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Maternal folate status and preterm birth in a high-risk US population

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Abstract

Objective: While maternal folate deficiency has been linked to poor pregnancy outcomes such as neural tube defects, anemia, and low birth weight, the relationship between folate and preterm birth (PTB) in the context of US post-folic acid fortification era is inconclusive. We sought to

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Authorship

BO, SA, and XW designed research; BO, RR, YL performed statistical analyses; GW, XH, and YJ performed lab assays; BO wrote the paper; and all the other coauthors have participated in data interpretation and presentation, critical review and revision of the manuscript. XW is the PI of the Boston Birth Cohort and had primary responsibility for final content. All authors approved the final manuscript for submission to PHN. None of the authors reported a conflict of interest related to the study.

Ethical Standards Disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by Institutional Review Boards of Boston University Medical Center and the Johns Hopkins Bloomberg School of Public Health. Written informed consent was obtained from all subjects/patients.

Conflict of Interest

All authors have no conflicts of interest to disclose.

explore the relationship between maternal folate status and PTB and its subtypes- spontaneous and medically indicated PTB.

Design: observational study

Setting: Boston Birth Cohort, a predominantly urban, low income, race-ethnic minority population at a high-risk for PTB.

Subjects: 7675 mother-infant dyads enrolled in the Boston Birth Cohort. A subsample (n=2,313) of these dyads had maternal plasma folate samples collected 24–72 hours after delivery.

Results: Unadjusted and adjusted logistic regressions revealed an inverse relationship between the frequency of multivitamin supplement intake and PTB. Compared to less frequent use, multivitamin supplement intake 3–5 times/week (adjusted odds ratio (aOR)= 0.78, 95% confidence interval (CI): 0.64, 0.96) or >5 times/week (aOR= 0.77, 95% CI: 0.64, 0.93) throughout pregnancy was associated with reduced risk of PTB. Consistently, higher plasma folate levels (highest versus lowest quartile) were associated with lower risk of PTB (aOR= 0.74, 95% CI: 0.56, 0.97). The above associations were similar among spontaneous and medically indicated PTBs.

Conclusions: If confirmed by future studies, our findings raise the possibility that optimizing maternal folate levels across pregnancy may help to reduce the risk of PTB among the most vulnerable US population in the post-folic acid fortification era.

INTRODUCTION

Preterm birth (PTB, birth before 37 completed weeks of gestation) has been recognized as one of the most pressing challenges to maternal and child health in the United States and the world (1). The role of maternal nutrition remains a promising but understudied area of investigation in the identification of important and modifiable risk factors for PTB.

Folates are a group of naturally occurring water-soluble B vitamins involved in biological reactions needed for fetal and placental growth such as DNA synthesis, repair and methylation (2). Maternal folate deficiency is a modifiable nutritional status that has been linked with adverse pregnancy outcomes such as neural tube defects, congenital anomalies, low birthweight, maternal megaloblastic anemia and preeclampsia (3–5). Folic acid is a synthetic form of folate that is used in multivitamin supplements and grain product fortification. Despite the establishment of national folate intake recommendations and mandatory folic acid fortification programs in the US since 1998 (6, 7), folate consumption is still of public health significance as data suggests that 25% of women of reproductive age have insufficient folate levels (8).

To date, much attention has been paid to the role of periconception folate intake to prevent neural tube defects (NTDs) in offspring (a first trimester event) (9–12). However, considerable knowledge gaps remain regarding the role of folate in PTB (a third trimester event). A population-based analysis demonstrated reduced PTB risk after the implementation of mandatory folic acid fortification in US (13). Some US studies have found an association between lower folate status and PTB, (14–18) while other studies found no association (19, 20). These mixed results may be due to variations across studies in terms

of sociodemographic characteristics of the study population, whether the studies were conducted prior to or after the mandatory folic acid fortification program, differences in definitions and measurement of folate status (self-reported intake versus biomarkers), and/or timing of folic acid administration (preconception versus specific trimesters). The optimal timing of folic acid intake in relation to PTB remains unclear i.e., whether there is a critical window of folic acid intake such as the periconception period as has been demonstrated in the folate-NTDs relationship versus during specific trimester of gestation, given preterm birth is a later event. To date, most relevant studies were based on self-report of folic acid supplementation, which is known to be imprecise and associated with large variation in plasma folate levels, a biomarker of folate nutritional status (21). There is a need for contemporary post folic-acid-fortification studies that examine the associations between folic acid intake as well as folate biomarkers and PTB and how the association between folate status and PTB varies by PTB sub types- spontaneous vs. medically indicated (induced) PTB in high-risk US populations.

Our study sought to address the aforementioned gaps in understanding of the association between maternal folate status and PTB in a large, predominantly urban low-income minority birth cohort in the US. Specifically, we examined the relationship between PTB and self-reported preconception (six months prior to pregnancy) and pregnancy multivitamin supplementation (during each trimester), as well as biomarker measures of maternal plasma folate at delivery. We also explored whether the associations differed for spontaneous vs. medically indicated PTB.

