To our knowledge, no experimental study has examined the effect of folic acid on fetal brain growth in women who smoke cigarettes during pregnancy.

Objectives: The aim of this study was to investigate the efficacy of higher-dose compared with standard-dose folic acid supplementation on prenatal fetal brain growth, measured by head circumference, brain weight, and brain-body weight ratio (BBR).

Design: In this randomly assigned, double-blind, controlled clinical trial, we recruited 345 smoking pregnant women attending a community health center in Tampa, FL between 2010 and 2014. Participants were randomly assigned in a 1:1 ratio to receive either 0.8 mg folic acid/d (standard of care at the study center) or 4 mg folic acid/d (higher strength). Participants were also enrolled in a smoking cessation program. A 2-level linear growth model was used to assess treatment effect and factors that predict intrauterine growth in head circumference over time. Multiple linear regression analyses were conducted to estimate the effect of higher-strength folic acid on head circumference at birth, fetal brain weight, and fetal BBRs.

Results: Mothers who received the higher dose of folic acid had infants with a 1.18 mm larger mean head circumference compared with infants born to mothers who received the standard dose, but this difference was not statistically significant ($P = 0.2762$). Higher-dose folic acid also had no significant effect on brain weight. The BBR of infants of mothers who received higher-dose folic acid was, however, 0.33 percentage points lower than that for infants of mothers who received the standard dose of folic acid ($P = 0.044$).

Conclusions: Infants of smokers in pregnancy may benefit from higher-strength maternal folic acid supplementation. We noted a decrease in the proportion of infants with impaired BBR among those on higher-dose folic acid. This trial was registered at clinicaltrials.gov as NCT01248260. *Curr Dev Nutr* 2019;3:nzz025.

Keywords: folic acid, brain growth, smokers, head circumference, fetal brain weight, brain-to-body weight ratio

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Abbreviations used: BBR, brain-body weight ratio; RBC, red blood cell; SGA, small for gestational age.

Introduction

The role of maternal smoking on fetal development is well documented (1–3). Nicotine readily crosses the placenta into the fetal serum and brain, (4) and has been found to be neurotoxic (5). Maternal tobacco exposure is associated with reduced head circumference (1, 2), altered indicators of infant body proportionality, such as brain-body weight ratio (BBR) (3), and infant neurocognitive developmental problems (6, 7). Although smoking cessation before midpregnancy may mitigate smoking-related deficits in infant head circumference and BBR (2, 3), many mothers find it difficult to quit, thereby exposing their unborn babies to the harmful effects of tobacco.

A likely mechanism of action of maternal smoking on adverse fetal outcomes is through a reduction in the concentration and activity of maternal folic acid reserve (8, 9). Smokers are more likely to consume diets deficient in folate and have impaired folate metabolism (9–11). Low maternal folate concentrations reduce the bioavailability of folate to the developing fetus, resulting in impaired growth (12). Previous studies suggest that prenatal folate deficiency might have an adverse effect on global fetal brain growth (13), and folate supplementation in early pregnancy may increase head circumference at birth (13) and prevent neurodevelopmental disorders in offspring (14, 15).

The role of folic acid supplementation and folate concentrations in fetal head growth among women of reproductive age is also well documented (13, 16). However, there is a dearth of research on the effect of folic acid supplementation among high-risk subgroups, such as women who smoke during pregnancy. To our knowledge, no experimental study has been conducted to examine this relation. In this study, we investigate the efficacy of high-strength compared with standard-dose folic acid on increasing prenatal brain growth. Study outcomes include head circumference, brain weight, and BBR. Head circumference is a noninvasive proxy for fetal brain growth and development (16), and is closely correlated to brain volume (17). We hypothesize that among mothers who smoke cigarettes in pregnancy, higher-dose folic acid treatment will be associated with increased fetal brain growth.

Methods

Trial population and design

This study is a randomized, double-blind, controlled clinical trial primarily conducted to assess the effect of high-strength folic acid on fetal body and brain size. Screening took place among 860 pregnant smokers; 345 met the eligibility criteria and were enrolled in the study. The eligible participants were randomly assigned in a 1:1 ratio to receive either 0.8 mg folic acid/d (standard of care at the study site) or 4 mg folic acid /d (higher-dose treatment). The folic acid tablets had the same color, shape, size, and were packed in identically coded bottles. Allocation concealment was achieved through the pharmacy. Based on the randomization card received from the patient, the pharmacist gave a bottle that contained either a 0.8 or 4 mg folic acid tablet. The code on the bottle was the only distinguishable feature which was used to determine what the bottle contained, and this was concealed with patient ID labels. The physicians, ultrasound technicians, laboratory staff, study investigators, and study participants were all blinded to the treatment groups. Randomization was performed through a computer-generated randomization schedule with the use of a permuted block design, with a block size of 12. This type of design was chosen to allow balance in the number of participants in each group at the end of the clinical trial.