SUBJECTS AND METHODS

Study population

We analyzed data from the Boston Birth Cohort (BBC) study which commenced in 1998 and is ongoing to date (22, 23). Our dataset included, 8494 mother-infant dyads enrolled in the study from 1998 to 2014. The BBC is registered at https://clinicaltrials.gov/ct2/show/NCT03228875. The initial and continuation of the study protocol were approved by the institutional Review Boards of Boston University Medical Center and Johns Hopkins Bloomberg School of Public.

Mothers who delivered at the Boston Medical Center (BMC), which serves a predominantly low-income, minority, inner-city patient population, were recruited 24–72 hours after delivery while still hospitalized. Of note, the length of stay in hospitals after childbirth in the US is 2 days for vaginal delivery and 4 days for caesarean section (24, 25). Cases were defined as mother-infant dyads with singleton, live, low birthweight (LBW; <2,500 grams) or preterm infants (<37 weeks of gestation) regardless of birthweight. Controls were defined as mother-infant dyads with singleton, live, term infants with birthweight 2,500g or more. Of note, this paper specifically examines PTB versus. term birth, regardless of birthweight.

Data collection

After informed consent was obtained, the study staff collected the epidemiological data, clinical data and maternal venous blood and placental samples. Epidemiological data were

collected within 24–72 hours postpartum via an in person maternal questionnaire interview. Clinical data were abstracted from medical records using a standardized form. Plasma folate levels were measured in a subsample of the maternal blood samples obtained within 24–72 hours postpartum from mothers who continued to receive care at BMC.

Definition of key variables

PTB was defined as delivery before 37 completed weeks of gestation. Gestational age was determined using an algorithm based on the first day of the last menstrual period and the results of early ultrasound (<20 weeks' gestation), as previously published (23).

Multivitamin supplement intake was determined during the maternal interview based on responses to the following questions: "Did you take prenatal vitamins prescribed by your doctor?" and "Did you take any over-the-counter multivitamins?" during pre-pregnancy (6 months prior to conception), 1st trimester (day 1 to day 90 of pregnancy), 2nd trimester (day 91 to day 180 of pregnancy), 3rd trimester (day 181 of pregnancy to birth)? Response categories included: none, 1 time per week, 2 times per week, 3–5 times per week, and almost daily. Based on responses to these two questions, preconception multivitamin intake was dichotomized (none vs. any). Intake for each trimester as well as across all trimesters was divided into the following categories: none, 1–2 times per week, 3–5 times, almost daily. In the US, prenatal or over-the-counter multivitamins typically contain 800 or 400 micrograms of folic acid respectively and are to be taken daily, however majority of mothers enrolled in the cohort received prenatal care and were advised to take prenatal vitamins which contain 800 micrograms of folic acid (26). A continuous measure of overall multivitamin supplement intake across all trimesters (henceforth referred to as the "multivitamin supplement intake index") was developed by adding multivitamin intake across the three trimesters to create a composite index of multivitamin supplement intake across pregnancy. For each trimester, frequency of intake was coded as none=0, 1–2 times per week=1, 3–5 times per week=3, almost daily=4. Thus, the composite index ranged from 0 to 12.

Plasma folate concentrations were measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd. China) using a Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) (27). Plasma folate levels were assessed as i) a continuous variable in nmol/L; ii) quartiles of plasma folate levels; and iii) categorizations per the World Health Organization (WHO) guidelines (folate deficiency/insufficiency (<13.5nmol/l); normal (13.5–45.3nmol/l) and elevated (> 45.3nmol/l)) (28).

Other covariates included sociodemographic factors such as: maternal age at delivery (<20, 20–34, 35+ years), maternal education (≤elementary, high school or ≥college), race/ethnicity (non-Hispanic Black, non-Hispanic White, Hispanic and Other), marital status (unmarried versus married), parity (nulliparous versus multiparous), receipt of public assistance including: WIC (Women Infants and Children), food stamps, AFDC (Aid to Families with Dependent Children now known as Temporary Aid to Needy Families), housing assistance or fuel assistance (yes versus no) and maternal nativity (US born versus foreign born). Behavioral risk factors included alcohol use (never versus any), smoking status (never used,

ever used, used in pregnancy) and stress (an indicator for mother's report of life or pregnancy as being 'very' stressful). Biomedical factors from abstracted records included preeclampsia disorders (preeclampsia, eclampsia, gestational hypertension, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome), maternal diabetes (presence of either gestational or pre-gestational diabetes), intrauterine infection/inflammation (presence of maternal fever or placenta pathology findings of villitis, deciduitis, chorioamnionitis, chorionitis, subchorionitis, funisitis, free membranitis) and prepregnancy BMI -from self-reported height and weight - was grouped into 4 categories: underweight (<18.5kg/m^2), normal weight (18.5–24.9kg/m^2), overweight (25–29.9kg/m^2), and obesity (>30kg/m^2).