Participants were recruited between 2010 and 2014 at the Genesis Clinic, Tampa, FL, a community health center affiliated with the Department of Obstetrics and Gynecology of the University of South Florida. Women were eligible to participate in the study if they fulfilled the following criteria: *1*) current smokers, *2*) aged 18–44 y; *3*) at <21 wk gestation, as confirmed by last menstrual period; and *4*) residents of Tampa, FL, or a surrounding area, to facilitate follow-up and reduce attrition. To identify pregnant women who smoked at baseline, screening for cotinine—a biological marker for nicotine—in saliva was performed. Women with detectable cotinine concentrations of ≥1 on a NicAlert test strip were confirmed eligible for the study. Women receiving chronic blood transfusion may have inaccurate measures of red blood cell (RBC) folate concentrations, due to transfused RBCs. Additionally, patients treated with anticonvulsants may have folate deficiency as a side effect of the medication (18). Participants were therefore excluded if there was evidence of chronic blood transfusion and generalized seizure disorder treated with anti-convulsant medication.

Fetal ultrasound assessments, study questionnaires, and salivary cotinine laboratory assessments were completed during the first 3 study visits. Participants were questioned regarding tablet intake and compliance, and the reported number of tablets consumed was cross-checked by observing the remaining number of tablets in each bottle. Women who adhered during their second and third visits were categorized as compliant, whereas those who did not adhere during ≥1 of the visits were grouped as noncompliant. At delivery, cotinine concentration was further evaluated, and fetal body measurements were taken. Written informed consent was obtained from all the participating women before trial-related procedures were initiated. Informed consent was available in both English and Spanish. Monetary compensation per clinical visit was estimated based on the median hourly wage for the community and the travel costs. For the total of 4 visits and completion of a dietary history questionnaire, a total of US$90 was reimbursed per participant as follows: first visit US$20; second visit US$20; third visit US$20; at delivery US$20; and dietary history questionnaire completion US$10.

Smoking cessation program

All women enrolled in the trial signed a contract stating they agreed to commit to quitting smoking and attended ≥1 smoking cessation session at the study site for smoking mothers, or called the Florida Quitline, a toll-free telephone-based tobacco use cessation service. The counseling sessions focused on the following: *1*) receipt of self-help materials and how to make a quit attempt; *2*) effects of secondhand smoke, partners who smoke, and tips on how to establish smoke-free homes and cars; *3*) stress management and benefits of not smoking; and *4*) prevention of smoking relapse. Women who persistently tested positive to salivary cotinine were referred by study personnel to Florida's Quitline. Additionally, all women were linked to community smoking cessation services, such as the Hillsborough County Healthy Start program, a free, voluntary, and intensive smoking cessation program.

Endpoint measures

The outcomes included prenatal fetal brain growth, defined as *1*) rates of growth in intrauterine ultrasound measure of head circumference (growth velocity); and *2*) 2 different measures of cumulative head circumference growth (fetal brain weight and fetal BBR). Serial ultrasound measurements of head circumference were obtained during 3 study visits according to standardized ultrasound procedures. Intrauterine head circumference was measured to the nearest millimeter on a transverse view of the fetal head in a plane showing both thalami and the third ventricle. The second outcome, fetal estimated brain weight, was derived from the National Institute of Neurological Disorders

and Stroke's Collaborative Perinatal Project and was calculated from the formula: [0.037 × head circumference (cm)$^{2.57}$] (19). Head circumference at birth was measured to the nearest centimeter with a measuring tape. The third outcome, the BBR, is the proportion of the body weight that resides in the brain (20, 21). The BBR was calculated as 100× the ratio of the infant's estimated brain weight to its birth weight and is expressed as 100 × [0.037 × head circumference (cm)$^{2.57}$]/birth weight (g). A high BBR indicates a higher percentage of birth weight residing in the brain, whereas a lower BBR suggests a lower fraction of birth weight residing in the brain (3). The typical values for healthy infants are estimated to be 9–10% (17). Furthermore, higher BBR indicates larger brain weight for a given head circumference and is associated with small-for-gestational-age (SGA) infants (17).