Statistics

All analyses were conducted using STATA version 14 (College Station, TX: StataCorp LP). Preliminary data analysis was performed in the full sample (N= 7,576) of all enrolled women with multivitamin supplement intake data and plasma folate subsample (n=2313) of these women that received follow- up pediatric care at BMC. Chi-squared tests for categorical variables and t-tests for continuous variables were used to compare maternal characteristics by PTB status. Cronbach's alpha was used to ensure the reliability of the scale "multivitamin supplement intake index" (Cronbach's alpha=0.87). Unadjusted and adjusted logit regressions were used to graph the probability of PTB by plasma folate level. Crude and adjusted logistic regressions were used to explore the relationship between PTB and self-reported multivitamin supplement intake and plasma folate level. Supplemental analyses were conducted for the PTB subgroups of spontaneous and medically indicated PTB. All P-values in the analyses were two-sided and the Type I error rate was set at 0.05.

RESULTS

This study was based on a full sample of 7,576 women with complete multivitamin supplement intake information from six months before conception to the third trimester of pregnancy and a sub-sample (n=2,313) with plasma folate samples collected at delivery. Those included in the full sample and those who had plasma folate data had similar baseline characteristics, except for a higher proportion of non-Hispanic Black mothers and PTBs in the subsample.

Table 1 displays the maternal characteristics for the total study population and plasma folate subsample (maternal characteristics by PTB subtype are presented in Supplemental Table 1). In the full sample, 27% of women had a preterm delivery. Compared to women with term births, women with PTB were more likely to be Non-Hispanic Black, older, US-born, unmarried, cigarette smokers, alcohol consumers and report a very stressful life or pregnancy. These women were also likely to have had preeclampsia disorders, intrauterine infection/inflammation, diabetes mellitus and be obese/overweight.

Within the plasma folate subsample, 31% of women experienced PTB. The mean (SD) and median plasma folate concentration at delivery was 32.3 (20.0) nmol/L and 28.5 nmol/L respectively for PTB, significantly lower than 36.3 (24.7) nmol/L and 31.5 nmol/L for term births. Significant associations between maternal characteristics and PTB that were seen in

the total supplement intake sample persisted in the plasma folate subsample. Plasma folate was mildly correlated with multivitamin supplement intake in the third trimester (rho: 0.10, p<0.001). The relationship between plasma folate concentrations and gestational age is presented graphically supplemental Figure 1. Every unit increase in plasma folate concentration was associated with a 0.01 week increase in gestational age (95% CI: 0.004, 0.016).

Table 2 displays the association between self-reported multivitamin supplement intake and PTB (analysis by PTB subtypes is presented in Supplemental Table 2). The overall multivitamin supplement intake across pregnancy was significantly associated with PTB. Specifically, each unit increase in the multivitamin supplement intake index reduced the odds of PTB (aOR= 0.98, 95% CI:0.97, 0.99). As a categorical variable, consistent multivitamin supplement intake (3–5 times/week or >5 times/week) reduced the odds of PTB compared to no intake across pregnancy (aOR= 0.78, 95% CI: 0.64, 0.96; aOR= 0.77, 95% CI: 0.64, 0.93, respectively). The relationship between multivitamin supplement intake across different time points and PTB showed that intake during the preconception period did not reduce PTB odds. Intake during the first trimester (>5 times/week) was associated with a reduction in PTB odds (aOR= 0.85, 95% CI: 0.73, 0.98). During the third trimester, intake of 3–5 times/week and >5 times/week was associated with lower odds of PTB (aOR= 0.75, 95% CI: 0.63, 0.85; aOR= 0.74, 95% CI: 0.64, 0.87, respectively). There was no significant difference in the odds of PTB among women with consistent multivitamin supplement intake of one to two times/week throughout pregnancy compared to no intake throughout pregnancy. Sensitivity analysis presented in Supplemental Table 3 showed a consistent pattern of multivitamin supplement intake at all time points with PTB among non-Hispanic Blacks only.

Table 3 displays the unadjusted and adjusted odds of PTB by plasma folate concentration (analysis by PTB subtypes is presented in Supplemental Table 3). Each unit and interquartile increase in plasma folate concentration reduced the odds of PTB (aOR= 0.99, 95% CI: 0.99, 1.00; aOR= 0.88, 95% CI:0.79, 0.97, respectively). This association persisted when plasma folate concentration was categorized. Compared to the lowest quartile (<19.4 nmol/L), the highest quartile of plasma folate concentration (>43.8 nmol/L) was associated with an over 25% reduction in PTB odds (aOR= 0.72, 95% CI: 0.54, 0.94). Similarly, excess plasma folate concentration (>45.3 nmol/L) was associated with reduced odds of PTB (aOR= 0.74, 95% CI: 0.56, 0.97) compared with normal plasma concentration (13.5–45.3 nmol/L).

Figure 1 displays the association between plasma folate concentration at delivery and probability of PTB, stratified by subtypes. Plasma folate concentration demonstrated a mild curvilinear relationship with overall PTB, a linear relationship with spontaneous PTB, and a curvilinear relationship with medically indicated PTB where higher concentrations of plasma folate were associated with a reduced probability of PTB or its subtypes.