Covariates

Based on prior knowledge, several maternal and infant characteristics were considered as potential confounding factors due to their demonstrated association with fetal brain development. These include sociodemographic factors (e.g., maternal age, race, marital status, and insurance status), lifestyle factors (cotinine concentration, alcohol consumption, dietary folate, and maternal BMI), perinatal factors (e.g., maternal depression, stress, gestational age), and maternal chronic diseases, such as hypertension and diabetes. Gestational age at delivery, a measure of duration of the pregnancy in weeks, was based on dating ultrasound at first prenatal visit and the date of delivery of the baby.

Sociodemographic and lifestyle factors were extracted from participants' medical records, specifically the American College of Obstetrics and Gynecology form. Dietary folate was assessed through the use of a proxy, maternal red blood cell (RBC) folate concentration at study baseline. RBC folate was measured by ELISA. This was the preferred folate biomarker because of its advantages of long-term stability and reduced susceptibility to sudden changes in diet (22). Depression was assessed with the use of the Edinburgh Postnatal Depression Scale. The Edinburgh Postnatal Depression Scale is a validated tool for assessing antenatal and postnatal depressive symptoms (23). Stress was measured with the use of the Perceived Stress Scale 14, which is a validated tool for comparisons between people in study samples (24).

Cotinine was measured by a test of maternal saliva for the presence of cotinine with NicAlert (Jant Pharmacal Corporation), a rapid semiquantitative screening test. Salivary cotinine analysis is the most sensitive and specific of the 3 types of cotinine measures (25).

Statistical analysis

We assessed differences in the baseline sociodemographic, lifestyle, and health-related characteristics of study participants by treatment group. Compliance rate between both groups was also examined. Means of continuous data were compared through the use of independent *t* tests or ANOVA, whereas categoric variables were compared with the chi-square test. Based on the intention-to-treat population, birth outcomes for all study participants were described in terms of frequencies and percentages. All primary analyses were conducted on a modified intention-to-treat basis and included participants who completed the trial with an observed endpoint, irrespective of compliance to protocol. Based on a power of 80%, a type 1 error rate of 5%, and a 50% reduction in the rate of loss of fetal brain growth, the estimated sample size required to detect a difference in the efficacy of high-strength compared with standard-dose folic acid on enhancing brain development was a total of 100 participants. Growth potentials for fetuses with congenital anomalies or those from multiple gestations may not be comparable to singleton pregnancies without abnormalities. Analyses therefore excluded all pregnancies that ended in a fetal loss (abortions, fetal demise, stillbirths, miscarriages), congenital anomalies, or multiple births.

Because there were 4 repeated measures of cotinine concentration from participants during the trial, we decided to identify the unique paths of cotinine concentrations throughout the pregnancy through the use of a group-based latent class trajectory model. This approach offers a data-driven method to identify distinct individual patterns of a variable of interest and the corresponding probability of falling into each pattern, also known as the posterior probability. Trajectory analyses were conducted, and participants were then classified based on their highest posterior probability. All statistical tests were 2-sided, with an α level set at $P < 0.05$. We used SAS version 9.4 for all analyses.

Fetal brain growth trajectory

The primary outcome was the rate of intrauterine growth in head circumference from the beginning of the second trimester of pregnancy until delivery. A 2-level linear growth model (multilevel model) was used to assess treatment effect and factors that predict intrauterine growth in head circumference over time. Multilevel modeling accounts for the dependency in observations when data have a nested, multilevel structure. In this study, the level 1 relation between gestational age and fetal head circumference was modeled individually for each participant, and the average relation across participants was reported. level 2 variables were then sequentially included in the model to account for differences between babies in average fetal brain growth. These subject-level covariates comprised maternal BMI, cotinine concentrations, and fetal sex. The primary exposure (treatment arm) was also included. The level 1 variable, repeated measures of gestational age, was centered at 13 wk because this period signifies the beginning of the second trimester of gestation. Furthermore, no ultrasound measurements of head circumference were recorded before 13 wk gestation.