In Table 4, the relationship between plasma folate concentration and spontaneous versus medically indicted PTB is presented. The final regression model for medically indicated PTB did not include biomedical risk factors to avoid the introduction of factors potentially in the causal pathway. Each unit increase in plasma folate concentration was associated with

reduced odds of spontaneous (aOR= 0.99, 95% CI: 0.99, 1.00) as well as medically indicated (aOR= 0.98, 95 % CI: 0. 0.98, 0.99) PTB. Among medically indicated preterm births, plasma folate concentration in the highest quartile was associated with a reduction in the odds of PTB; this relationship did not reach statistical significance for spontaneous PTB. Plasma folate concentrations greater than 45.3 nmol/L were associated with a 30% reduction in the odds of spontaneous (aOR: 0.72; 95% CI: 0.55, 0.95) and medically indicated PTB (aOR: 0.58; 95% CI: 0.40, 0.82).

DISCUSSION

In our full sample, multivitamin supplement intake and plasma folate concentrations were generally adequate or high, as expected in this era of mandatory folic acid fortification of the food supply. Still, about a quarter of women had a relatively low plasma folate concentration (<19.4nmol/L), which was associated with an increased risk of PTB. While there is no national data, an association between folate fortification and reduced PTB rates was observed in California among live births that occurred from January 1990 through December 2000 (13). After adjusting for maternal age, parity, race/ethnicity, education, year of birth, and fortification period, fortification was shown to reduce PTB risk (relative risk ratios (RR) = 0.96; 95% CI 0.94, 0.97).

After controlling for confounding factors, our analysis shows that multivitamin supplement intake of at least 3 times/week throughout pregnancy was significantly associated with a reduction in the odds of PTB, consistent with other US based prospective studies that have assessed dietary folate intake (14, 16). A study in a low-income minority population showed that low (≤240μg/day) and intermediate (241–400μg/day) dietary folate intake were associated with an increased risk of PTB, respectively, as compared with women who had a folate intake >400μg/day (14). In another study, dietary folate intake ≤500μg was associated with an almost two times greater risk of preterm delivery (16).

We note that there was no significant association between preconceptional supplement intake and PTB, contrary to the findings of Bukowski et al., which demonstrated a 50% −70% related reduction in the incidence of early spontaneous PTB. However, in our study, preconception supplement intake was very low (7.1%) and may have resulted in reduced statistical power to detect significant associations. Multivitamin supplementation in the first and third trimester were both significantly associated with reduced PTB odds, with use during the third trimester associated with a greater reduction in PTB odds compared to use during the first trimester. It is unclear why second trimester multivitamin supplement intake is not associated with PTB. While the association observed in the third trimester may be a reflection of intake in other trimesters given the high correlation across trimesters, these findings suggest that the third trimester may be a critical time window in the folate-PTB relationship. While folate is needed for maternal tissue and fetal and placental growth throughout pregnancy, the rapid fetal development occurring during the third trimester is associated with maximum folate catabolism and thus increased requirements during this critical period (29, 30). Studies show that women who stopped multivitamin or folate supplementation after the first trimester had lower concentrations of maternal serum and red blood cell folate concentrations (29–31). The association between multivitamin supplement

intake and PTB was corroborated using plasma folate concentration at delivery. Increasing plasma folate concentrations significantly reduced the odds of PTB. Our findings are consistent with other US-based studies with folate measurements during pregnancy wherein each 1 nmol/L increase in serum folate concentration at 28 weeks of gestation was also associated with reduced risk of PTB (14) and serum folate concentration less than 36.9 nmol/L in the second trimester (24–29 weeks of gestation) led to a nearly twofold increased risk of PTB (16). These findings demonstrating the relationship between maternal folate status and PTB are particularly important given that the national PTB rates have remained high, 10.4% in 2007, and 9.8% in 2016 despite research and intervention efforts (32). Our research on a predominantly minority population is also appropriate given that national PTB rates was highest among non-Hispanic black births (13.8%) (32). The patient population from which we derived the Boston Birth Cohort have a high rate of preterm birth (15–17%) and the higher rates of PTB seen in the study sample are because the study over-sampled preterm birth at the enrollment. Among this population at a particular high risk of preterm birth and inadequate folate intake, and we have shown that in such a setting, the lower levels of plasma folate with preterm delivery may be a reflection of the duration of folic acid supplementation and that maternal adequate folate status can reduce the risk of preterm birth (33).

Maternal factors well demonstrated in the literature to be associated with, but not necessarily in the causal pathway of PTB, include demographic, obstetric, medical, and psychosocial risk factors (34). However, the underlying mechanisms for the link between folate and PTB are not well-understood, but appear to be biologically plausible. For example, variations in key genes involved in folate metabolism such as dihydro folate reductase (DHFR) and serine hydroxy-methyl transferase (SHMT1) appear to increase the risk for spontaneous PTB (35). Other mechanistic pathways that may explain the folate – PTB relationship include hyperhomocysteinemia, placental implantation and intrauterine infection and inflammation (36, 37). Low folate status is associated with hyperhomocysteinemia, which has been linked with increased arterial stiffness, insulin resistance and endothelial dysfunction. Folate may also affect placental implantation and vascular remodeling through its role as a superoxide scavenger in antioxidant defenses (36). Folate deficiency is also associated with abnormal inflammatory responses, which could conceivably trigger premature parturition in the context of intrauterine infection (38, 39).