The treatment effect, i.e., the difference between the 2 groups, was determined from the multilevel models. In the first model, the unconditional growth in head circumference was modeled as a function of gestational age. The intercept and slopes were fit as random effects, which varied across fetuses. An unstructured covariance matrix had the best fit and was utilized in modeling. Variables that were either associated with folic acid folate treatment or head circumference at birth in our bivariate analysis or the literature were included in our model. Based on the log-likelihood ratio, only 3 of the likely confounding variables improved the model fit, namely cotinine group, sex, and BMI. In addition to the unconditional model (model 1), results from the following models were also reported: (model 2) model 1 + treatment; (model 3) model 2 + cotinine group trajectory; (model 4) model 3 + fetal gender; and (model 5) model 4 + maternal BMI. Model 5 had the best fit. Thus, the overall treatment effect and other fixed- and random-effects parameter estimates were reported from this model.

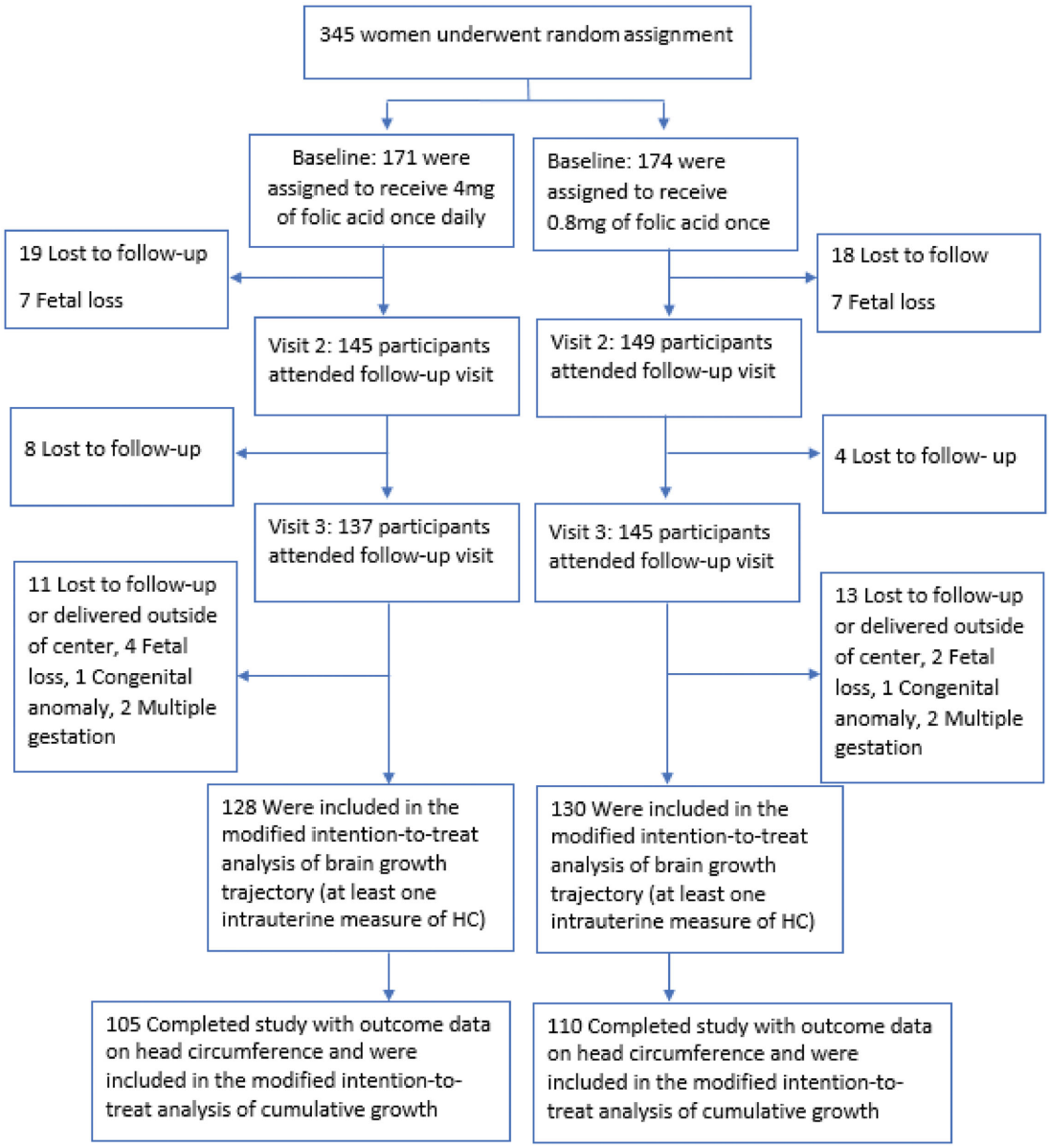

FIGURE 1 Enrollment, randomization, and follow-up of participants.