Strengths of our study include the use of complementary measures of folate status- from maternal self-report (capturing pattern of long-term use) and more objective biomarkers, providing multi-measure consistent evidence to support the folate-PTB relationship. The study is the largest investigation with recent birth cohort data on plasma folate and PTB published to date and is the first one that performed PTB subtype analyses. While maternal folate status has been linked with spontaneous PTB (18), its relationship with medically indicated PTB has only been demonstrated in animal studies (40). Again, such research is particularly relevant among high risk populations such as Non-Hispanic Blacks which have lower folate levels compared to other race/ethnic groups (8) and higher proportion of medically indicated PTB (41).

Our study contributes new knowledge to the field by exploring specific patterns of multivitamin supplementation during the preconception period and across trimesters. This facilitates identifying the critical period over the course of pregnancy to reinforce adequate folate intake in order to reduce PTB. Finally, our study focuses on the high-risk non-Hispanic black US populations in need for interventions to address both PTB and lower folate status.

We, however, acknowledge some limitations of this study. While plasma or serum folate is a common inexpensive measurement used in clinical and research setting, it only reflects short term folate status within the past few days in contrast to red cell folate concentrations which measure long-term folate status. Thus, plasma folate concentration at delivery can only be used as a proxy for third trimester folate concentration (42). As shown in a previous publication(21), maternal self-reported multivitamin intake during 3rd trimester was positively associated with maternal plasma folate levels. While folate measurements during early pregnancy would be more ideal, plasma folate concentration at delivery can be used as a proxy for third trimester folate concentration. In addition, multivitamin supplement intake was based on self-report, which is subject to recall bias as well as the fact that there was no information on the actual dosage of folic acid consumed. Also, the determination of folate status based on the frequency of supplement intake may be incomplete since folate status may also be influenced by dietary intake of folate rich/fortified foods and other factors affecting folate metabolism. Due to the high correlations between multivitamin supplement intake across all trimesters (rho: 0.58– 0.85, $p<0.001$), further adjustments for intake during other trimesters were not conducted when we explored the associations in each trimester. This was an observational study enriched by PTB, and by its nature cannot enable causal inference (43) as unobserved or uncontrolled confounding remains a threat to validity. Additional detail on some of the confounding variables, such as the level of cigarette and alcohol consumption in each trimester would have been helpful. While no randomized controlled trial has been conducted in the US and is unlikely given the advantageous role of folate on pregnancy outcomes, study findings need to be confirmed in other nationally representative prospective longitudinal studies. This is a US urban low income population in a post-folate fortification context and caution is needed in generalizing study findings to other populations with different characteristics. We also acknowledge that ours is a high-risk (low income, minority, urban) US population in a post-folate fortification context and that our findings may be reproducible in other populations in other countries.

There are important implications to be gleaned from this study. The association between folate and PTB among this predominantly minority, urban low-income population is important as studies have shown that women who were non-white (Non-Hispanic Black and Hispanic women), aged 18–24 years, and had less than a high school education or had a household income of <$25,000 are the least likely to report daily consumption of a supplement containing folic acid (44). In addition, minority populations are less likely to have heard about folic acid, to know it can prevent birth defects, and to consume foods fortified with folic acid or take vitamins containing folic acid (45, 46).

Lastly, our study supports the importance of consistent folate intake throughout pregnancy to mitigate adverse pregnancy outcomes, including PTB (9, 29, 47). This study demonstrated

minimal difference in PTB mitigation related to multivitamin supplement intake of 3–5 times/week versus >5 times/week, suggesting a possible threshold dosing schedule of 3 times/week. If corroborated by other studies, this finding may impact the recommendations for frequency of multivitamin supplement intake before and during pregnancy. Specifically, this finding suggests that the same protective benefit can be derived from a 3 times weekly dose compared to a daily dose. Finally, folate has a broad biological function, and there is increasing recognition that folate supplementation during pregnancy may affect both short-term and long-term health of the offspring. For example, in the same cohort, we demonstrated beneficial effects of adequate maternal plasma folate levels on offspring obesity (48, 49). Furthermore, our recent study (21) along with that of others (50) raised concern about the potential risk of extremely high levels of folate on autism. Therefore, more work remains to be done to determine optimal range of maternal folate levels throughout pregnancy for major organs and systems in the offspring. Ultimately, we need to define an optimal range of folate levels (neither too low nor too high) preconception and during pregnancy, which can maximize its health benefits and minimize its risk. This may require careful consideration of a woman's health conditions, dietary intake and folic acid supplementation, and measurement of plasma folate levels as needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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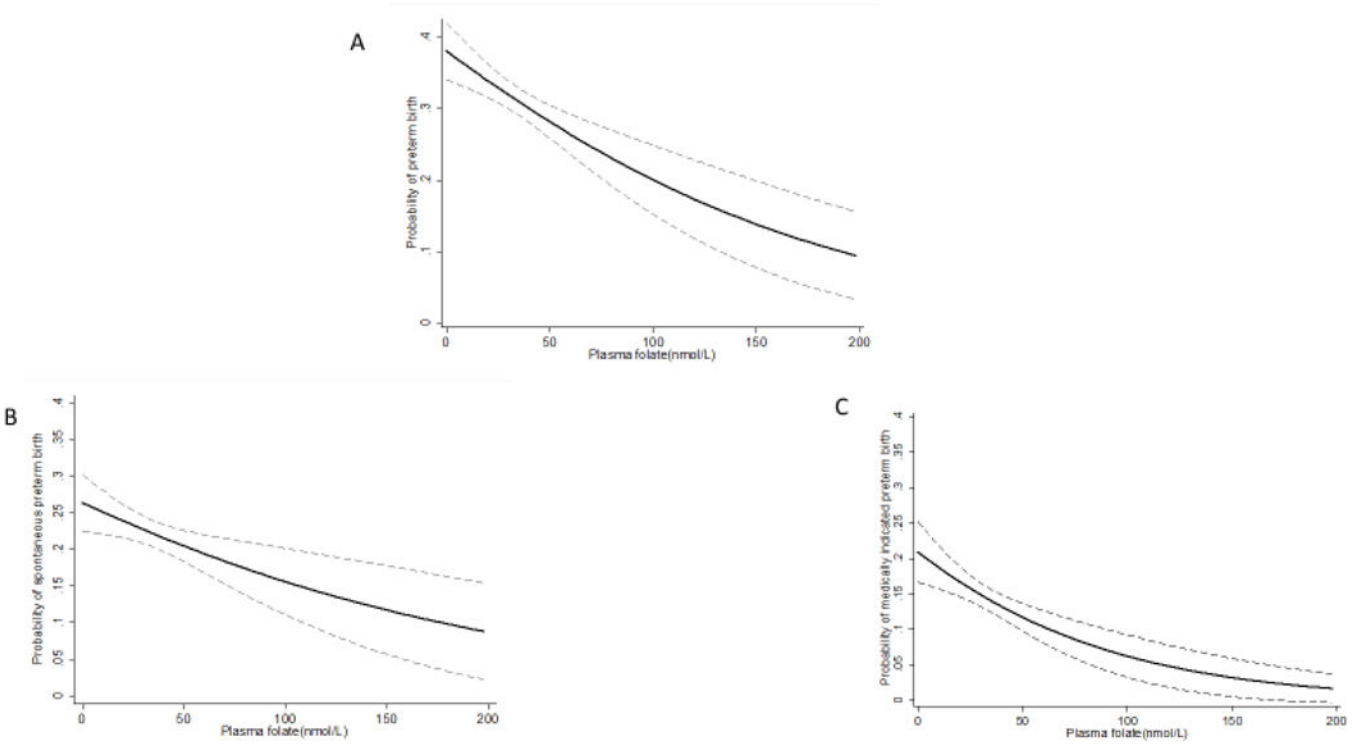

FIGURE 1:

Probability of Overall (A), Spontaneous (B) and Medically Indicated (C) Preterm Birth by Plasma Folate Concentration

Table 1:

Maternal Characteristics of Study Population (N=7, 576)

Maternal Characteristics	Multivitamin Supplement sample				Plasma folate subsample			
	Term		PTB		Term		PTB	
	N	%	N	%	N	%	N	%
	5,507	73	2,069	27	1,593	69	720	31
Race/ethnicity								
Non-Hispanic Black[a]	2,764	50.2	1,083	52.3	1,133	71.1	555	77.1
Non-Hispanic White	645	11.7	275	13.3	65	4.1	27	3.8
Hispanic	1,614	29.3	536	25.9	323	20.3	115	16.0
Other	451	8.2	163	7.9	72	4.5	23	3.2
Missing	33	0.6	12	0.6	0	0.0	0	0.0
Age in years								
<20	608	11.0	195	9.4	159	10.0	67	9.3
20–34	4,053	73.6	1,446	69.9	1,171	73.5	494	68.6
35+	813	14.8	416	20.1	263	16.5	159	22.1
Missing	33	0.6	12	0.6	0	0.0	0	0.0
Nativity (US born)								
Foreign born	3,403	61.8	1,118	54.0	970	60.9	377	52.4
US born	2,029	36.8	935	45.2	596	37.4	338	46.9
Missing	75	1.4	16	0.8	27	1.7	5	0.7
Education								
Less than high school	1,680	30.5	623	30.1	434	27.2	204	28.3
High School/GED	1,800	32.7	741	35.8	575	36.1	279	38.8
Some/beyond College	1,970	35.8	684	33.1	578	36.3	234	32.5
Missing	57	1.0	21	1.0	6	0.4	3	0.4
Marital Status								
Married	2,038	37.0	698	33.7	564	35.4	225	31.3
Unmarried	3,339	60.6	1,339	64.7	1,019	64.0	488	67.8
Missing	130	2.4	32	1.5	10	0.6	7	1.0
Receipt of public assistance [b]								
No	856	15.5	354	17.1	208	13.1	112	15.6
Yes	4,651	84.5	1,715	82.9	1,385	86.9	608	84.4
Parity								
Multiparous	3,119	56.6	1,180	57.0	933	58.6	425	59.0
Nulliparous	2,374	43.1	885	42.8	657	41.2	294	40.8
Missing	14	0.3	4	0.2	3	0.2	1	0.1
Cigarette smoking								
Never	4,449	80.8	1,534	74.1	1,321	82.9	545	75.7
Ever	376	6.8	175	8.5	114	7.2	73	10.1
Continued in pregnancy	611	11.1	336	16.2	145	9.1	99	13.8