Cumulative growth in the fetal brain

We examined the effect of the intervention on fetal brain weight and fetal BBRs at birth. Multiple linear regression analyses were conducted separately for the outcomes. To account for potential confounders, we controlled for race because of the imbalance across treatment groups after randomization. Cotinine group trajectory was also included in the model to account for possible changes in cotinine concentrations during the course of pregnancy. Mean differences in the outcomes and their corresponding standard errors were reported.

Results

Study participants

Figure 1 describes participant enrollment and follow-up. A total of 345 smoking pregnant women were enrolled in the trial; 171 women were randomly assigned to the high-dose folic acid group and 174 to the standard-dose folic acid group. Of these women, 258 (74.8%) had ≥2 ultrasound measurements of head circumference, and 215 (62.3%) had outcome data on head circumference at delivery. These numbers represent the count of participants included in the modified intention-to-treat analysis of brain growth trajectory and cumulative brain growth, respectively.

Sociodemographic, lifestyle, and health-related characteristics of study participants by treatment arm are shown in Table 1. The baseline characteristics of the participants in the 2 groups were similar, except for race. Women of "other" races, including Hispanic women, were more likely to be assigned to the standard of care. The mean ± SD age of participants was 26.7 ± 5.6 y. The mean gestational age at enrollment and the mean baseline folate concentration were 12.3 ± 3.9 wk and 718.5 ± 187.0 ng/mL, respectively. The trial population predominantly

TABLE 1 Baseline sociodemographic, lifestyle, and health-related characteristics of all randomly assigned study participants by folic acid treatment ($n = 345$)[1]

Variable	All subjects	High-dose folic acid group ($n = 171$)	Standard-dose folic acid group ($n = 174$)	P value[2]
Age, y	26.7 ± 5.6	26.4 ± 5.1	27.3 ± 5.8	0.10
Gestational age, wk	12.3 ± 3.9	12.4 ± 3.9	12.2 ± 3.9	0.57
Baseline cotinine[2]	2.9 ± 1.4	3.0 ± 1.5	2.9 ± 1.4	0.35
Baseline RBC folate, ng/mL	718.5 ± 187.0	731.3 ± 180.9	706.0 ± 192.3	0.21
Race				0.03*
White	148 (42.9)	78 (45.6)	70 (40.2)	
Black	140 (40.6)	74 (43.3)	66 (37.9)	
Other	47 (16.5)	19 (11.4)	38 (21.8)	
Marital status				0.25
Married	34 (9.8)	20 (11.7)	14 (8.05)	
Single/divorced	311 (90.1)	151 (88.3)	160 (91.9)	
Spanish speaking				
Yes	21 (6.1)	8 (4.7)	13 (7.5)	0.28
No	324 (93.9)	163 (95.3)	161 (92.5)	
Insurance				0.43
Public/none	328 (95.1)	161 (94.1)	167 (96.0)	
Private	17 (4.9)	10 (5.9)	7 (4.0)	
BMI				
Not overweight (<25.0 kg/m^2)	111 (32.4)	53 (31.0)	58 (33.7)	0.12
Overweight (25.0–29.9 kg/m^2)	85 (24.8)	36 (21.0)	49 (28.5)	
Obese (≥30.0 kg/m^2)	147 (42.9)	82 (47.9)	65 (37.7)	
Alcohol use				
Yes	30 (9.5)	18 (11.2)	12 (7.6)	0.27
No	287 (90.5)	142 (88.7)	145 (92.4)	
Illicit drug use				
Yes	94 (31.0)	50 (32.9)	44 (29.1)	0.48
No	209 (69.0)	102 (67.1)	107 (70.9)	
Stress score				
High	191 (55.4)	95 (55.6)	96 (55.2)	0.95
Low	154 (44.6)	76 (44.4)	78 (44.8)	
Depression score				
High	63 (18.3)	31 (18.1)	32 (18.4)	0.95
Low	282 (81.7)	140 (81.9)	142 (81.6)	

[1]Values are n or means ± SDs. Some cell numbers do not sum up to 345 due to missing data. *Significant P values. RBC, red blood cell.
[2]NicAlert cotinine test strips display seven levels (0–6), each of which represents a range of salivary cotinine concentrations. Higher levels on the strip indicate a higher range of cotinine.

comprised single/divorced women (90.1%) and without insurance or on public insurance plans (95.1%). Approximately two-thirds of the population was either overweight or obese (67.7%). There were no statistical differences in the rate of excluded outcomes due to fetal loss by treatment group. Compliance rate was also not significantly different in the higher-dose folic acid group (87.7%) compared with the standard of care group (92.7%) ($P = 0.1741$).

Figure 2 illustrates the cotinine trajectories for trial participants. We discriminated 3 distinct groups of maternal cotinine velocities: one with a consistently high concentration of cotinine, another with a low cotinine concentration, and a third group with moderate cotinine concentration but a marked decline towards the end of gestation. Almost half of the trial participants (48.3%) belonged to the group with a consistently low cotinine concentration. At the end of the study, only 3.0% of the women with cotinine data at delivery ($n = 8$) had stopped smoking (zero cotinine concentration).

Fetal brain growth trajectory

Table 2 shows the results of multilevel linear growth models for the longitudinally measured fetal head circumference based on the modified intention-to-treat population. In the adjusted model, the mean head circumference at 13 wk (due to centering at 13 wk) for all fetuses was 109.2 mm ($P < 0.001$). This time corresponds to the beginning of the second trimester of pregnancy. The average rate of growth in head circumference per week starting at the onset of the second trimester of gestation was 9.66 mm, and this finding was statistically significant ($P < 0.001$). Although infants of participants who received the higher-strength dose of folic acid had a 1.18 mm larger head circumference than the infants of those who received the standard folic acid dose, this difference was not statistically significant ($P = 0.28$). The interindividual variance in the outcome was 67.7 mm ($P < 0.001$), indicating that the initial head circumference at a gestational age of 13 wk across fetuses was significantly different. This finding was mainly explained by differences in maternal BMI and fetal sex. The intraindividual variance was 70.1 mm and was statistically significant ($P < 0.001$), indicating significant variability in brain growth over time across children. Maternal cotinine group concentration accounted mainly for this difference. The cotinine group trajectory, however, had no significant effect on the rate of brain growth. Fetuses with a smaller head circumference at the beginning of the second trimester had a

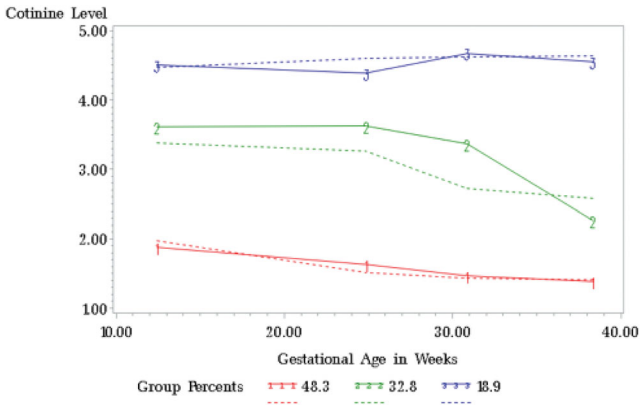

FIGURE 2 Longitudinally derived cotinine trajectories in the modified intention-to-treat population ($n = 319$). Each straight line reflects an individual cotinine trajectory: 1, low level; 2, moderate level; and 3, high level.

faster rate of growth than those with a larger head circumference ($\tau_{10} = -6.00$). Compared with male fetuses, female fetuses had a 3.2 mm slower growth rate in head circumference ($P = 0.003$).

Cumulative growth of the fetal brain

The mean ± SD head circumference of babies at birth was 33.8 ± 2.0 cm. **Table 3** shows multivariable linear regression results of the effect of folic acid treatment on fetal brain weight at birth. Higher-dose folic acid had no significant effect on brain weight in our participants (mean ± SE difference: 6.90 ± 5.85 g; $P = 0.24$). Compared with infants of mothers in the low-cotinine groups, infants of mothers in the high- or moderate-cotinine groups had ∼30 g lower brain weight ($P < 0.001$). Infants of black mothers also had smaller brain weight at birth than infants of white mothers ($P = 0.01$).