Maternal Characteristics	Multivitamin Supplement sample				Plasma folate subsample			
	Term		PTB		Term		PTB	
	N	%	N	%	N	%	N	%
	5,507	73	2,069	27	1,593	69	720	31
Missing	17	1.3	24	1.2	13	0.8	3	0.4
Alcohol consumption								
No	4,858	88.2	1,782	86.1	1,431	89.8	644	89.4
Yes	474	8.6	218	10.5	124	7.8	67	9.3
Missing	175	3.2	69	3.3	38	2.4	9	1.3
Stress[c]								
No	4,404	80	1,544	74.6	1,270	79.7	535	74.3
Yes	1,078	19.6	516	24.9	317	19.9	183	25.4
Missing	25	0.5	9	0.4	6	0.4	2	0.3
Body Mass Index categories								
Underweight	227	4.1	97	4.7	62	3.9	27	3.8
Normal	2,494	45.3	859	41.5	701	44.0	269	37.4
Overweight/obese	2,367	43	973	47	745	46.8	386	53.6
Missing	419	7.6	140	6.8	85	5.3	38	5.3
Preeclampsia disorders [d]								
No	5,047	91.6	1,582	76.5	1,483	93.1	534	74.2
Yes	460	8.4	487	23.5	110	6.9	186	25.8
Intrauterine infection/inflammation								
No	4,371	79.4	1,557	75.3	1,361	85.4	560	77.8
Yes	690	12.5	432	20.9	169	10.6	151	21.0
Missing	446	8.1	80	3.9	63	4.0	9	1.3
Diabetes Mellitus								
None	5,162	93.7	1,850	89.4	1,498	94.0	632	87.8
Gestational	220	4.0	124	6	53	3.3	49	6.8
Pre-gestational	75	1.4	79	3.8	31	1.9	34	4.7
Missing	50	0.9	16	0.8	11	0.7	5	0.7
Multivitamin supplement intake								
Preconception	9							
None	5,137	93.3	1,940	93.8	1,504	94.4	671	93.2
Any	370	6.7	129	6.2	89	5.6	49	6.8

Abbreviation: GED-General Equivalency Diploma; N-number; PTB-preterm birth; SD-standard deviation; US-United States; WHO-World Health Organization.

[a]Non-Hispanic Black includes Black, African American, Haitian, Cape Verdian.

[b]Public assistance is defined as receipt of any of the following: WIC (Women Infants and Children), food stamps, AFDC (Aid to families with dependent children), housing assistance or fuel assistance.

[c]Mother's self-report of life or pregnancy being very stressful.

[d] Preeclampsia disorders are defined as the presence of preeclampsia, gestational hypertension and hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome

Table 2:

Relationship Between Multivitamin Supplement Intake and Preterm Birth (N=7,576)

Period	Term (N=5507)		Preterm (N=2,069)		Unadjusted		Adjusted OR[a]	
	N of cases	(%)	N of cases	(%)	OR	95% CI	OR	95% CI
Multivitamin supplement intake in pregnancy (1st to 3rd trimester)								
Continuous (index) [b]	n/a	n/a	n/a	n/a	0.97	0.96, 0.98	0.98	0.97, 0.99
Categorical								
None (ref)	436	7.9	219	10.6	1.00	n/a	1.00)	n/a
1–2x a week	933	16.9	406	19.6	0.87	0.71, 1.06	0.91	0.74, 1.12
3–5x a week	1373	24.9	476	23.0	0.69	0.57, 0.84	0.78	0.64, 0.96
>5x a week	2765	50.2	968	46.8	0.70	0.58, 0.83	0.77	0.64, 0.93
Multivitamin supplement intake in specific time periods								
Preconception								
None (ref)	5137	93.3	1,940	93.8	1.00	n/a	1.00	n/a
Any	370	6.7	129	6.2	0.92	0.75, 1.14	0.90	0.72, 1.12
First trimester								
None (ref)	914	16.6	402	19.4	1.00	n/a	1.00	n/a
1–2x a week	204	3.7	97	4.7	1.08	0.83, 1.41	1.08	0.82, 1.43
3–5x a week	1582	26.9	539	26.1	0.83	0.71, 0.96	0.90	0.76, 1.06
>5x a week	2907	52.8	1,031	49.8	0.81	0.70, 0.92	0.85	0.73, 0.98
Second trimester								
None (ref)	642	11.7	290	14.0	1.00	n/a	1.00	n/a
1–2x a week	220	4.0	108	5.2	1.09	0.83, 1.42	1.19	0.90, 1.57
3–5x a week	1558	28.3	570	27.6	0.81	0.68, 0.96	0.92	0.77, 1.10
>5x a week	3087	56.1	1,101	53.2	0.79	0.68, 0.92	0.86	0.73, 1.02
Third trimester								
None (ref)	655	11.9	345	16.8	1.00	n/a	1.00	n/a
1–2x a week	255	4.6	99	4.8	0.74	0.56, 0.96	0.80	0.61, 1.06
3–5x a week	1558	28.3	543	26.2	0.66	0.56, 0.78	0.75	0.63, 0.90
>5x a week	3039	55.2	1,082	52.3	0.68	0.58, 0.78	0.75	0.64, 0.87