The fetal BBR for infants of the study participants ranged from 7.4% to 13.9%. The BBR of infants of mothers who received high-dose folic acid treatment was 0.33 percentage points lower than for infants of mothers who received the standard dose of folic acid ($P = 0.04$; **Table 4**). Unlike other measures of cumulative brain growth, high cotinine group and black race were associated with higher BBR. Compared with women who had low cotinine concentrations, mothers with high cotinine concentrations had 0.93% higher BBRs ($P < 0.001$). Infants of black mothers had ∼0.5% higher BBRs than infants of white mothers ($P = 0.007$).

Discussion

Our randomized clinical trial compared the efficacy of a combination of higher-strength folic acid supplementation versus standard-of-care folic acid dose on prenatal brain growth among smokers in pregnancy. Higher-strength folic acid supplementation in combination with smoking cessation had no effect on intrauterine brain growth from the beginning of the second trimester of gestation through delivery. We observed a significant effect of maternal folic acid treatment on BBR, but no effects on brain weight at birth. The absence of a difference in the rate of intrauterine brain growth and brain weight at birth by trial arm might be explained by the initiation period of folic acid supplementation. The mean gestational age at study enrollment and commencement of supplementation was 12.3 wk, approximately the end of the first trimester of pregnancy.

The infants of women on higher-strength folic acid experienced a 0.33 percentage point reduction in BBR compared with their counterparts on the standard treatment ($P = 0.04$). This finding suggests

TABLE 2 Multilevel linear growth models for the effect of folic acid treatment on growth in fetal head circumference ($n = 258$)[1]

	Estimate				
Parameter	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Fixed effects					
Intercept (γ_{00})	108.16*	107.72*	107.67*	108.19*	109.20*
Gestational age[2] (γ_{01})	9.70*	9.70*	9.70*	9.69*	9.66*
Treatment (γ_{10})					
High dose	—	1.02	0.98	1.38	1.18
Standard dose	—	—	—	—	—
Group					
Low	—	—	0.94	0.90	0.78
Moderate	—	—	1.30	1.73	1.84
High	—	—	—	—	—
Gender					
Female	—	—	—	−3.10*	−3.21*
Male	—	—	—	—	—
BMI					
Not overweight (<25.0 kg/m^2)	—	—	—	—	−1.61
Overweight (25.0–29.9 kg/m^2)	—	—	—	—	−0.75
Obese (≥30.0 kg/m^2)	—	—	—	—	
Deviance (–2LL)	8188.6	8185.7	8180.4	7775.2	7736.6

[1]Model 1 was used as baseline model for computing the explained individual variance (σ^2) in head circumference growth accounted for by folic acid treatment. Model 1 was used as the baseline model for computing the explained intercept variance (τ_{00}) in head circumference growth accounted for by folic acid treatment. *Values are significant at $P < 0.05$.
[2]Centered variable (centering done with the use of the value at 13 wk).

TABLE 3 Effect of folic acid treatment on fetal brain weight (g) at birth ($n = 215$)[1]

Variable	$\beta \pm$ SE	*P* value
Intercept	328.55 ± 10.74	<0.001
Treatment		0.24
High dose	6.90 ± 5.85	
Standard dose	Ref.	—
Cotinine group		
Low	Ref.	
Moderate	−25.81 ± 6.54	<0.001*
High	−29.56 ± 8.20	<0.001*
Race		
White	Ref.	
Black	−16.16 ± 6.48	0.01*
Other	−8.79 ± 9.00	0.33

[1]Values are adjusted for race and cotinine group trajectory. *Values are significant at $P < 0.05$. β, estimate; Ref., reference group.

that folic acid does not favor the fetal brain over fetal body growth. A high BBR signifies a larger brain weight for a given head circumference, and this is commonly observed in SGA infants (17) and infants with intrauterine growth restriction (25). Therefore, the decrease in BBR found in this trial correlates with a lower risk of SGA birth among the higher-dose folic acid arm (26). Harel et al. (27) reported that a higher BBR was associated with a more severe intrauterine growth restriction process and a greater risk that the fetal brain would be affected (27). Our finding has potential clinical implications: it demonstrates that high-dose folic acid will be beneficial in terms of optimal brain growth development among infants of smokers.

Significant relations between cotinine trajectory group and the cumulative measures of brain growth were also observed. A dose-response relation was observed for all outcomes. Higher cotinine concentration was associated with worse outcomes, i.e., reduced brain weight and higher BBR. Lindley et al. (3) found similar effects of smoking on head circumference and BBR. In their observational study, nonsmokers were compared with mothers who stopped smoking by 32 wk of gestation, light smokers who continued to smoke, and heavy smokers who continued to smoke during pregnancy. A dose-response gradient was observed with these self-reported smoking levels. It is well documented that maternal smoking affects fetal brain development and results in neurocognitive issues, such as deficits in intelligence quotient in the offspring (28, 29). Because infants born to mothers with lower cotinine concentrations had a reduced risk of suboptimal fetal brain growth than those born to mothers with higher concentrations, having women quit smoking will have significant implications for brain development. It is noteworthy that despite being enrolled in smoking cessation programs, only 3% of our participants quit smoking. Other findings in this trial are the increased risk of adverse cumulative brain growth outcomes among blacks. Our study confirms previous reports of racial disparities in birth outcomes, with black mothers having worse birth outcomes, including infants with lower birth weight and smaller head circumference, than white mothers (30).

Our study has some limitations. We lost a relatively high proportion of our participants to follow-up. For instance, in our multilevel modeling, we had data on only 82.7% ($n = 258$) of the 319 women eligible to be included in the modified intention-to-treat analysis. There were no significant differences in demographic characteristics between the participants lost to follow-up and those who remained in the study. Therefore, we do not expect loss to follow-up to bias our results. We did not directly assess or control for the effect of diet, a possible confounder in this study. However, there is a reduced likelihood of dietary differences by treatment arms because of randomization. Further, the baseline comparison of RBC folate, a proxy for long-term folate diet, supports this claim. As with most clinical trials, the generalizability of our findings may be an issue. Voluntary participants in studies can be different from nonparticipants. Our study sample also included low-income women with a high proportion of minorities, further limiting the generalizability of our findings. On the other hand, this is a study strength because minority populations are understudied in clinical trials. Therefore, our result can enrich the literature on the effect of folic acid in minorities.

Another strength of our study is the use of a double-blind, randomized clinical trial, which allows for causal inference. We also conducted modified intention-to-treat analysis, and therefore our findings likely closely represent the effectiveness of higher-strength folic acid in improving prenatal brain growth under real-world conditions. To our knowledge, this is the first randomized controlled trial to report on the efficacy of high-strength folic acid in combination with enrollment in a smoking cessation program in preventing adverse fetal brain outcomes among smokers in pregnancy.

The vulnerability of the developing fetal brain is dependent on whether an exposure or its active metabolites reach the developing nervous system and the period of exposure (29). In our trial, we cannot say with certainty that folic acid supplementation was commenced at the critical period of brain growth, and that folic acid reached the brain at the dose at which we supplemented. Future experimental studies should investigate the role of early folic acid supplementation among smokers, starting from before conception until delivery. Doing so may help in identification of the critical period of development associated with maximum folate-associated brain growth. It is also crucial to understand how folic acid supplementation relates to blood folate concentrations in the fetal brain. Blood folate concentrations, including RBC and serum folate, are better proxies for the assessment of folate status (31). These biomarkers are recommended for assessing folate bioavailability for optimal growth and development of neural cells.

TABLE 4 Effect of folic acid treatment on fetal BBR at birth ($n = 215$)[1]

Variables	$\beta \pm$ SE	*P* value
Intercept	10.44 ± 0.30	<0.001
Treatment		0.04*
High dose	−0.33 ± 0.16	
Low dose	Ref.	—
Cotinine group		
Low	Ref.	
Moderate	0.21 ± 0.18	0.25
High	0.94 ± 0.23	<0.001*
Race		
White	Ref.	
Black	0.49 ± 0.18	0.007*
Other	0.30 ± 0.25	0.23

[1]Values are adjusted for race and cotinine group trajectory. *Values are significant at $P < 0.05$. β, estimate; BBR, brain-body weight ratio; Ref., reference group.

In conclusion, we demonstrated a reduction in BBR with the use of higher-strength folic acid initiated during early-mid pregnancy. However, no treatment effects were found on intrauterine brain growth rate and brain weight. Our findings show that smokers in pregnancy may benefit from folate supplementation in reducing the risk of having infants with impaired brain-body proportionality.

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