Abbreviations: CI-confidence interval; N-number, OR-odds ratio, ref: reference

[a]Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, cigarette use, alcohol use, stress, body mass index (BMI), preeclampsia disorders, intrauterine infection/inflammation and diabetes mellitus.

[b]Composite measure of supplement intake from 1st to 3rd trimester

Table 3:

Relationship between maternal plasma folate levels and preterm birth (n=2313)

Plasma folate sample	Term (n=1593)		Preterm (n=720)		Unadjusted		Adjusted OR[a]	
	N of cases	%	N of cases	%	OR	95% CI	OR	95% CI
Continuous plasma folate concentration(nmol/L)								
Each unit increase[1]	n/a	n/a	n/a	n/a	0.991	0.986, 0.995	0.994	0.990, 0.999
Each interquartile increase in folate level[2]	n/a	n/a	n/a	n/a	0.81	0.73, 0.90	0.88	0.79, 0.97
Quartiles of plasma folate concentration(nmol/L)								
Lowest quartile: 6.6 to 19.4 (ref)	378	23.7	201	27.9	1.00	n/a	1.00	n/a
Second quartile: 19.4– 30.0	386	24.2	192	26.7	0.94	0.73, 1.19	1.02	0.79, 1.33
Third quartile: 30.0–43.8	393	24.7	185	25.7	0.89	0.69, 1.13	1.09	0.83, 1.42
Highest quartile: 43.8–185.5	436	27.4	142	19.7	0.61	0.47, 0.79	0.74	0.56, 0.97
WHO classification								
Insufficiency/deficiency: <13.5	155	9.7	82	11.4	1.07	0.80, 1.43	0.86	0.63, 1.18
Normal: 13.5–45.3 (ref)	1030	64.7	508	70.6	1.00	n/a	1.00	1.00 (ref)
Excess: ≥45.3	408	25.6	130	18.1	0.65	0.52, 0.81	0.70	0.55, 0.89

Abbreviations: CI-confidence interval; N-number, OR-odds ratio, ref: reference, WHO: World Health Organization.

[a]Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, preeclampsia disorders, intrauterine infection/inflammation, and diabetes.

[1]Mean (SD) for term and preterm births: 36.6 (24.7) and 32.3 (20.0) nmol/L respectively.

[2]Median (interquartile range) for term and preterm births: 31.5 (20.8–45.6) and 28.5 (19.0–38.5) nmol/L respectively.

Table 4:

Plasma folate levels and unadjusted and adjusted odds of PTB subtypes

	Spontaneous PTB				Medically indicated PTB			
Plasma folate levels	**N of cases**	**(%)**	**aOR** [a]	**95% CI**	**N of cases**	**(%)**	**aOR** [b]	**95% CI**
Continuous plasma folate concentration(nmol/L)								
Each unit increase	n/a	n/a	0.995	0.990, 1.000	n/a	n/a	0.99	0.s98, 0.99
Each interquartile increase in folate level	n/a	n/a	0.90	0.80,1.01	n/a	n/a	0.73	0.62, 0.87
Quartiles of plasma folate concentration(nmol/L)								
Lowest quartile: 6.6–19.4 (reference)	114	23.2	1.00 (ref)	n/a	87	18.7	1.00 (ref)	n/a
Second quartile: 19.4–30.0	119	23.6	1.12	0.82, 1.53	73	15.9	0.85	0.60, 1.21
Third quartile: 30.0–43.8	124	24.0	1.27	0.93, 1.73	61	13.4	0.68	0.47, 0.99
Highest quartile: 43.8–185.5	96	18.1	0.86	0.62, 1.18	46	9.5	0.46	0.31, 0.69
WHO classification (nmol/L)								
Insufficiency/deficiency: <13.5	45	22.5	0.77	0.53, 1.12	37	19.3	1.25	0.83, 1.86
Normal: 13.5–45.3 (reference)	321	23.8	1.00 (ref)	n/a	187	15.4	1.00 (ref)	n/a
Excess: ≥45.3	87	17.6	0.72	0.55, 0.95	43	9.5	0.58	0.40, 0.82

Abbreviation: aOR-adjusted odds ratio; n/a-not applicable; N-number; ref-reference; PTB: preterm birth; WHO-World Health Organization

[a]Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, preeclampsia disorders, intrauterine infection/inflammation, diabetes.

[b]Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress