MATERNAL FOLATE STATUS AND PRETERM BIRTH IN THE BOSTON BIRTH COHORT

by

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Abstract

Preterm birth (PTB) is one of the most pressing challenges to maternal and child health in the United States (US). There remains an urgent need to identify important and modifiable risk factors for PTB among those most at risk- such as urban low income, Non-Hispanic blacks. This dissertation aimed to: i) evaluate the relationship between maternal folate status and risk of PTB and ii) investigate the biologic plausibility of the folate-PTB association by evaluating the role of folate on major pathogenic pathways leading to PTB –namely preeclampsia and intrauterine infection/inflammation (IUI). The analyses included 7565 mother-newborn dyads and a subsample (n=2313) with plasma folate assay at delivery in Boston Birth Cohort.

There was 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. Multivitamin supplement intake of three or more times a week in the 3rd trimester was associated with reduced odds of preeclampsia (aOR=0.77, 95% CI: 0.65, 0.93). Each interquartile increase in plasma folate reduced the odds of preeclampsia by 20% (aOR=0.80, 95% CI: 0.68,0.95). Preeclampsia mediated 62% of the relationship between multivitamin supplement intake and medically indicated PTB. Multivitamin supplement intake of three or more times a week in the 3rd trimester (aOR=0.74, 95% CI: 0.63, 0.87) was associated with reduced odds of IUI. Each interquartile increase in plasma folate reduced the odds of IUI by 15% (aOR=0.85, 95% CI: 0.73, 0.98). Joint analysis of IUI and multivitamin supplement intake showed that multivitamin intake attenuated the relationship between IUI and PTB.

Findings from this study are relevant to policies and interventions, specifically among vulnerable populations. The results of this dissertation have a potential impact on ongoing research, clinical and programmatic efforts to improve prenatal nutrition and birth outcomes. Findings may inform the design and implementation of nutrition-based screening and interventions to prevent PTB and associated short-term and long-term consequences.

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Table of Contents

List of Tables

List of Figures

Chapter One: Introduction

Background

Preterm birth (PTB) has been recognized as one of the most pressing challenges to maternal and child health in the US (1). It is associated with high neonatal, infant and childhood morbidity and mortality as well as cardiovascular and metabolic diseases in adulthood (2). National PTB rates have declined only slightly from 2007 to 2016 (10.4% and 9.8% respectively) (3), with the PTB rate in Non-Hispanic blacks (13.8%) highest compared to Hispanics (9.4%) and Non-Hispanic Whites (9.04%) (3, 4). One major hurdle in reducing the intractable national PTB rate is the multi-factorial etiology involved in both spontaneous and medically indicated PTBs (5-7). Major biomedical risk factors for PTB include preeclampsia disorders and intrauterine infection inflammation (IUI) which are major causes of medically indicated and spontaneous PTB respectively (8, 9). There remains an urgent need to identify important and modifiable risk factors for PTB among those most at risk- such as urban low income, Non-Hispanic blacks (10, 11).

Low maternal folate status has been linked with adverse pregnancy outcomes such as neural tube defects, congenital abnormalities, low birth weight, anemia and preeclampsia (12-14). National recommendations regarding folate intake and supplementation were instituted in 1992 while mandatory folic acid fortification programs were implemented in 1998 to improve pregnancy outcomes (15, 16). The 2012 National Health and Nutrition Examination Survey (NHANES), however, suggests that 25% of women of reproductive age have insufficient folate levels (17). Non-Hispanic Black and Hispanic women are more likely to have lower folate levels than non-Hispanic white women (18). To date, attention has been paid to the role of folate intake during preconception and early pregnancy on neural tube defects (occurring during first

trimester). Considerable knowledge gaps remain on the role of folate in PTB (a third trimester event).

Maternal folate status has been linked to PTB in many studies (19-25), however some studies have found no relation (26-30). These mixed results are likely due to variations across studies in terms of sociodemographic characteristics of the study population (31-33), presence of folic acid fortification programs (31, 32), population folate status (34), folate measurement (self-reported intake versus biomarkers) (30-32) or timing of folic acid supplementation (preconception versus specific trimesters of pregnancy) (34).

It is biologically plausible that folate may affect PTB (35). Folate is essential for DNA synthesis and repair, gene expression, fetal organ/system formation, normal metabolism and immune function (36) and possesses anti-oxidative and anti-inflammatory properties (37, 38). Folate is needed for normal endothelial function (39, 40) and its deficiency is associated with hypertension and cardiovascular diseases in adults (41). Thus, folate deficiency may be linked to preeclampsia- defined as pregnancy induced hypertension with proteinuria- and a major cause of medically indicated PTB. A few studies suggest that the relationship between preeclampsia and PTB is moderated by folate status (42, 43). In addition, folate deficient individuals are susceptible to infections and have increased circulating biomarkers of inflammation (44). IUI has been associated with folate deficiency and is hypothesized to lead to 25-40% of PTB cases (9, 35). These plausible pathways through which folate may influence PTB are described later in detail.

This dissertation examined the association between maternal folate status and PTB (as well as two major pathogenic pathways (preeclampsia and intrauterine infection/inflammation (IUI))

using data from the Boston Birth Cohort (BBC)- a US urban low-income minority (about 50% African American) population. The BBC is a well-established cohort study, specifically designed to study PTB, with sufficient sample size of PTB subtypes (spontaneous or medically indicated) allowing for the exploration of the association between folate and PTB subtypes. In addition, the presence of data on pregnancy complications (such as preeclampsia and IUI) in the BBC allows for a research of plausible biological pathways underlying the folate-PTB relationship- specifically: the inflammation and vascular pathological pathways leading to PTB. Maternal folate status was assessed using both self-reported multivitamin supplement intake from preconception to each trimester of pregnancy via standard questionnaire interview, as well as folate biomarkers in archived maternal plasma samples obtained at delivery (a proxy of 3rd trimester folate status). These data allowed for the investigation of the temporal association of folate status in relation to PTB, and further delineating dose-response relationships using objective biomarkers.

Study Aims

The dissertation is organized along the following aims and hypotheses:

Aim 1. Evaluate the relationship between maternal folate status and risk of PTB (overall and subtypes of PTB).

Maternal folate status was assessed using complimentary measures of self-reported multivitamin supplement intake from preconception to third trimester and plasma folate concentrations at delivery.

Null hypotheses include:

1a Self-reported frequency of multivitamin supplement intake during the preconception (6-month prior to preconception) period is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)

1b Self-reported frequency of multivitamin supplement intake during pregnancy (first, second and third trimester) is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)

1c Maternal plasma folate levels at delivery (a proxy of 3rd trimester folate status) is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)

Aim 2. Investigate the biologic plausibility of the folate-PTB association by evaluating the role of folate on major pathogenic pathways leading to PTB.

Null hypotheses include:

2a Maternal folate status (assessed by self-report and biomarker) does not moderate the relationship between preeclampsia in pregnancy and PTB (including overall and medically indicated PTB).

2b Preeclampsia does not mediate the relationship between maternal folate status and PTB (including overall and medically indicated PTB).

2c Maternal folate status (assessed by self-report and biomarker) does not moderate the relationship between IUI and PTB (including overall and spontaneous PTB).

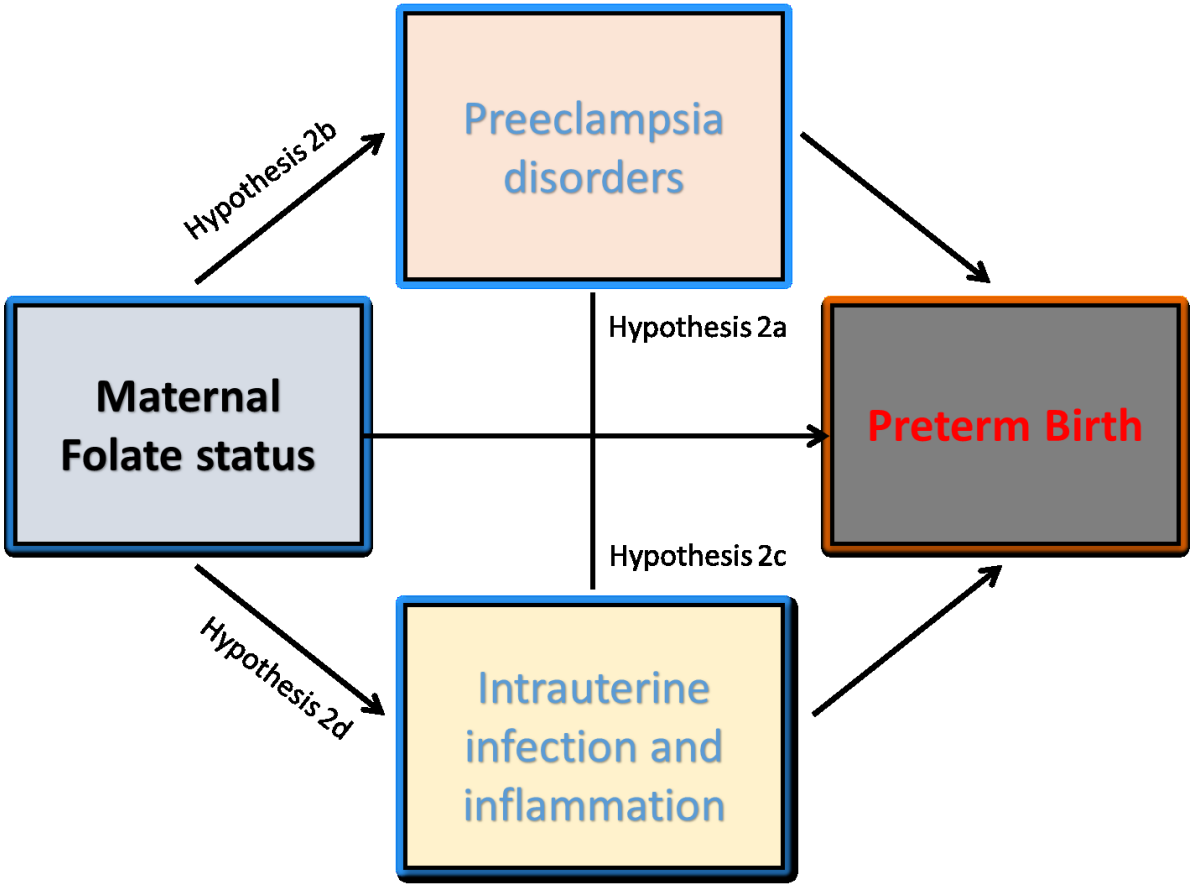
2d IUI does not mediate the relationship between maternal folate status and PTB (including overall and spontaneous PTB).

Findings from this study are directly relevant to vulnerable US- urban low-income minority populations. The results of this dissertation have a potential impact on ongoing research, clinical and programmatic efforts to improve prepregnancy and prenatal nutrition and birth outcomes and may help inform the design and implementation of nutrition-based screening and interventions to prevent PTB and its consequences. Globally, mandatory folic acid fortification of grain products has been implemented in some countries including the US, Canada, and Australia, while many other countries have not. Our findings may also have implications for populations in the developing countries with low folate intake, but caution is needed in extrapolating our findings to populations with different demographic and clinical characteristics and contexts.

Conceptual Framework

A schematic representation of the conceptual framework highlighting the analytical approach for testing the study hypotheses is shown in the figure below. The framework reflects the study aims to elucidate the direct and indirect relationships between maternal folate status and PTB as well as to explore the potential moderating and mediating effects of two major causes of PTB (preeclampsia as well as IUI) on the folate-PTB relationship. These interrelationships were explored using complementary measures of maternal folate status across multiple time points from preconception to delivery, leveraging extensive clinical and epidemiological data relevant to this study.

Figure 1-1: Conceptual Framework



The exploration of maternal folate status across multiple time points is based on the life course approach to health. The life course theory achieved prominence in health with research conducted by Barker which demonstrated that PTB has a causal relationship to the origins of hypertension, coronary heart disease, and non-insulin-dependent diabetes later in life (45-47). In the life course health development model developed by Halfon et al, health is defined as an emergent capacity of human beings that dynamically develops over time, in response to multiple nested, and ever changing genetic, biological, behavioral, social and economic contexts (48). With this model, the goal of health practice is reiterated as the promotion of positive health at all stages of life and not just the avoidance of disease (49). This life course health development model feeds directly into the conceptual framework of this study as it spans from the

preconception to the peripartum period incorporating distal and proximal factors influencing PTB.

Dissertation Overview

The dissertation is presented in seven chapters. This chapter introduces the study aims and presented relevant background information. The second chapter presents a literature review and a study rationale, followed by the conceptual framework that guided the research. The third chapter presents the study design, data collection procedures, sample size and power calculations, definition of key variables and statistical analysis methods. Chapter four presents the first manuscript on maternal folate status and PTB in the BBC. Chapter five presents the second manuscript on interrelationships between maternal folate status, preeclampsia and PTB while Chapter six presents the third manuscript on interrelationships between maternal folate status, IUI and PTB. Chapter seven summarizes this dissertation, with a discussion of the clinical, public health, policy and research implications and conclusion.

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Chapter Two: Literature Review

Overview

This chapter provides an in-depth review of the literature on PTB and folate status in the US. Systematic literature reviews conducted on the relationship folate and PTB are summarized. Then, prospective and retrospective studies specific to the US population are presented, given the unique context conferred by the presence of mandatory folic acid fortification programs, the racial/ethnic composition and low national prevalence of folate deficiency (1). This chapter also discusses potential mechanisms for the relationship between folate and PTB.

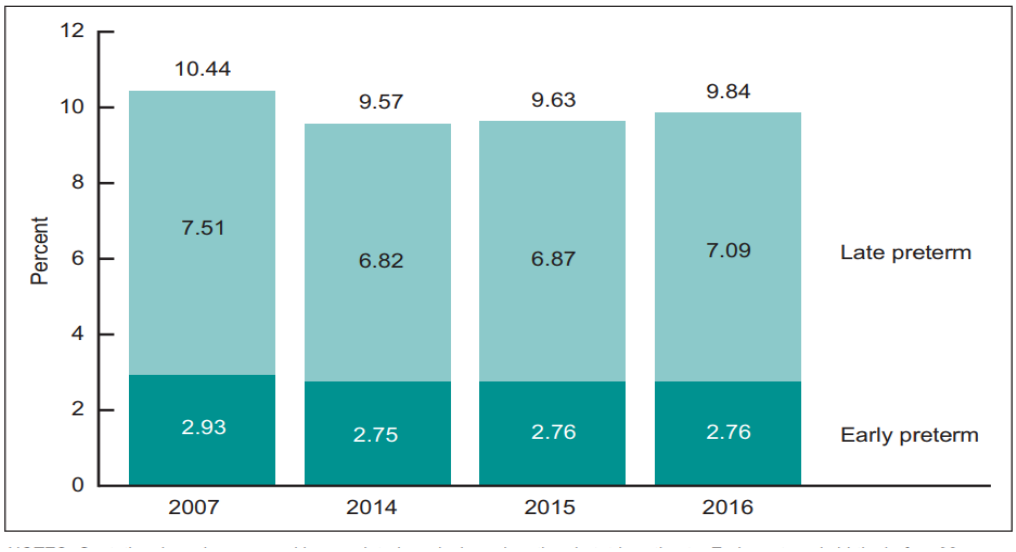
Preterm Birth Review

A review of the literature on PTB in the US is highlighted below. This includes the definition, distribution, etiology and measurement of PTB as well as a description of PTB subtypes in the US.

Definition and Distribution

In 2016, close to 1 of every 10 infants born in the US was born preterm, defined as birth before 37 completed weeks of gestation (2). US PTB rates remain much higher than other developed countries (3).

Figure 2-1: Trends in Preterm Birth rates in the United States.
Data source- final 2007, 2014, and 2015 and provisional 2016 natality data



After decades of rising annual PTB rates, rates have slowly declined from 10.4% in 2007 to 9.6% in 2014, but has increased since then to 9.8% in 2016 (4), presumably due to reduced birth rates in teenagers (5). Natality data from the Division of Vital Statistics (6), show considerable racial/ethnic variation in PTBs in the US. The rate of PTB among non-Hispanic black women (13.8%) was about 50% higher than the rate of PTB among non-Hispanic white women (8.9%) (4) (2). PTB also varies by maternal age (7-9), and socioeconomic status (10-12).

Subtypes

Based on gestational age, natality data shows that about 70% of all PTBs are "late" (occurring between 34-36 weeks of gestation) with the remaining classified as "early" (before 34 weeks of gestation) - a trend which has persisted since 2007 (6). On the basis of the clinical presentation, PTB may be classified as (i) spontaneous PTB when preterm labor starts spontaneously, (ii) preterm prelabor rupture of membranes (pPROM), or (iii) medically indicated PTB when delivery is indicated for pregnancy complications (induction of labor or elective caesarean birth before 37 completed weeks of gestation for maternal or fetal indications (both "urgent" or "discretionary"), or other non-medical reasons) (3). PTBs are sometimes referred to as either spontaneous (first 2 clinical conditions are often combined) or medically indicated (13). Data on the national prevalence of spontaneous or medically indicated sub groups of PTB are not reported though it appears that spontaneous PTB are more prevalent than medically indicated PTB (14). Between 1987 and 2012, medically indicated PTB demonstrated an increasing trend with a concomitant decrease in the incidence of spontaneous PTB (15).

Measurement

Until 2014, natality data calculated gestational age based on the date of the last normal menstrual period (LMP). Alternative measures of gestational age, termed the "clinical estimate"

(1989 revision) and the "obstetric estimate" (2003 revision) based on the birth attendant's final estimate of gestational age, have also been collected (16, 17). A comparison of these estimates of gestational age suggests that LMP-based data may overestimate the PTB rate. Early ultrasound dating (<20 weeks of gestation) is considered to be a more accurate method of determining gestational age (18, 19). The obstetric estimate, which incorporates all perinatal factors including ultrasound data, may have higher validity than LMP data, which may be less accurate due to poor maternal recall and individual variation in menstrual cycle length (20). Based on the obstetric estimate, the national PTB rate was only 9.6% in 2013 compared to 11.4% based on maternal LMP.

Etiology

PTB has been described as a complex condition resulting from multiple etiologic pathways (16). A precise mechanism cannot be established in most cases; therefore, factors associated with PTB, but not necessarily in the causal pathway, have been sought to explain preterm delivery (21).

Etiology of spontaneous preterm birth

Spontaneous PTB may be considered a clinical syndrome, arising from different pathological processes that activate prematurely one or more mechanisms leading to parturition (13). These processes include infection or inflammation, uteroplacental ischemia or hemorrhage, uterine over-distension, stress, and other immunologically mediated processes (22).

Infection and its related activation of inflammatory responses have been suggested as leading risk factors of spontaneous PTB with infection/inflammation detected in at least 25% of all PTBs according to a recent study (23). Increased production of proinflammatory cytokines has been

linked with uterine activation of contraction and PTB, whereas production of anti-inflammatory cytokines has been associated with uterine quiescence during gestation (24, 25). Other identified risk factors for spontaneous PTB include young or advanced maternal age, short inter-pregnancy intervals and low maternal body mass index (21, 26). Obstetric factors include prior PTB and uterine over distension with multiple pregnancy (27). Medical risk factors include infections such as urinary tract infections (28), malaria (29), bacterial vaginosis (28), HIV (29) and syphilis (30). Some lifestyle factors include stress and excessive physical work or long times spent standing (26), smoking, excessive alcohol consumption and periodontal disease (31). Spontaneous PTB have been found to vary by maternal age and socio-environmental context (29) but the cause for spontaneous PTB remains idiopathic in up to 50% of all cases (32). The role of maternal nutrition remains a promising area of investigation.

Etiology of medically indicated preterm birth

A US study published in 2011 showed that more than half of all medically indicated "late" PTB did not have a definitive medical indication (33). Unintended medically indicated PTB also can occur with the elective delivery of a baby thought to be term due to errors in assessment of gestational age (34). However, such unintended PTB have reduced with the advent of routine ultrasounds during prenatal care. Clinical conditions that may lead to medically-indicated PTB include placental abruption, uterine rupture, fetal distress and fetal growth restriction (35) and preeclampsia (5, 36, 37). Preeclampsia, has been shown to account for as much as 15% of PTB cases, due to medically indicated PTB (38). Studies have shown that the association between preeclampsia and medically indicated PTB is more than two times stronger than the preeclampsia- spontaneous PTB association (39). Medically indicated preterm deliveries of women with preeclampsia are justified because of the concerns about maternal and fetal safety

with continued gestation. The American College of Obstetricians and Gynecologists (ACOG) recommends that in the presence of severe preeclampsia, delivery be induced after 34 weeks of gestation (40).

Medically indicated PTB in the US have been linked with a variety of behavioral risk factors such as prenatal care (41), substance abuse (42) as well as stress, depression and anxiety (43-48). Again, the role of vitamin/micronutrient levels (49-51) merits further investigation.

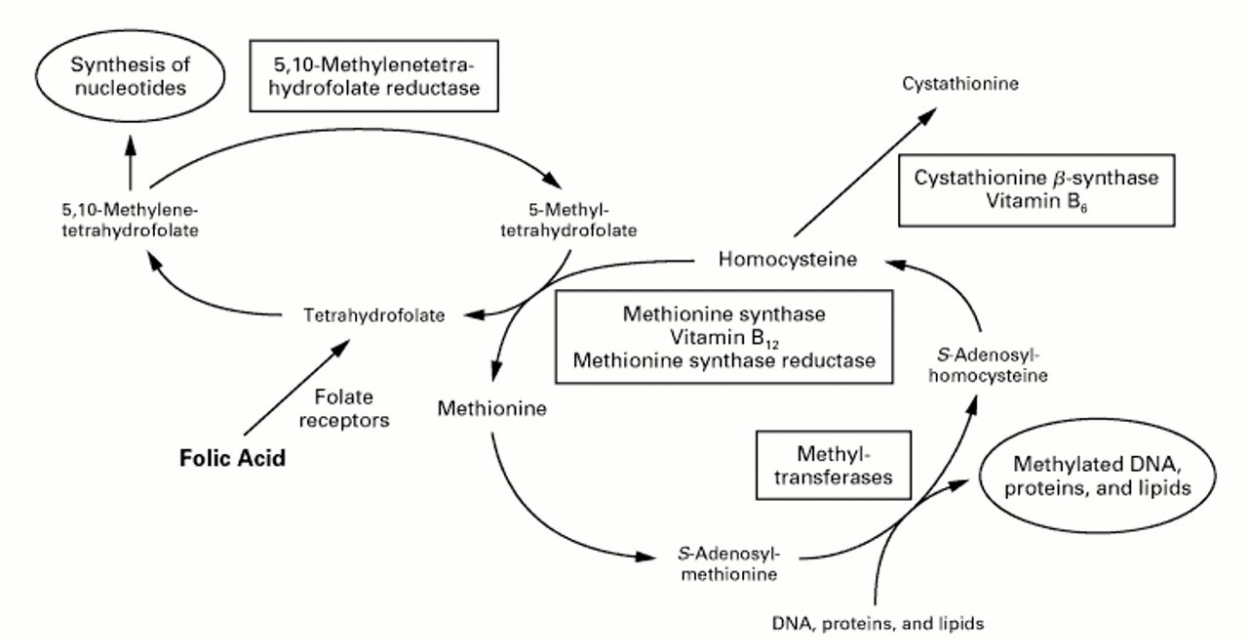
Folate Status Review

A detailed introduction to folate status in the US is highlighted below. This includes the definition and function of folate as well as the measurement of folate status at a population level. In addition, normal and pathological folate status in the US is discussed.

Definition

Folate, also known as vitamin B_9, is a naturally occurring water-soluble B vitamin involved in many cellular pathways and biological reactions needed for fetal and placental growth. Sources of folate include: vegetables, fruits, nuts, beans, peas, grains, brewer's yeast and liver. Folate is a generic term for a group of compounds including folic acid and its derivatives. Folic acid is a synthetic form of folate which does not exist in nature, although oxidation of folates to folic acid is seen in stored or cooked foods. 5-Methyl Tetrahydrofolate (5-MeTHF) is a biologically active form of folate and is the most abundant form found in plasma, representing over 90% of folate and is the predominant active metabolite of ingested folic acid (52). The bioavailability of naturally occurring folate is less than that of folic acid. A schematic of folate derivatives and folate metabolism is presented below.

Figure 2-2: Folate Metabolism



Function

Folate plays an essential role in one-carbon metabolism, facilitating the transfer of one-carbon units in reactions required for metabolism of nucleic acids, amino acids and methylating agents required for normal metabolism and gene regulation. Its role in prevention of neural tube defect is via its function as an essential cofactor in folate-mediated one-carbon metabolism and in the epigenetic regulation of the transcription of genes that control neural closure (53). Folate also plays a role in the prevention of oxidative stress and inflammation (54, 55). Recently, the epigenetic effect of folate in its role in methylation has been identified as a crucial link between nutrition and health manifesting at both the individual and trans-generational level (56-58). Among adults, folate has been associated with hypertension (59, 60) and subsequent cardiovascular diseases (61) via its effect on endothelial function.

Measurement

Folate status can be assessed using biomarkers in urine, serum, plasma or the red blood cells using a variety of techniques including microbiological methods, radioisotope competitive binding, and enzyme linked or chemiluminescence assays. Red blood cell (rbc) folate concentrations measure long-term folate status as they respond very slowly to changes in folate intake. This slow response is because erythrocytes, which have a 120-day lifespan, accumulate folate only during erythropoiesis.

Plasma or serum folate reflects recent folate intake and low levels are an early indicator of inadequate folate status. A single plasma folate measurement cannot be used to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. Repeated low values of plasma folate within an individual over the course of a month, however, are indicative of low folate status or folate depletion (62, 63). Measurement of plasma folate may be preferred over rbc-folate because it can be reliably measured using immunological or chromatography methods, shows faster response after supplementation, and reflects the amount available for transfer via the placenta (64). Alternative approach for assessing folate status using biomarkers involves surrogate measurement of metabolites known to increase in folate deficiencies (65). One of these, total homocysteine (tHcy) is used as a surrogate functional marker for folate status (66).

Folate status can also be estimated based on folate intake. This approach includes the use of food frequency questionnaires (FFQ), 24-hour dietary recall and intake of supplements (66). These methods are non- invasive, cheaper and easier to implement compared with biomarker assessments. As they are self-reported assessments, they are sometimes flawed with multiple errors such as interviewer bias or recall bias. Though they have been validated as correlated with

biomarker measurements (67, 68), they often require an exhaustive list of various sources of dietary intake and may involve complex computation to determine dietary folate equivalents (DFE)- the measure of daily intake which takes into account the higher bioavailability of synthetic folic acid compared to naturally occurring folate in food (69).

A US based study found the correlation coefficients between total folate intake and biological markers to be r=0.41 for serum and 0.25 for red blood cell folate ($P < .05$ for both). Serum folate correlated with rbc folate, $r = 0.50$ *(*$P < .05$). Their results suggest that the estimate of folate from the food frequency questionnaire, and the pregnancy supplement use questions reflect short-term as opposed to long-term folate status in the population (70).

Abnormal folate level

In the US, early NHANES results defined low red cell folate as less than 140 ng/mL and low plasma folate levels as less than 3 ng/mL (63). More recently, the World Health Organization (WHO) established guidelines for utilising serum and red blood cell folate concentrations to assess folate status in populations. Their guidelines discuss two major measurement methods in defining folate status. The first involves the use of macrocytic anemia as a hematological indicator and defines folate deficiency as serum/plasma folate levels less than 6.8 nmol/L; possible folate deficiency as 6.8-13.4nmol/L; normal folate values as 13.5-45.3nmol/L and elevated levels as higher than 45.3nmol/L (62). Red cell folate levels less than <226.5nmol/L are also indicative of folate deficiency.

The second assessment defines folate deficiency based on the folate concentration below which homocysteine concentrations start to rise. With this guideline, folate deficiency corresponds to serum/plasma folate levels less than 10 nmol/L; or red cell folate levels less than 340ng/L.

Folate deficiency may occur due to low dietary intake, poor gastrointestinal absorption of ingested folate and increased need (such as in physical activity, pregnancy); it can also be caused by pathological liver conditions, and a dysfunction in folate metabolism which may be due to genetics or drug interactions (71, 72). Excessive folate is linked with vitamin B12 deficiency, interaction with drugs that inhibit folate metabolism, decreased zinc absorption, hypersensitivity reactions, association with cancer, neurotoxicity and epileptogenic effects and increased susceptibility to malaria (73).

Folate status in the US Female Population

Higher doses of folate are recommended for pregnant (600 mcg DFE) and lactating (500 mcg DFE) mothers to prevent neural tube defects (74). However, NHANES data from 2000 to 2010 suggests that 25% of women of reproductive age in the US have insufficient folate levels to prevent neural tube defects (75). Other findings from the NHANES show that minority women are more likely to have lower folate levels than non-Hispanic white women(76). Also, women who were non-white, aged 18-24 years, had less than a high school education, or had a household income of <$25,000 were the least likely to report daily consumption of a supplement containing folic acid (77). In addition, minority populations are less likely to have heard about folic acid, to know it can prevent birth defects or pregnancy complications, consume foods fortified with folic acid, or take vitamins containing folic acid before pregnancy (78-81).

Review of the Relationship between folate status and PTB

Systematic literature reviews

A systematic review of recent observational studies identified a link between reduced maternal folate levels and PTB while three reviews of randomized control trials (RCTs) did not corroborate this relationship.

The review by Mantovani et al. (82), examined the results from recent observational studies (2009 onwards) on the effect of periconceptional (started before conception and continued until 12 weeks of gestational age) folic acid supplementation on PTB. Their search was restricted to Medline and involved a manual search of observational studies from 2009 onwards that analyzed the rate of PTB in patients who received supplementation with folic acid before and/or throughout pregnancy. Their findings from 7 selected studies (two of which were conducted in the US) suggest a slight reduction of PTBs.

Fekete et al. (83) conducted a systematic review of the MEDLINE, EMBASE and Cochrane Library CENTRAL databases from inception to February 2010 for RCTs in which folate intake and health outcomes in pregnancy were investigated. Their analysis of six studies from five publications involving only 380 participants showed no relation between maternal folate intake (folic acid plus dietary folate) and PTB. Of the six studies included in their analysis, three were published over 25 years ago and none was based in US.

Lassi et al. (84) broadened the scope of their systematic review to all randomised, cluster-randomised and cross-over controlled trials evaluating supplementation of folic acid alone or with other micronutrients versus no folic acid (placebo or same micronutrients but no folic acid) in pregnancy. They included all pregnant women regardless of age and parity but excluded studies in which women were supplemented during the periconception period. Three trials involving 2959 women are included in their meta-analysis of folate on PTB risk. They found no impact of folic acid on PTB (risk ratio (RR) 1.01, 95% confidence interval (CI) 0.73 to 1.38). However, they acknowledged that only one of the studies defined PTB correctly- two of these studies defined PTB as births before 38 weeks of gestation. Furthermore, none of the studies were conducted in the US.

Saccone and Berghella's review (85) in 2016 evaluated the efficacy of folic acid supplementation during pregnancy to prevent PTB. Five randomized trials including 5,332 asymptomatic singleton gestations without prior PTB who were randomized to prophylactic treatment with either folic acid supplementation or control were included in the analysis. Women who received folic acid supplementation had a similar rate of PTB (22.6% vs 22.9%; RR 0.99, 95% CI 0.82-1.18), PTB<34 weeks (7.1% vs 8.7%; RR 0.77, 95% CI 0.55-1.09). However, three of the included studies were conducted during the 1970s and the authors found poor compliance with random allocation in these studies. Moreover, only one RCT had PTB as primary outcome, none of the studies randomized folic acid preconceptionally and none was conducted in the US.

In summary, differences in the systematic review findings might relate to quality of the studies reviewed- majority of the RCTs included in the meta-analysis were conducted four decades ago and PTB was poorly defined. In addition, the systematic reviews did not provide information on the folate status of participants and the folic acid fortification context in the included studies. Also, it is possible that the timing of folate administration may play a role as only the review by Mantovani limited the timing to the preconception period. There is also the possibility that folic acid supplementation is only beneficial in women with folic acid deficiency. Finally, it could also be that folic acid supplementation is truly not effective in preventing PTB.

Review of US studies on the relationship between folate status and PTB

A major gap in the study of the folate-PTB association is the paucity of US based studies. Studies have been conducted across varying populations such as China (86), Greece (87), Venezuela (88), Hungary (89), Netherlands (90) and Norway (91). These populations are not comparable with the US due to differences in racial composition, implementation of national folic acid fortification programs and folate deficiency levels in women (1). While no RCT has

been conducted in the US, prospective studies conducted in the US demonstrated that a low dietary intake or plasma level of folate were associated with increased risk of PTB, controlling for other known risks whereas retrospective studies showed conflicting results. A review of seven US studies demonstrated a reduction in PTB with increased folate or national folic acid fortification programs in five studies, while one study did not find an association and another found mixed results. These studies were conducted among vulnerable as well as nationally representative populations and included different methods of folate assessment as well as timing of folate administration as seen in the table 1 below.

Table 2-1: Summary of US studies exploring the relationship between folate and preterm birth

Design	Authors	Pre/post fortification	Location	Study population	Sample size	Timing of folate	Folate measure	Association
Prospective	Scholl, Theresa O., et al. (1996).	1995-Pre-Fortification	New Jersey	Urban, poor, predominantly African American	N=827	Blood-28 weeks of gestation Intake-prenatal Diet-3 24-hour diet recall	-plasma folate -self reported intake of folic acid supplement -self reported intake of dietary folate	RR 1.9, 95% CI 1.0–3.6
	Siega-Riz, Anna Maria, et al. (2004)	1995-2000 Peri-fortification	North Carolina	Low to mid income women	N=2,026	24-29 weeks of gestation	-self reported intake of dietary folate -plasma folate -red cell folate	RR 1.8, 95% CI 1.3–2.5
	Bukowski, Radek, et al. (2009)	1999-2002 Post fortification	Multistate	Predominantly White	N=34,480	periconception-one-year prepregnancy to delivery	-self reported intake of FA supplement	aHR 0.31, 95% CI 0.11-0.90
	Bodnar, Lisa M., et al. (2010).	2003-2007 Post fortification	Pittsburg, Pennsylvania	Low income, predominantly African American	N=313	<16 weeks of gestation	-plasma folate	aRR (95% CI): 0.4 (0.1, 0.9);
	Shaw, et al. (2004)	1990-2000 Pre-and post-	California	White and Hispanic	N=5,916,630	Population level data from pre-and post-fortification period. No additional information on diet or supplements. No individual level assessment of folate status		RR: 0.96; 95% CI

Design	Authors	Pre/post fortification	Location	Study population	Sample size	Timing of folate	Folate measure	Association
		fortification						0.94, 0.97
Retrospective	Dunlop, Anne L., et al. (2012)	2003-2006 Post fortification	Nashville	White and black	N=155	Delivery	-plasma folate	None
	Shaw, Gary M., et al. (2011)	1998-2005 Post fortification	Multistate	Nationally representative	N= 5,952	periconception- 12 wks. prepregnancy to delivery	-self reported intake of FA supplement -self reported intake of dietary folate -self reported intake of dietary folic acid	-aOR: 0.54; 95% CI: 0.31, 0.93
aHR: adjusted hazard ratio; aRR: adjusted relative risk; CI: confidence interval; FA: folic acid; N/A: not applicable; RR: relative risk;								

Prospective studies

A study in New Jersey was conducted in 1995 prior to the national mandatory folic acid fortification program among an urban poor, predominantly African American population (N=827) (92). Compared with women who had a folate intake >400μg/day, those with intermediate folate intake (241–400μg/day) and low folate intake (≤240μg/day) had a progressively increased risk of PTB (RR 3.4, 95% CI 1.9–6.1 and RR 1.9, 95% CI 1.0–3.6, respectively). In addition, each 1 nmol/L increase in serum folate concentrations at 28 weeks of gestation was also associated with reduced risk of PTB (aOR: 0.984; 95% CI: 0.969, 0.998). Their analyses controlled for gestation at entry, age, parity, ethnicity, cigarettes smoking, gestational weight gain, plasma vitamin B-12 and dietary intake of energy, zinc, fiber.

Between 1995 and 2000- around the timing of the folic acid fortification program- 2648 middle to low income women from 4 prenatal centers were recruited between 24 and 29 weeks' gestation in a North Carolina study (70). Women completed a questionnaire that provided data on sociodemographic characteristics and health behaviors both before and during the pregnancy,

as well as provided blood at the time of the interview (2314 women had dietary data, 2026 specimens were available for the serum folate analysis, and 1034 specimens for the red blood cell analysis). Those with low serum folate levels in the second trimester had a nearly twofold increased risk of PTB (RR 1.8, 95% CI 1.3–2.5) controlling for prenatal supplement use and time of storage of folate sample. However, only covariates that changed the coefficient of the maternal folate exposure variables by greater than 10% were retained in the final model. Thus, other sociodemographic and dietary covariates were not included in the final model.

In the years following the folic acid fortification program- 1999-2002, Bukowski, Radek, et al. (93) conducted secondary data analysis from a multicenter cohort aneuploidy risk study in the US and explored the link between preconceptional folic acid supplementation and PTB. They studied data from 34,480 women from 15 US centers who delivered singleton pregnancies at 20 to 42 weeks. The association between preconceptional folic acid supplementation and the risk of spontaneous PTB was evaluated using survival analysis. Adjusting for maternal age, race, body mass index, education, marital status, smoking, parity, and history of prior preterm, folic acid supplementation for 1 y or longer was associated with spontaneous PTB between 20 and 28, and 28 to 32 weeks (adjusted HR 0.31, 95% CI 0.11-0.90, p = 0.031 and 0.53, 0.28-0.99, p = 0.046, respectively). However, preconceptional folic acid supplementation was not significantly associated with the risk of spontaneous PTB beyond 32 wk. These hazard rates were comparable to those seen in the unadjusted analysis. The authors concluded that, folic acid supplementation for 1 y or longer prior to conception decreased the risk of spontaneous PTB, and that this association was strong, specific, dose-dependent, consistent with other studies, biologically plausible due to the role of IUI, and essentially unchanged after adjustment for potential confounders.

Between 2003-2007, years after the implementation of the fortification program, a prospective cohort study in Pittsburg, Pennsylvania attempted to identify specific folate subtypes related to PTB (94). They analyzed nonfasting blood samples from 313 pregnant women enrolled before 16 weeks of gestation. Study participants were from antepartum clinics serving a predominantly publicly insured, low-income population that was 55% black and 44% white. Eligible women had singleton pregnancies, were non-Hispanic whites or non-Hispanic blacks based on self-reports, and had no known preexisting conditions, vaginal bleeding, fetal anomalies, or current or planned cervical cerclage. At enrollment, women completed an interviewer-administered questionnaire to collect data on sociodemographic characteristics, medical, reproductive, and sexual history, and maternal behaviors. Women also provided a nonfasting blood sample that was banked for later analysis. After adjustment for race-ethnicity, education, smoking, and obesity, 1-SD increases in serum total folate and serum 5-methyltetrahydrofolate (5MeTHF) concentrations were associated with significant reductions in the risk of spontaneous PTB ($P < 0.05$). Study findings also demonstrated that the relationship between serum total folate concentrations and spontaneous PTB were driven by serum 5MeTHF concentrations.

A population based study was conducted by Shaw et al. (95) to explore the frequencies of low birthweight and preterm delivery among approximately 6 million California infants before and after compulsory food fortification with folic acid. They designated the period "before fortification" as January 1, 1990, through December 31, 1997, and the period of "fortification" as October 1, 1998, through December 31, 2000. The unadjusted prevalence of preterm delivery did not substantially vary across birth years. However, Poisson regression analyses adjusting for maternal age, parity, race/ethnicity, education, year of birth, and fortification period revealed a

reduced risk for PTB (relative risk ratios (RR) = 0.96; 95% CI 0.94, 0.97) for preterm delivery. There was no individual level information on dietary intake, supplement use, folate status or timing of consumption of folate fortified foods.

Retrospective studies

US based retrospective studies, have shown mixed results. Shaw, Gary M., et al. (96) investigated periconceptional intake of multiple sources of folate and folic acid (dietary folate, folic acid intake and folate containing supplement use respectively) to determine their association with preterm delivery. They conducted telephone interviews with 5952 women enrolled in the multi-state National Birth Defects Prevention Study who delivered in the post folic acid fortification period from 1998 to 2005. These interviews took place between 6 weeks to 24 months postpartum. In their analysis of folic acid supplement use, their reference group was women with preconception supplement use. Folate use in the first as well as second/third trimester was associated with reduced odds of PTB (first trimester-aOR:0.69; 95% CI: 0.52, 0.99 and second/third trimester-aOR: 0.54; 95% CI: 0.31, 0.93). However, sporadic or non-use was not associated with significantly different odds of PTB. There was also no association between dietary folate and dietary folic acid intake and PTB.

In Nashville, Dunlop and colleagues explored maternal micronutrient status and PTB for Black and White US Women (97). Biospecimens and medical record data for this study were derived from a subsample of women enrolled into the Nashville Birth Cohort during 2003-2006. The study randomly selected 160 women (stratified by race- non-Hispanic black and non-Hispanic white- as well as PTB status) out of 1547 eligible women as this was a pilot investigation. There was no significant difference in the mean concentration of folate collected at

delivery for women with PTB versus term birth. Their adjusted logistic regressions might have been hampered by insufficient power as seen with the very wide confidence intervals.

In summary, although many studies on the folate- PTB relationship have been done in various settings, the results have been inconclusive. Systematic reviews revealed important limitations of previous studies, including dated studies, inaccurate definition of PTB and small sample sizes. US based studies suggest a significant reduction in PTB risk with folic acid supplementation or increased plasma folate, particularly among low income minority women. However, some gaps highlighted include the small sample size of studies of plasma folate assays. This present study is one of the few studies that explore the relation between folate and PTB using a relatively large number of plasma folate samples along with self-reported multivitamin supplementation.

Review of potential mechanistic pathways in the folate-PTB- relationship

Specific pathways that may explain the folate – PTB relationship include preeclampsia and intrauterine infection/inflammation (IUI) summarized in Table 2-2 below.

Table 2-1: Summary of studies on intrauterine inflammation or preeclampsia in the folate-PTB relationship

Study	Design	Methods	Findings
Simhan et al. (2011)	Prospective cohort study in Pittsburgh, PA.	Pregnant women (n = 417) at <16 weeks' gestation had serum samples that were analyzed for folate species and cervical fluid that was assayed for cytokine concentrations. Analyses include spearman correlation and locally weighted regressions.	After confounder adjustment, maternal serum folate level had a strong negative association with elevated anti-inflammatory scores; (P< .05). Maternal serum folate was associated with cervical cytokine concentrations, which suggests a possible mechanistic link between folate and PTB risk.
Banhidy et al. (2011)	Case control study in Hungary	Birth outcomes of newborns were evaluated in 1,017 (2.7%) pregnant women with medically recorded pre-eclampsia and	There was a lower risk of PTB (6.8%) of newborn infants born to pregnant women with early onset pre-eclampsia after

		37,134 pregnant women without pre-eclampsia	folic acid supplementation from early pregnancy. There was no significant reduction in the rate of PTB and low birthweight in pregnant women with late onset pre-eclampsia after folic acid supplementation.
Dhobale et al. (2012)	Cross sectional study in India	The levels of folate, vitamin B12 and homocysteine were measured in mothers delivering preterm (PT; gestation <37 weeks; n = 67), those delivering preterm due to preeclampsia (PT-PE; n = 49) and women delivering at term (control group; n = 76).	Plasma folate levels were significantly reduced in the PT group (8.01 ± 5.1 ng/ml, p = 0.014), while there was no difference in the PT-PE group (9.2 ± 5.8 ng/ml) as compared to the control group (9.2 ± 4.8 ng/ml).

Preeclampsia

The link between folate and PTB may also be possibly explained by preeclampsia-which complicates about 4-5% of pregnancies and occurs when physiologic expansion of the maternal circulation and hormonal changes leading to vasodilation and reduced blood pressure fail to occur (98-100).

Few studies have explored the role of hypertension in the relation between folate and PTB. In Hungary, researchers evaluated the rate of PTB in the newborns of pregnant women with early and late onset pre-eclampsia according to folic acid supplementation (89). The study was conducted prior to folic acid fortification programs in 1998 and study participants include 1,017 (2.7%) preeclamptic women and 37,134 pregnant women without pre-eclampsia enrolled in a National surveillance system between 1980 and 1996. The study groups were differentiated per supplementation of high dose of folic acid alone from early pregnancy. Pregnant women with pre-eclampsia had a higher rate of PTB compared to those without preeclampsia (10.2% versus 9.1%). There was a lower risk of PTB (6.8%) of newborn infants born to pregnant women with early onset pre-eclampsia after folic acid supplementation from early pregnancy. Folic acid

significantly reduced the rate of PTB (OR with 95% CI: 0.41, 0.18–0.94) in pregnant women with early onset PE.

A cross sectional study in India assessed the relation between folate, preeclampsia and PTB (101). The levels of folate, vitamin B_{12} and homocysteine were measured in mothers enrolled in 2007-2010, delivering preterm (PT; gestation <37 weeks; n = 67), delivering preterm due to preeclampsia (PT-PE; n = 49) and delivering at term (control group; n = 76). Women were given tablets of iron (100 mg per tablet) and folic acid (1 mg per tablet) during the 1st trimester of pregnancy. A food frequency questionnaire was used to estimate the frequency of intake of foods rich in folic acid and all women had limited access to folate fortified foods. Blood samples were collected just before delivery. Reduced folate levels were observed only in PT and not in PT-PE women as compared to the controls. Plasma folate levels were significantly reduced in the PT group (8.01 ± 5.1 ng/ml, p = 0.014), while there was no difference in the PT-PE group (9.2 ± 5.8 ng/ml) as compared to the control group (9.2 ± 4.8 ng/ml).

Although recent systemic reviews (102, 103) did not demonstrate a direct relation between folate and preeclampsia, other studies have shown that folate may prevent preeclampsia among high risk women. Similar patterns of associations were observed in analysis by RBC and serum folate levels and in dose-response analysis. A 2016 prospective cohort study in Canada (104) demonstrated that folate is associated with preeclampsia in high risk women. Specifically, the rate of PE was lower in the supplementation group (either supplementation with multiple vitamins containing folic acid or folic acid alone) than in the no supplementation group, but this difference was only statistically significant in high risk women- obese, with prior preeclampsia, chronic hypertension, diabetes or multiple pregnancy- (aOR: 0.42; 95% CI: 0.18, 0.98) for

supplementation with folate containing multivitamins and aOR: 0.17; 95% CI: 0.03, 0.95 for folic acid supplementation alone).

In a 2016 retrospective study (105) a cohort of women who received daily oral 5-MeTHF 15 mg supplementation as prophylactic treatment since the first trimester for recurrent preeclampsia were compared with women who did not. All asymptomatic singleton gestations with prior preeclampsia (in the previous pregnancy) were included and women with chronic hypertension were excluded. The study showed that women with prior preeclampsia who received daily oral 5-MeTHF 15 mg supplementation had a significantly lower incidence of overall preeclampsia, severe preeclampsia and early-onset preeclampsia.

Intrauterine infection/inflammation

There is evidence from the literature that IUI is a primary cause of 25%-40% of PTB cases (23). Specifically: (i) amniotic fluid of women with preterm labor have higher rates of microbial colonization and levels of inflammatory cytokines than women with preterm deliveries not associated with PPROM or term patients in labor (106); (b) intrauterine or systemic administration of microbes or microbial products to pregnant animals can result in preterm labor and delivery (107, 108); (c) subclinical intrauterine infections are associated with preterm labor and delivery (109); (d) patients with intra-amniotic infection (110) or intrauterine inflammation (i.e. elevation of amniotic fluid cytokines (111) and matrix-degrading enzymes (112) identified as early as the mid-trimester are at risk for subsequent preterm delivery.

Studies suggest that PTB is associated with abnormal inflammatory responses, which may be triggered by intrauterine infection or hemorrhage (113). Folate is needed for normal immune function and folate-deficient individuals demonstrate immune dysfunction at both the cell-mediated and humoral levels (114). In addition, the phagocytic and bactericidal role of

polymorphonuclear leukocytes have been shown to be decreased in individuals with folate deficiency, thereby increasing their susceptibility to infections such as asymptomatic bacteriuria and PTB (114-116). Inadequate maternal folate levels in pregnancy increases serum-C reactive proteins and inflammatory cytokine concentration which in turn is associated with shortened gestational age at delivery(117).

Only one study has specifically explored the possibility of infection/inflammation as a mechanistic pathway in the folate-PTB relationship. Simhan, Bodnar and colleagues (118) conducted a follow up study in Pittsburgh in 2011 using the same population presented in Bodnar et al. The authors sought to explore the role of lower genital tract infection as a mechanistic link between folate and PTB. Their analysis included 417 women with plasma folate samples. In addition, their cervical fluid was analyzed for cytokine concentrations. Like Bodnar et al., study participants were patients of resident antepartum clinics in Pittsburgh, PA. The antepartum clinics served a predominantly publicly insured, low-income population that is approximately 55% black and 44% white. Eligible women were <16 weeks' gestation, non-Hispanic white or non-Hispanic black (based on self-report), and had singleton pregnancies with no known preexisting conditions, vaginal bleeding, fetal anomalies, or current or planned cervical cerclage. At enrollment, a mean of 9.5 weeks of gestation, women underwent a standard pelvic examination and provided a nonfasting blood sample and completed a questionnaire on sociodemographic characteristics; medical, reproductive, and sexual history; and maternal behaviors. Results demonstrated that anti-inflammatory cytokine scores were negatively associated with serum 5MeTHF concentrations ($r = –0.11$; $P < .05$) and were positively associated with serum 5FoTHF ($r = 0.19$; $P < .001$).

The authors' conclusions drew from their previous research (94) which showed that women with low 5MeTHF and high 5FoTHF had the highest risk of PTB. They identified this same subgroup of women as having a unique environment in their lower genital tract. The authors however alluded to some of the limitations of their study such as the sample size and study design limited their ability to study the relation between folate status and infection/inflammation in the upper genital tract.

This dissertation aims to contribute significantly to the scant literature on the mechanistic pathways of the folate-PTB relationships. By using a relatively large sample of PTB, preeclampsia, as well as folate and placental pathology samples, this research is better poised to explore the role of preeclampsia and IUI as potential pathways.

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Chapter Three: Methodology

Overview

This chapter presents research methodology of this dissertation, including the study design, data collection procedures, sample size and power calculations, definition of key variables, statistical analysis methods and ethical considerations.

Study Population

The data analyzed was from the ongoing Boston Birth Cohort (BBC) study on PTB, which commenced in 1998. To date, about 8500 mother-infant dyads have been enrolled at birth at the Boston Medical Center (BMC), a large urban hospital serving a predominantly low-income, minority, inner-city patient population. The overall rates of PTB were approximately 17% in this population compared with the national average of 9.6% (1). Eligibility for the BBC study included delivery of a live singleton birth at BMC. Exclusion criteria included pregnancies that are a result of in vitro fertilization or that involve multiple gestations, fetuses with chromosomal abnormalities or major birth defects, preterm deliveries due to maternal trauma, and women with congenital or acquired uterine lesions or incompetent cervix. The BBC oversampled PTB and/or low birthweight, using an approximately 1:2 case-control ratio: Cases were defined as women who delivered singleton, live, LBW (<2500 grams) or preterm infants (<37 weeks of gestation) regardless of birth weight. Controls were matched for maternal age and ethnicity and were defined as women who delivered singleton, live, term infants with birth weight 2500 g or more.

Data Collection

All eligible mothers were approached within 72 hours postpartum by research staff, who were fluent in Spanish, English or Haitian creole; and who were trained in research ethics as well as the study protocol to ensure data collection quality. Among eligible cases and controls who were approached by the research staff, about 90% agreed to participate in the study. After informed

consent was obtained, the study staff collected the following: epidemiologic data, clinical data and maternal venous blood and placental samples.

Epidemiologic data was collected via maternal questionnaire interview with a standard questionnaire including the following modules: demographic characteristics, cigarette smoking, alcohol consumption, diet, medical and reproductive history. The interviews were typically conducted in the post-delivery ward and lasted on average 40 minutes.

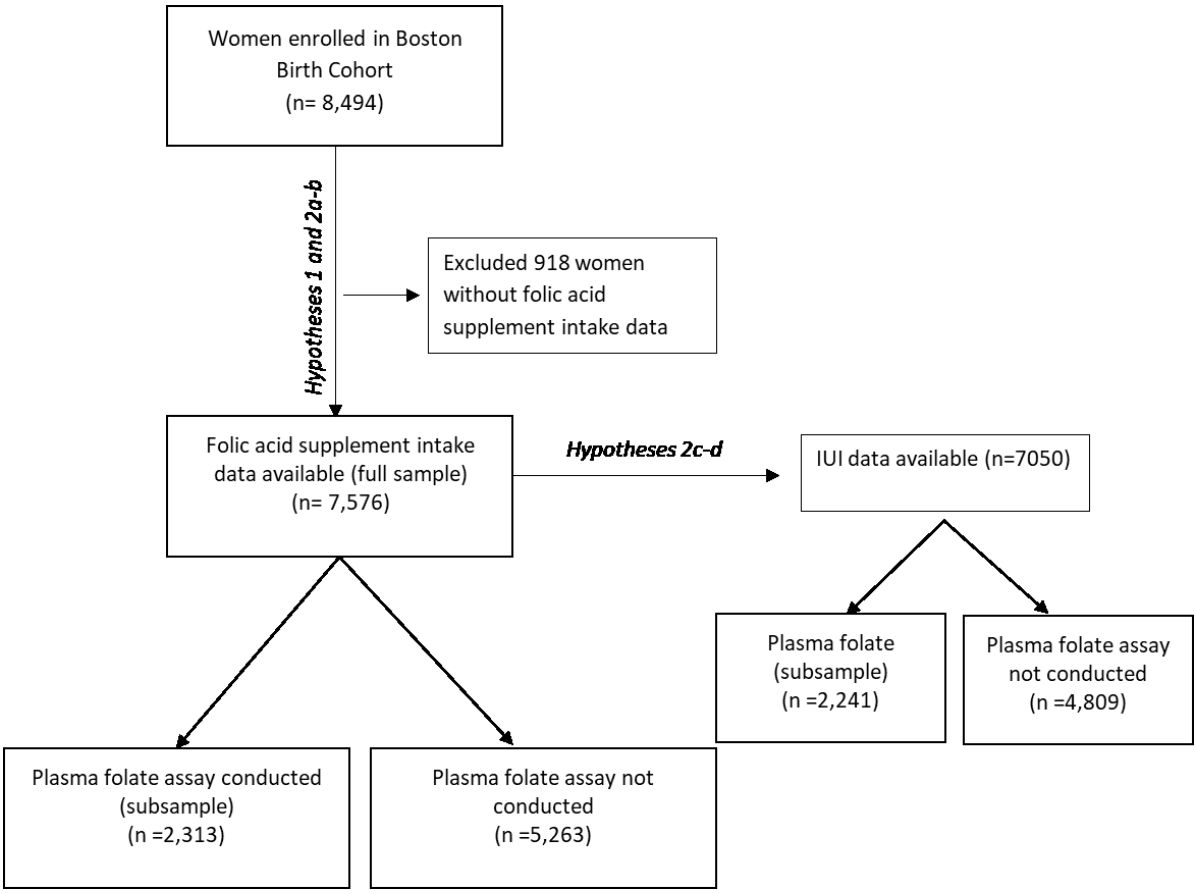
A standardized abstraction form was used to record data from medical records review, including prenatal and intrapartum clinical care, pre-pregnancy conditions, pregnancy complications, birth outcomes, ultrasonographic findings and laboratory test results.

Placenta samples for histopathology, as well as maternal venous blood samples, were obtained according to standard protocol (2). A perinatal pathologist, not blinded to clinical information, examined all placentas (in accordance with College of American Pathologists guidelines (3) when clinically indicated, per BBC protocol. Pathologic placental lesions were diagnosed according to standard criteria (4). To verify reliability of diagnoses, a second perinatal pathologist independently reviewed a subset of placentas. Placental pathology diagnoses were recoded by a third perinatal pathologist into eight predominantly inflammatory and vascular categories, based upon the classification proposed by Redline (5). To establish reliability, the second perinatal pathologist confirmed the placental coding. Categories of the placental pathology include chorioamnionitis, chronic villitis, Maternal Vascular Malperfusion, marginal (venous) abruption, umbilical cord obstruction, fetal vascular malperfusion, villous stromal-vascular abnormalities, and a miscellaneous group. A detailed description of the placental pathology coding has been published (6).

Study Sample

This dissertation employed data from 8,494 mother-infant dyads enrolled in the BBC. The final sample for analysis was 7,576 women with information on folic acid intake as well as a subset of the BBC (N=2,560) with measured folate biomarkers of folate at delivery. This subset of mothers was selected purposefully because their children were enrolled in the postnatal follow-up at the BMC. Of the 7,576, a total of 7,050 had data on IUI and thus for the hypotheses 2 c-d analyses, the sample size was 7,050 women and a subsample of 2,241 with folate biomarkers.

Figure 3-1: Study Participants Flowchart



As the study consists of a high-risk urban minority population and employs a case-control study design, the table below summarizes differences in the racial composition, socioeconomic status and key outcomes of interest between the BBC and national estimates.

Table 3-1: Comparison of the BBC study population with national estimates (N=7576)

Characteristic	Study sample (%)	National estimates (%)
Race/ethnicity		
Non-Hispanic Black	51	13 (7)
White	12	62
Hispanic	28	18
Other	09	07
Government assistance	84	21 (7)
Preterm birth	27	9.6
Preeclampsia	13	3-5
Intrauterine infection/Inflammation	15	N/A
Abbreviations: N/A: not applicable		

Key Variables

The key outcome, PTB was defined as delivery before 37 completed weeks of gestation. Gestational age was determined using an algorithm based on last menstrual period and the result of early ultrasound dating (<20 weeks' gestation) as previously published (1). The last menstrual period estimate was used only if confirmed by an ultrasound within 7 days or if no ultrasound estimate was obtained; otherwise, the ultrasound estimate was used.

Subtypes of Preterm birth: Spontaneous PTB was defined as PTB when preterm labor starts spontaneously or because of preterm premature rupture of membranes (pPROM). Medically indicated PTB was defined as delivery due to induction of labor or caesarean birth before 37 completed weeks of gestation (8).

The main predictor, maternal folate status is based on two complementary assessments: self-reported multivitamin supplement intake and plasma biomarkers of folate status.

Self-reported supplement intake*:* The data source is the postpartum questionnaire which includes the following questions: “Did you take prenatal vitamins prescribed by your doctor?” and “Did you take any over the counter vitamins?”.

Folic acid 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 options for both questions is yes or no. Follow up questions assess frequency of use in the prepregnancy (6 months prior to conception), 1st-, 2nd-and 3rd- trimester among those who responded yes. Response options include: less than once a week, one to two times a week, three to five times a week, 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 400 or 800 micrograms of folic acid and are instructed to be taken daily (9). A continuous measure of overall multivitamin supplement intake across all trimesters (referred to as “multivitamin supplement intake index”) was developed using principal component analysis to derive a composite index of multivitamin supplement intake across the three trimesters. 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 assay is a more objective measurement of folate status in maternal blood (10). Plasma folate levels were measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd. China) (11) using a Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) (11). Plasma folate levels were assessed as i) a continuous variable in nmol/L; ii) a transformed

continuous variable for each interquartile unit in nmol/L; iii) quartiles of plasma folate levels; and iv) 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)).(12).

Preeclampsia was defined as hypertension of new-onset during pregnancy and proteinuria ≥300 mg protein in 24 h. Gestational hypertension was defined as new onset hypertension during pregnancy without proteinuria. Eclampsia was the occurrence of seizures in a woman with preeclampsia that could not be attributed to other causes. The constellation of hemolysis, elevated liver enzymes and low platelets developing during pregnancy defined HELLP syndrome [32]. Data sources include abstracted medical records for all study participants. In the data analyses, the term preeclampsia includes all four conditions: preeclampsia, gestational hypertension, eclampsia, and HELLP syndrome.

Intrauterine infection/inflammation (IUI) is defined as presence of maternal intrapartum fever as documented in medical records and/or placental pathology evidence of IUI: specifically, the presence of villitis, deciduitis, chorioamnionitis, chorionitis, subchorionitis, funisitis, and free membranitis from placental histology (13).

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), housing assistance or fuel assistance (yes versus no) and maternal nativity (US born versus non-

US born). Behavioral risk factors included alcohol use (never versus any), smoking status (never used, ever used, used in pregnancy) and stress (mother's report of life or pregnancy as being 'very' stressful). The above variables were based on maternal questionnaire interview.

Other maternal factors from medical records include maternal diabetes (presence of either gestational or pre-gestational diabetes) and prepregnancy BMI grouped into 4 categories: normal weight (18.5-24.9kg/m^2), underweight (<18.5kg/m^2), overweight (25-29.9kg/m^2), and obesity (>30kg/m^2).

The table below highlights the variables included in the analytical models.

Table 3-2: Description of Variables Included in Analysis

Variables of interest	Data source	Definition	Value labels
Key variables			
Preterm birth	Medical Records	Gestational age derived from LMP and early ultrasound <20 weeks	Binary: <37 weeks yes or no (reference).
Plasma folate levels	Plasma folate assay	Plasma folate levels nmol/L	Continuous: unit increase, interquartile unit increase Categorical: into quartiles, per WHO categorization (Folate insufficiency, normal (reference) and excess levels)
Folic acid supplement intake	Postpartum questionnaire	Intake of supplement in pre-pregnancy, 1st, 2nd and 3rd trimester	Categorical: by time periods and by frequency of use
Preeclampsia-eclampsia disorders	Abstracted Medical Records	gestational hypertension, preeclampsia, eclampsia, HELLP syndrome.	Binary: yes or no (reference)
Intrauterine infection/inflammation	Abstracted Medical Records Placenta pathology	Presence of maternal intrapartum fever, villitis, deciduitis, chorioamnionitis,	Binary: yes or no (reference)

Variables of interest	Data source	Definition	Value labels
		chorionitis, subchorionitis, funisitis, free membranitis	
Other covariates of interest			
Other medical conditions			
Maternal diabetes	Medical Records	Presence of pregestational or gestational diabetes	Categorical: none, pregestational diabetes and gestational diabetes
Prepregnancy Obesity	Postpartum questionnaire	BMI (pre-pregnancy weight in kg/height in meters squared) greater than 30kg/m^2	Categorical: underweight, normal, overweight/obese
Demographic factors			
Maternal age at delivery	Postpartum questionnaire	Maternal age in years	Categorical: <20, 20-34, 35+ years)
Race/ethnicity	Postpartum questionnaire	Self-reported race/ ethnicity	Categorical: non-Hispanic Black, non-Hispanic White, Hispanic and Other
Marital status	Postpartum questionnaire	Marital status at delivery	Binary: Unmarried or married (reference)
Education	Postpartum questionnaire	Highest grade of school completed	Categorical: ≤elementary, high school or ≥college
Public assistance	Postpartum questionnaire	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	Binary: Yes, versus no
Parity	Postpartum questionnaire	(# of live births NOT INCLUDING index case	Binary: multiparous (≥1) or primiparous
Behavioral risk factors			
Tobacco use in pregnancy	Postpartum questionnaire	Use of cigarettes, cigars, tobacco or snuff 6 months pre-pregnancy and by trimester	Categorical: never used, ever used, used in pregnancy

Variables of interest	Data source	Definition	Value labels
Alcohol use in pregnancy	Postpartum questionnaire	Alcohol consumption 6 months pre-pregnancy and by trimester	Categorical: never used, ever used, used in pregnancy
Stress	Postpartum questionnaire	Self-reported categorization of the amount of stress in life or pregnancy as being 'very', 'average' or 'not' stressful.	Categorical: no stress (reference), stress in pregnancy/life

Analytical Methods

Data Preparation: All analyses were conducted using STATA version 14. Preliminary data analysis was performed in the total sample and plasma folate subsample. Exploratory data analysis included descriptive statistics of all variables included in the analytic model - frequencies, means, standard deviations as well as histograms, box- and scatter- plots were used to identify invalid responses, outliers, and missing values. Chi-squared tests for categorical variables and t-tests for continuous variables were used to compare maternal characteristics by PTB status.

Aim 1: Evaluate the relationship between maternal folate status (using complementary measures and assessed from preconception to delivery) and risk of PTB.

Hypothesis:

- Self-reported frequency of multivitamin supplement intake during the preconception (6-month prior to preconception) period is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)
- Self-reported frequency of multivitamin supplement intake during pregnancy (first, second and third trimester) is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)
- Maternal plasma folate levels at delivery (a proxy of 3rd trimester folate status) is not associated with PTB (including PTB subtypes- spontaneous and medically indicated PTB)

Principal component analysis was used to create a "multivitamin supplement intake index" during 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 (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. Additional 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.

An example of the multivariate logistic regression model (full model) used to explore the relationship between PTB and multivitamin supplement intake is presented as

$$\log_e\left(\frac{\Pr(preterm\ birth = yes)}{\Pr(preterm\ birth = no)}\right) = \beta_0 + \beta_1 . X_{preconception\ folate\ intake} + \beta_2 . X_2 + \cdots + \beta_{15} . X_{15}$$

In the equation above, other covariates include maternal race, age, nativity, education, marital status, receipt of public assistance, parity, cigarette use, alcohol use, stress, body mass index (BMI), preeclampsia, IUI and diabetes mellitus.

Similar logistic regressions were conducted using maternal plasma folate levels as i) a continuous variable in nmol/L; ii) a transformed continuous variable for each interquartile unit in nmol/L; iii) quartiles of plasma folate levels; and iv) 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)). An example of the multivariate logistic regression model used to explore the relationship between PTB and plasma folate concentration at delivery is presented as

$$\log_e\left(\frac{\Pr(\,preterm\ birth = yes)}{\Pr(\,preterm\ birth = no)}\right) = \beta_0 + \beta_1 . X_{plasma\ folate\ concentration} + \beta_2 . X_2 + \cdots + \beta_{15} . X_{15}$$

Aim 2: Investigate biologic plausibility of the folate-PTB association by evaluating the role of folate on major pathogenic pathways leading to PTB (including PTB subtypes- spontaneous and medically indicated PTB).

Hypotheses 2a and 2c:

- Maternal folate status (assessed by self-report and biomarker) does not moderate the relationship between preeclampsia in pregnancy and PTB (including overall and medically indicated PTB).
- Maternal folate status (assessed by self-report and biomarker) does not moderate the relationship between IUI and PTB (including overall and spontaneous PTB).

Potential interaction of preeclampsia or IUI on the relationship between folate status and PTB can include either additive or multiplicative effects. Additive effects can be described as the extent to which the effect of two factors on an outcome together exceeds the effect of each considered individually. On the other hand, the multiplicative effects measure the extent to which the effect of both exposures together exceeds the **product** of the effects of the two exposures considered separately. Thus, in a logistic model, additive effects would be equal to OR_{11} - OR_{10} - OR_{01} +1 while the multiplicative effects would be OR_{11}/ OR_{10} x OR_{01} where OR_{11} would be interpreted as the effect of both factors together and the expressions OR_{10} and OR_{01} would be the effects of the first factor and the second factor, respectively. In a hypothetical model:

$$logit\ 1\{P(D = 1|\ G = g, E = e, C = c)\} = \gamma_0 + \gamma_1 g + \gamma_2 e + \gamma_3 eg + \gamma_4 c$$

The main effects, γ_1 and γ_2, when exponentiated, simply give the odds ratios for each of the two exposures. The coefficient γ_3, when exponentiated, gives the measure of multiplicative interaction for odds ratios while the additive effects would be equal to:

$$e^{\gamma_1+\gamma_2+\gamma_3} - e^{\gamma_1} - e^{\gamma_2} + 1.$$

Thus, the use of logistic regressions in data analysis will help to explore both additive and multiplicative effects of preeclampsia and IUI on the relationship between folate status and PTB.

Hypotheses 2b and 2d:

- Preeclampsia does not mediate the relationship between maternal folate status and PTB (including overall and medically indicated PTB).
- IUI does not mediate the relationship between maternal folate status and PTB (including overall and spontaneous PTB).

To explore the potential mediating influence of preeclampsia or IUI on the relationship between plasma folate levels and PTB, mediation analysis was done using the Baron and Kenny approach (14) with a series of logistic regressions. According to Baron and Kenny (15), mediation is demonstrated when the following conditions are met: (a) the main independent variable is significantly associated with the main dependent variable; (b) the independent variable is significantly related to the mediator variable; and [3] the mediator variable is significantly associated with the dependent variable when the independent variable is controlled for. Thus, the total effect is the sum of the direct effect between the independent and dependent variable and indirect effect that occurs via the mediator variable. This can be represented as:

$$\boldsymbol{c = c' + ab}$$

Where c is the total effect, *c'* is the direct effect and *ab* is the indirect effect which is the product of the two path coefficients from the independent to the mediator and from the mediator to the dependent variable. Following the Baron and Kenny analysis strategy to explore preeclampsia mediating the relationship between folate status and PTB, the mediation analysis first identified the relationship between folate status (causal variable) and PTB (dependent variable). Next, the relationship between folate status and preeclampsia (mediating variable), and the relationship of preeclampsia with PTB were explored. Finally, the association between folate status and PTB controlling for preeclampsia was conducted.

In Stata, the *binary_mediation* command was used to perform the mediation analysis because the mediating variable- preeclampsia disorder- was dichotomous. In addition, the *bootstrap* command was used to obtain standard errors and confidence intervals of direct and indirect effects while the *medeff* command was used to provide standard errors and confidence intervals for the mediation effect.(15). All analyses were conducted using software STATA version 14. All P-values in the analyses were two-sided and Type I errors were set at 0.05.

Sample size/power calculation

The sample size and power calculation for the proposed research was influenced by the recommendations of Hsieh (16, 17) who suggested the following: 1) The use of a simplified sample size formulae for comparing proportions in order to calculate the required sample size for a simple logistic regression model and 2) Subsequently, one can then adjust the required sample size for a multiple logistic regression model by a variance inflation factor that takes into account the multiple correlation coefficient of covariates included in the model. This method requires no assumption of low response probability in the logistic model.

The sample size and power calculation for this dissertation was calculated for interactions between folate status and preeclampsia (Aim 2c) presented below. As this aim uses a sub-group of the sample with plasma folate assays (N=2313), it is chosen as an example to demonstrate power and sample sizes.

First, the sample size was calculated for univariate logistic regressions (17) having an overall PTB proportion (P=0.31, from the plasma folate data) and an odds ratio of PTB due to folate insufficiency ranging from 1.1-1.3 with an alpha of 0.05 (one tailed) and power ranging from 0.7-0.9. The resulting sample size was then adjusted for multivariate logistic regressions by dividing the sample size by a variance inflation factor of $1-\rho^2$ where ρ is the multiple correlation coefficient relating folate to the other confounding variables included in the analysis ($1-\rho^2$ is estimated at 0.9811 from the data).

Table 3.3: Sample size and power calculations

Alpha (α)	**Proportion of PTB (P)**	**Power**	**OR of preterm and folate**	**Variance inflation factor $(1-\rho^2)^{-1}$**	**Final sample size**
0.05	0.31	0.9	1.1	0.9811	5139
		0.8	1.1		3711
		0.9	1.2		1413
		0.8	1.2		1022
		0.9	1.3		689
		0.8	1.3		499
Abbreviations: OR: odds ratio; PTB: preterm birth; ρ: multiple correlation coefficient Note: All sample size figures are rounded to the nearest whole number.					

Ethical Considerations

This dissertation conducted secondary data analysis using BBC study data. The BBC study protocol was approved by the institutional review boards of Boston University Medical Center,

the Ann & Robert H. Lurie Children's Hospital of Chicago (formerly Children's Memorial Hospital of Chicago), and the Johns Hopkins Bloomberg School of Public Health (JHSPH). All research conformed to data procurement, management and analysis procedures set forth by the JHSPH IRB. Furthermore, I completed all the necessary training of ethics of health research and have been approved as a student investigator to use the BBC data for my dissertation research by JHSPH IRB.

The main potential risk involved in this study is the breach of confidentiality of Health Information Portability and Accountability Act (HIPAA) protected patient information for study participants. All data used in this study did not contain personal identifiers and was stored on encrypted secure network servers.

Table 3-4: Study Timeline

	ACTIVITY	2017								2018		
		04	05	06	07	08	09	10	12	01	02	03
	Department and School-wide exams											
Data analysis	**Data cleaning, variable creation and imputation**											
	Dataset merging											
	Aim 1 analyses											
	Aim 2 a-b analyses											
	Aim 2 c-d analyses											
	Dissertation preparation and manuscript development											
	Final Defense											

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Chapter Four: Manuscript I

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

This paper is under review by American Journal of Clinical Nutrition (AJCN)

Abstract

Background: 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.

Objective: To explore the relationship between maternal folate status and PTB and its subtypes– spontaneous and medically indicated PTB.

Design: This analysis included 7,675 mother-infant dyads enrolled in the Boston Birth Cohort, an observational study of a predominantly urban, low income, race-ethnic minority population at a high-risk for PTB. We examined complementary measures of folate status: the frequency of multivitamin supplement intake prior to and during pregnancy and maternal plasma folate concentration at delivery. A subsample (n=2,313) of these dyads had maternal plasma folate assays.

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: 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 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). Some US studies have found an association between lower folate status and PTB, (13-17) while other studies found no association. (18, 19). 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 PTB 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 (20). 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 ongoing Boston Birth Cohort (BBC) study which commenced in 1998 (21, 22). The BBC is registered at https://clinicaltrials.gov/ct2/show/NCT03228875.

To date, over 8,500 mother-infant dyads have been enrolled in the study. 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. 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 2,500 g 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 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 (22).

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 400 or 800 micrograms of folic acid and are to be taken daily (23). 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) (24). 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)) (25).

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 (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 (IUI, defined as presence of maternal fever or placenta pathology findings of villitis, deciduitis, chorioamnionitis, chorionitis, subchorionitis, funisitis, free membranitis) and prepregnancy BMI 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).

Ethics

The study protocol was approved by the Institutional Review Boards of Boston University Medical Center and the Johns Hopkins Bloomberg School of Public Health.

Statistical analysis

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, IUI, diabetes mellitus and be obese/overweight.

Within the plasma folate subsample, 31% of women experienced PTB. The mean plasma folate concentration at delivery was 32.3 nmol/L for PTB, significantly lower than 36.3 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, $p<0.001$).

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 2** 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 PTB, 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.

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 (13, 15). 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 (13). In another study, dietary folate intake ≤500μg was associated with an almost two times greater risk of preterm delivery (15).

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. While it is unclear why second trimester multivitamin supplement intake is not associated with PTB, 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 (26, 27). Studies show that women who stopped multivitamin or folic acid supplementation after the first trimester had lower concentrations of maternal serum and red blood cell folate concentrations (26-28). 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 wherein each 1 nmol/L increase in serum folate concentration at 28 weeks of gestation was also associated with reduced risk of PTB (13) and serum folate concentration less than 36.9 nmol/L in the second trimester led to a nearly twofold increased risk of PTB (15). 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 (29). Our research on a predominantly minority population is also appropriate given that PTB rates was lowest among non-Hispanic Asian births (8.6%) and highest among non-Hispanic black births (13.8%) (29).

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 (30). 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 (31). Other mechanistic pathways that may explain the folate – PTB relationship include hyperhomocysteinemia, placental implantation and IUI (32, 33). 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 (32). Folate deficiency is also associated with abnormal inflammatory responses, which could conceivably trigger premature parturition in the context of IUI (34, 35).

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 (17), its relationship with medically indicated PTB has only been demonstrated in animal studies (36). 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 (37).

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 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. Plasma folate concentration at delivery can only be used as a proxy for third trimester folate concentration as it reflects short term folate status within the past few days (38). In addition, multivitamin supplement intake was based on self-report, which is subject to recall bias. 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 (39) as unobserved or uncontrolled confounding remains a threat to validity. 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 prospective longitudinal studies.

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 (40). 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 (41, 42).

Lastly, our study reaffirms the importance of consistent folate intake throughout pregnancy to mitigate PTB risk (9, 26, 43). 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 folic acid 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 (44, 45). Furthermore, our recent study (20) along with that of others (46)

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.

Tables and figures

Table 3-1: 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[1]	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 [2]								
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
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[3]								
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 [4]								
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		93.3						
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
First trimester								
None	914	16.6	402	19.4	248	15.6	137	19.0
1-2x a week	204	3.7	97	4.7	60	3.8	28	3.9
3-5x a week	1,482	26.9	539	26.1	514	32.3	232	32.2

>5x a week	2,907	52.8	1,031	49.8	771	48.4	323	44.9
Second trimester								
None	642	11.7	290	14	183	11.5	96	13.3
1-2x a week	220	4.0	108	5.2	67	4.2	33	4.6
3-5x a week	1,558	28.3	570	27.5	518	32.5	242	33.6
>5x a week	3,087	56.1	1,101	53.2	825	51.8	349	48.5
Third trimester								
None	655	11.9	345	16.7	193	12.1	126	17.5
1-2x a week	255	4.6	99	4.8	75	4.7	32	4.4
3-5x a week	1,558	28.3	543	26.2	512	32.1	227	31.5
>5x a week	3,039	55.2	1,082	52.3	813	51.0	335	46.5
Pregnancy (1st to 3rd trimester)								
None	436	7.9	219	10.6	122	7.7	78	10.8
1-2x a week	933	16.9	406	19.6	267	16.8	134	18.6
3-5x a week	1,373	24.9	476	23	471	29.6	200	27.8
>5x a week	2,765	50.2	968	46.8	733	46.0	308	42.8
Plasma folate concentration(nmol/L)								
Mean (SD)	n/a		n/a		36.3	24.7	32.3	20.0
WHO classification								
Insufficiency/deficiency: <13.5 nmol/L	n/a		n/a		155	9.7	82	11.4
Normal: 13.5-45.3	n/a		n/a		1,030	64.7	508	70.6
Excess: ≥45.3	n/a		n/a		408	25.6	130	18.1

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

[2] 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.

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

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

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

Table 4-2: Relationship Between Multivitamin Supplement Intake and Preterm Birth (N=7,576)

Period	Preterm (N=2,609)		Unadjusted		Adjusted OR[1]	
	N of cases	(%)	OR	95% CI	OR	95% CI
Multivitamin supplement intake in pregnancy (1st to 3rd trimester)						
Continuous (index) [2]	n/a	n/a	0.97	0.96, 0.98	0.98	0.97, 0.99
Categorical						
None (reference)	219	33.4	1.00 (reference)	n/a	1.00 (reference)	n/a
1-2x a week	406	30.3	0.87	0.71, 1.06	0.91	0.74, 1.12
3-5x a week	476	25.7	0.69	0.57, 0.84	0.78	0.64, 0.96
>5x a week	968	25.9	0.70	0.58, 0.83	0.77	0.64, 0.93
Multivitamin supplement intake in specific time periods						
Preconception						
None (reference)	1,940	27.4	1.00 (reference)	n/a	1.00 (reference)	n/a
Any	129	25.9	0.92	0.75, 1.14	0.90	0.72, 1.12
First trimester						
None (reference)	402	30.6	1.00 (reference)	n/a	1.00 (reference)	n/a
1-2x a week	97	32.3	1.08	0.83, 1.41	1.08	0.82, 1.43
3-5x a week	539	26.7	0.83	0.71, 0.96	0.90	0.76, 1.06
>5x a week	1,031	26.2	0.81	0.70, 0.92	0.85	0.73, 0.98
Second trimester						
None (reference)	290	31.1	1.00 (reference)	n/a	1.00 (reference)	n/a
1-2x a week	108	32.9	1.09	0.83, 1.42	1.19	0.90, 1.57
3-5x a week	570	26.8	0.81	0.68, 0.96	0.92	0.77, 1.10
>5x a week	1,101	26.3	0.79	0.68, 0.92	0.86	0.73, 1.02
Third trimester						
None (reference)	345	34.5	1.00 (reference)	n/a	1.00 (reference)	n/a
1-2x a week	99	28.0	0.74	0.56, 0.96	0.80	0.61, 1.06
3-5x a week	543	25.8	0.66	0.56, 0.78	0.75	0.63, 0.90
>5x a week	1,082	26.3	0.68	0.58, 0.78	0.75	0.64, 0.87

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

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

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

Table 4-3: Relationship between maternal plasma folate levels and preterm birth (n=2313)

Plasma folate sample	Preterm (n=720)		Unadjusted		Adjusted OR[1]	
	N of cases	%	OR	95% CI	OR	95% CI
Continuous plasma folate concentration(nmol/L)						
Each unit increase	n/a		0.991	0.986, 0.995	0.994	0.990, 0.999
Each interquartile increase in folate level	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 (reference)	201	34.7	1.00 (reference)	n/a	1.00 (reference)	n/a
Second quartile: 19.4-30.0	192	33.2	0.94	0.73, 1.19	1.02	0.79, 1.33
Third quartile:30.0-43.8	185	32.0	0.89	0.69, 1.13	1.09	0.83, 1.42
Highest quartile: 43.8-185.5	142	24.5	0.61	0.47, 0.79	0.74	0.56, 0.97
WHO classification (nmol/L)						
Insufficiency/deficiency: <13.5	82	34.6	1.07	0.80,1.43	0.86	0.63, 1.18
Normal: 13.5-45.3 (reference)	508	33.0	1.00 (reference)	n/a	1.00 (reference)	1.00 (reference)
Excess: ≥45.3	130	24.2	0.65	0.52, 0.81	0.70	0.55, 0.89

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

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

Table 4-4: Plasma folate levels and unadjusted and adjusted odds of PTB subtypes

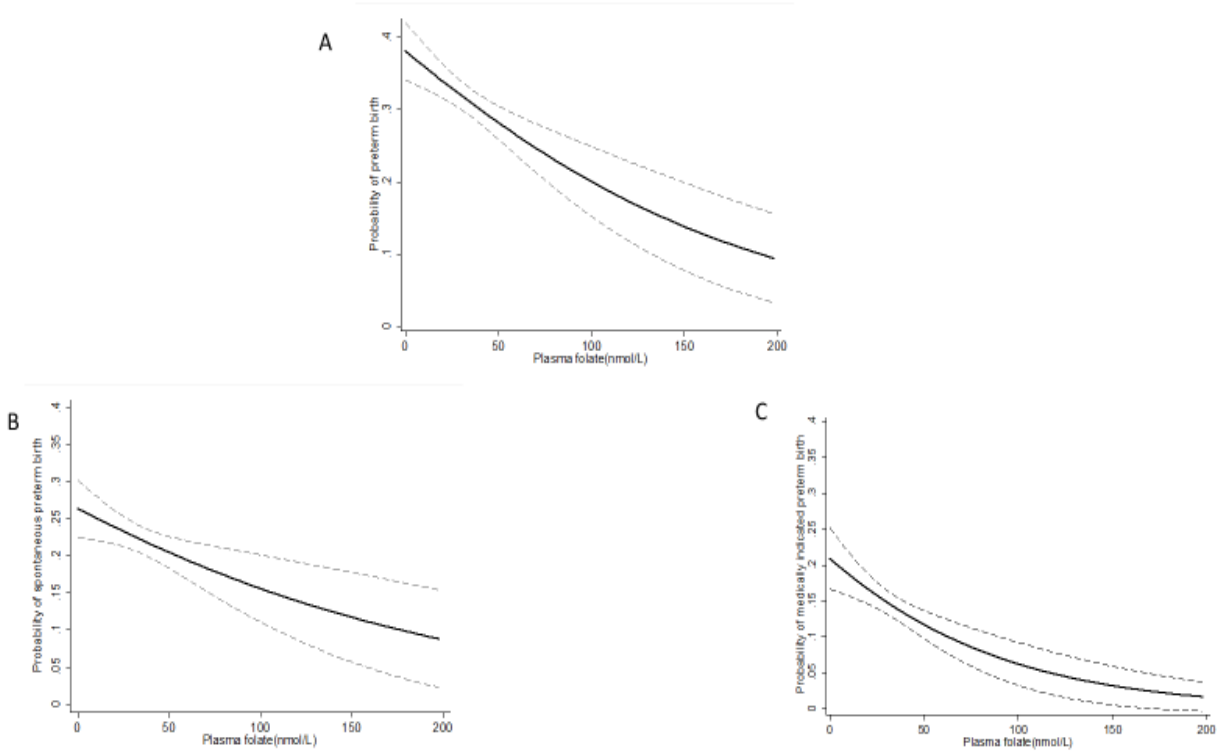
Plasma folate levels	Spontaneous PTB				Medically indicated PTB			
	N of cases	(%)	aOR [1]	95% CI	N of cases	(%)	aOR [2]	95% CI
Continuous plasma folate concentration(nmol/L)								
Each unit increase	n/a	n/a	0.995	0.990, 1.000	n/a	n/a	0.99	0.98, 0.99
Each interquartile increase in folate level	n/a	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

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

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

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

Figure 4-1: Probability of Overall (A), Spontaneous (B) and Medically Indicated (C) Preterm Birth by Plasma Folate Level



Supplemental Table 4-1: Maternal characteristics by overall PTB and PTB subtypes

Maternal Characteristics	Term (N=5507)	PTB (N=2069)	Spontaneous PTB (N=1353)	Medically indicated PTB (N=716)
	%	%	%	%
Maternal race/ethnicity				
Non-Hispanic Black[1]	71.8	28.2	17.6	10.6
Non-Hispanic White	70.1	29.9	21.3	8.6
Hispanic	75.1	24.9	16.9	8.0
Other	73.5	26.5	18.2	8.3
Missing	73.3	26.7	13.3	13.3
Age in years				
<20	75.7	24.3	18.3	6.0
20-34	73.7	26.3	17.7	8.6
35+	66.2	33.8	18.6	15.2
Missing	73.3	26.7	13.3	13.3
Nativity (US born)				
Not born in US	75.3	24.7	15.6	9.1
Born in US	68.5	31.5	21.5	10.1
Missing	82.4	17.6	13.2	4.4
Education				
Less than high school	72.9	27.1	18.5	8.6
High School/GED	70.8	29.2	18.8	10.4
College or more	74.2	25.8	16.4	9.3
Missing	73.1	26.9	17.9	9.0
Marital Status				
Married	74.5	25.5	15.8	9.8
Unmarried	71.4	28.6	19.3	9.3
Missing	80.2	19.8	11.1	8.6
Receipt of public assistance [2]				
No	70.7	29.3	17.8	11.5
Yes	73.1	26.9	17.9	9.1
Parity				
Multiparous	69.1	30.9	20.5	10.3
Nulliparous	74.0	26.0	16.9	9.1
Missing	77.8	22.2	16.7	5.6
Cigarette smoking				
Never	74.4	25.6	16.2	9.4
Ever	68.2	31.8	21.1	10.7
Continuous in pregnancy	64.5	35.5	26.7	8.8

Missing	74.7	25.3	12.6	12.6
Alcohol consumption				
No	73.2	26.8	17.6	9.2
Yes	68.5	31.5	20.8	10.7
Missing	71.7	28.3	16.0	12.3
Stress [3]				
No	74.0	26.0	17.3	8.6
Yes	67.6	32.4	19.8	12.5
Missing	73.5	26.5	17.6	8.8
BMI				
Underweight	70.1	29.9	23.5	6.5
Overweight	74.4	25.6	17.9	7.8
Obese	70.9	29.1	17.8	11.3
Missing	75.0	25.0	15.0	10
Preeclampsia [4]				
No	76.1	23.9	19.3	4.6
Yes	48.6	51.4	7.7	43.7
Intrauterine infection/inflammation				
No	73.7	26.3	15.9	10.3
Yes	61.5	38.5	31.0	7.5
Missing	84.8	15.2	11.6	3.6
Diabetes Mellitus				
No	73.6	26.4	17.6	8.8
Gestational Diabetes Mellitus	64.0	36.0	22.1	14.0
Diabetes Mellitus	48.7	51.3	22.7	28.6
Missing	75.8	24.2	16.7	7.6
Folate intake in preconception				
None	72.6	27.4	17.9	9.5
Any	74.1	25.9	16.6	9.2
First trimester				
None (ref)	69.5	30.5	21.6	9.0
1-2x a week	67.8	32.2	21.3	11.0
3-5x a week	73.3	26.7	17.2	9.5
>5x a week	73.8	26.2	16.7	9.5
Second trimester				
None (ref)	68.9	31.1	21.7	9.4
1-2x a week	67.1	32.9	23.2	9.8
3-5x a week	73.2	26.8	17.4	9.4
>5x a week	73.7	26.3	16.8	9.5
Third trimester				
None (ref)	65.5	34.5	23.4	11.1

1-2x a week	72.0	28.0	19.8	8.2
3-5x a week	74.2	25.8	16.9	8.9
>5x a week	73.7	26.3	16.8	9.4
Folate intake in pregnancy				
None (ref)	66.6	33.4	22.7	10.7
1-2x a week	69.7	30.3	21.5	8.8
3-5x a week	74.3	25.7	16.5	9.2
>5x a week	74.1	25.9	16.4	9.6

[1] Non-Hispanic Black includes Black, African American, Haitian, Cape Verdian;

[2] Public assistance is defined as receipt of any of the following: food stamps, WIC, AFDC, fuel or housing assistance.

[3] Mother's report of index pregnancy or life being very stressful.

[4] Preeclampsia is defined as the presence of preeclampsia, gestational hypertension and hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome.

Abbreviations: BMI-body mass index; N-number; PTB-preterm birth

Supplemental Table 4-2: Relationship Between Multivitamin Supplement Intake and Preterm Birth among Non-Hispanic Blacks only (N=3,847)

Period	Preterm (N=1,803)		Unadjusted		Adjusted OR[1]	
	N of cases	(%)	OR	95% CI	OR	95% CI
Multivitamin supplement intake in pregnancy (1st to 3rd trimester)						
Continuous (index)[2]	n/a	n/a	0.97	0.95, 0.99	0.97	0.97, 0.99
Categorical						
None (reference)	125	34.8	1.00	n/a	1.00	n/a
1-2x a week	229	30.9	0.81	0.62, 1.05	0.86	0.65, 1.15
3-5x a week	242	27.5	0.71	0.55, 0.92	0.79	0.58, 1.04
>5x a week	487	26.4	0.67	0.53, 0.85	0.75	0.58, 0.97
Multivitamin supplement intake in specific time periods						
Preconception						
None (reference)	1,014	28.2	1.00	n/a	1.00	n/a
Any	69	27.9	0.99	0.74, 1.32	0.99	0.73, 1.34
First trimester						
None (reference)	224	30.4	1.00	n/a	1.00	n/a
1-2x a week	59	32.4	1.10	0.77, 1.55	1.12	0.77, 1.62
3-5x a week	279	28.4	0.91	0.74, 1.12	0.95	0.76, 1.19
>5x a week	521	26.8	0.84	0.69, 1.01	0.87	0.71, 1.06
Second trimester						
None (reference)	164	32.9	1.00	n/a	1.00	n/a
1-2x a week	66	32.4	0.97	0.69, 1.38	1.09	0.75, 1.57
3-5x a week	301	26.7	0.82	0.65, 1.03	0.91	0.71, 1.16
>5x a week	552	26.3	0.73	0.59, 0.90	0.80	0.64, 1.00
Third trimester						
None (reference)	202	36.9	1.00	n/a	1.00	n/a
1-2x a week	59	27.8	0.66	0.47, 0.93	0.73	0.51, 1.05
3-5x a week	282	27.4	0.64	0.52, 0.80	0.72	0.57, 0.91
>5x a week	540	26.2	0.61	0.50, 0.74	0.68	0.55, 0.84

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

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

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

Supplemental Table 4-3: Unadjusted and adjusted odds ratios estimated from logistic regressions showing relationship between multivitamin supplement intake and PTB subtypes

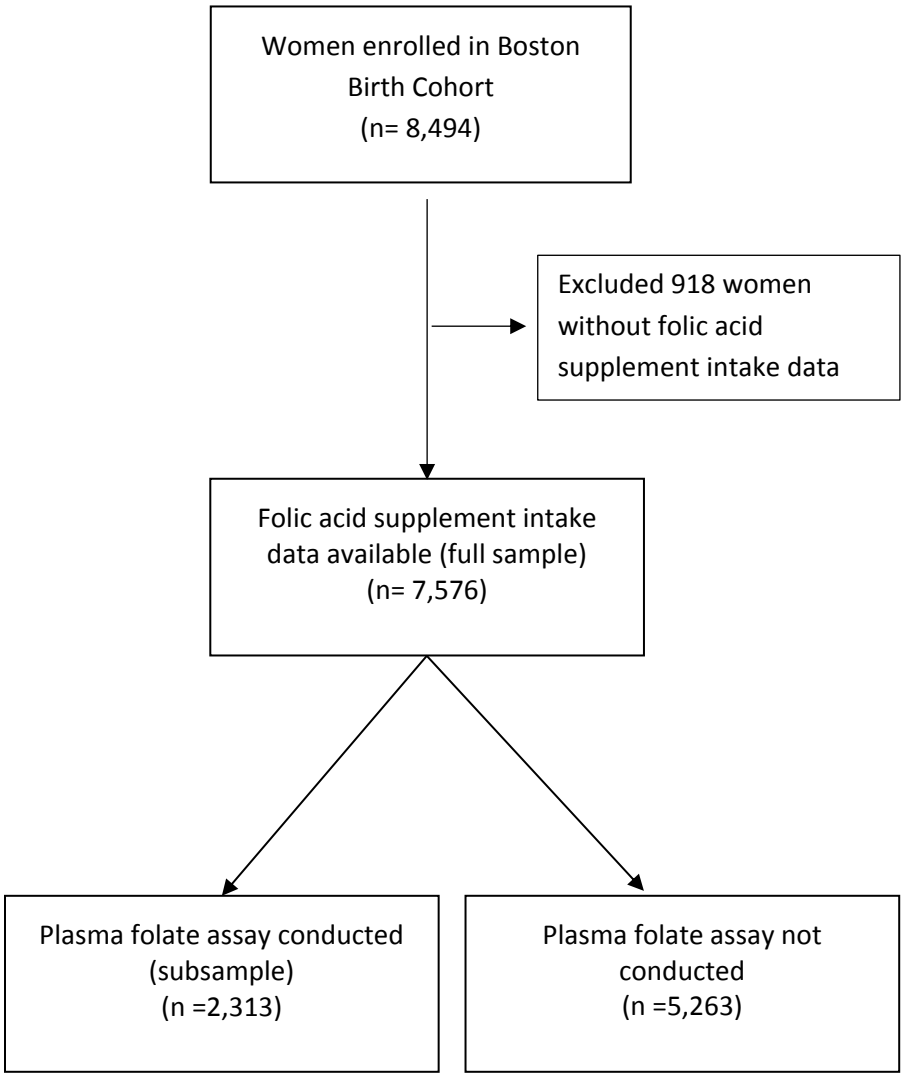
Multivitamin supplement intake sample	Spontaneous PTB				Medically indicated PTB			
	No of cases	(%)	aOR [1]	95% CI	No of cases	(%)	aOR [2]	95% CI
Preconception								
None (ref)	1,270	19.8	1.00	n/a	670	11.5	1.00	n/a
Any	83	18.3	0.96	0.75, 1.24	46	11.1	0.84	0.60, 1.17
First trimester								
None (ref)	284	23.7	1.00	n/a	118	11.4	1.00	n/a
1-2x a week	64	23.9	1.04	0.76, 1.43	33	13.9	1.29	0.85, 1.97
3-5x a week	348	19.0	0.82	0.68, 0.99	191	11.4	1.07	0.83, 1.38
>5x a week	657	18.4	0.78	0.66, 0.92	374	11.4	1.02	0.81, 1.28
Second trimester								
None (ref)	202	23.9	1.00	n/a	88	12.1	1.00	n/a
1-2x a week	76	25.7	1.11	0.81, 1.53	32	12.7	1.14	0.73, 1.77
3-5x a week	371	19.2	0.83	0.67, 1.01	199	11.3	1.00	0.76, 1.31
>5x a week	704	18.6	0.79	0.65, 0.95	397	11.4	0.96	0.74, 1.23
Third trimester								
None (ref)	234	26.3	1.00	n/a	111	14.5	1.00	n/a
1-2x a week	70	21.5	0.82	0.60, 1.12	29	10.2	0.69	0.45, 1.08
3-5x a week	355	18.6	0.71	0.58, 0.86	188	10.8	0.75	0.58, 0.97
>5x a week	694	18.6	0.71	0.59, 0.85	388	11.3	0.76	0.60, 0.96
Pregnancy (1st to 3rd trimester)								
None (ref)	149	25.5	1.00	n/a	70	13.8	1.00	n/a
1-2x a week	288	23.6	0.95	0.75, 1.20	118	11.2	0.83	0.60, 1.15
3-5x a week	305	18.2	0.73	0.58, 0.92	171	11.1	0.85	0.62, 1.15
>5x a week	611	18.1	0.72	0.58, 0.89	357	11.4	0.83	0.63, 1.11

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

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

Abbreviation: aOR-adjusted odds ratio; CI- confidence interval; n/a-not applicable; No-number; ref-reference; PTB: preterm birth

Supplemental Figure 4-1: Study Participants Flow Chart



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Chapter Five: Manuscript II

Inter-relationships of Folate, Preeclampsia, and Medically Indicated Preterm Birth: New Insight from the Boston Birth Cohort

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Abstract

Background: Previous studies suggest that adequate maternal folate status decreased the risk of preterm birth (PTB), but its role in preeclampsia and to what degree preeclampsia mediates the folate-PTB association are not well studied.

Objective: We aimed to explore the inter-relationships between maternal folate, preeclampsia, and PTB, in particular, the potential role of preeclampsia as a mediator of the folate-PTB relationship in a predominantly urban, low-income, minority population in the US.

Study Design: The analyses included 7,565 mother-infant dyads, enrolled in the Boston Birth Cohort study. Folate status was assessed by both maternal self-reported frequency of multivitamin supplement intake during preconception and each trimester of pregnancy, as well as maternal plasma folate concentrations in a subset (n=2,313) with plasma samples obtained 24-72 hours after delivery. Simple and multiple logistic regressions were used to explore the interrelationships between maternal folate, preeclampsia and PTB, including subtypes (spontaneous and medically indicated).

Results: Intake of multivitamin supplements containing folate, three or more times a week in the 3rd trimester was associated with reduced risk of preeclampsia (aOR=0.77, 95% CI: 0.65, 0.93). Similar results were obtained for multivitamin supplement intake in the 2nd trimester and among subgroups of non-Hispanic Black women as well as all nulliparous women. Each interquartile increase in plasma folate reduced the odds of preeclampsia by 20% (aOR=0.80, 95% CI: 0.68, 0.95). Compared to plasma folate concentrations in the first quartile, the highest quartile was associated with 38% reduced odds of preeclampsia (aOR=0.62, 95% CI: 0.43, 0.90). Preeclampsia mediated 10% of the multivitamin supplement intake-PTB relationship. Most of the mediation occurred in medically indicated PTB where preeclampsia mediated 62% of the

multivitamin supplement intake-PTB relationship. Preeclampsia did not mediate the relationship between folate status and spontaneous PTB.

Conclusion: In this high-risk urban, low income US population, adequate maternal folate appeared to protect against preeclampsia; such association was consistent across strata of parity and race/ethnicity. Preeclampsia mediated the beneficial effect of maternal folate against medically indicated PTB. Our findings have important clinical and public health implications that warrant additional investigation.

Key words: Folate, preeclampsia, preterm birth, multivitamin supplementation, Boston Birth Cohort

Introduction

Preeclampsia, a major cause of maternal morbidity and mortality, currently affects 3-6% of pregnancies in the US (1) and is more common among non-Hispanic Black women, in parallel with a high prevalence of chronic hypertension in this population (2, 3). Preeclampsia and associated complications or sequelae have enormous long-term health and societal consequences as well as economic implications. For example, the costs associated with preterm birth (PTB) from early-onset preeclampsia are approximately 40 to 100 times higher than for a term pregnancy (4), and both preeclampsia and PTB have short-and long-term health consequences on the child (5, 6).There is a critical need to identify safe and cost-effective ways to prevent preeclampsia and mitigate its consequences, including PTB.

Folate, an essential B vitamin, has received great attention in public health and clinical arena over the past two decades. It is the only nutrient that is under mandatory folic acid fortification of grain products in the U.S. and many countries in the world, aiming to prevent neural tube defects. However, both NHANES (a national representative sample) and our recent data showed individual folate status varied greatly from insufficiency to excess, likely due to differences in dietary intake, use of folic acid supplementation, and metabolic factors (7). To date, studies on maternal folate in relation to preeclampsia are limited and the findings are inconclusive. Some studies suggest that folate containing multivitamins or folic acid supplementation (8-11), as well as maternal serum and red blood cell folate levels (12), were associated with a lower risk of preeclampsia while other studies did not demonstrate any association (13-15). These inconsistent results across studies may be attributed to differences in study design, population characteristics, assessment of folate status, and adjustment for confounders. This topic is worth of further exploration because folic acid is attractive as a potential safe and inexpensive intervention, and

importantly, its biological plausibility and relevance to clinical and public health practice. Folate, in addition to its many other important biological functions for maternal and fetal health, improves endothelial function and plays an important role in improving cardiovascular health (16). Previous studies including our own found that adequate maternal folate status can reduce the risk of overall PTB (17-19).

This study sought to address several important research gaps regarding the inter-relationships of maternal folate status, preeclampsia, and PTB. First, the American College of Obstetricians and Gynecologists currently recommends that women with prior preeclampsia use folic acid supplementation in the preconception period and first trimester (20). Interestingly, despite this, there is lack of data on the role of adequate folate status during the 2nd and 3rd trimester, when most cases of preeclampsia develop. There is also limited understanding about the role of folate in preeclampsia among nulliparous mothers as compared to multiparous mothers.

Second, given that preeclampsia is a major cause of medically indicated PTB, it could be the mediator in the relationship between maternal folate and PTB. If true, it is expected that this mediation should occur primarily in medically indicated PTB. A few international studies explored the role of preeclampsia in the relation between folate and overall PTB in Hungary (21) and in India (22). In Hungary, there was a lower risk of PTB among pregnant women with early onset preeclampsia after folic acid supplementation in early pregnancy, while in India, folate levels were comparable among mothers with PTB from other causes and mothers with PTB due to preeclampsia. Thus, it is still unclear whether preeclampsia plays a role in mediating the association between maternal folate and PTB, especially medically indicated PTB, and how this reveals itself in US populations.

Third, the reasons for the racial/ethnic disparities in preeclampsia and medically indicated PTB are not fully understood. Recent NHANES data suggests that 25% of women of reproductive age in the US have insufficient folate levels (23) and that non-Hispanic Black women are more likely to have lower folate levels than non-Hispanic white women (24). As such, it is important to study the inter-relationships of folate status, preeclampsia and PTB (including medically indicated PTB) among non-Hispanic Black populations as they remain a high-risk group for these health conditions (2, 25, 26) for reasons that we need to understand.

The objective of this study was to investigate 1) the association between maternal folate status and preeclampsia in the Boston Birth Cohort, a large sample of a predominantly urban, low-income, non-Hispanic Black US population. We further explored if the association is similar in multiparous vs. nulliparous mothers; and to what degree the relationship between maternal folate and PTB is mediated by preeclampsia. We also explored whether the mediation is stronger in medically indicated compared to spontaneous PTB.

Materials and Methods

Study population

The data are from the ongoing Boston Birth Cohort (BBC) study which commenced in 1998 (27, 28). To date, over 8500 mother-infant dyads have been enrolled at delivery at the Boston Medical Center (BMC), a large urban hospital serving a predominantly low-income, minority, inner-city patient population. Eligible cases were defined as mother-infant dyads with singleton, live, LBW (<2500 grams) or preterm infants (<37 weeks of gestation) irrespective of birthweight; controls were defined as mother-infant dyads with singleton, live, term infants with birthweight 2500 g or more. 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 Health.

Data Collection

After obtaining informed consent, epidemiologic data was collected by trained research assistants using a face-to-face maternal questionnaire interview administered between 24-72 hours postpartum. Clinical data were collected from medical records with a standardized abstraction form. Maternal blood samples were obtained for plasma folate assay within a few days after delivery from a subset of the sample who planned to have their children followed at BMC for primary care and consented to enroll their children in a follow up study at BMC (5). Complete multivitamin supplement intake information from the preconception period (six months before conception) to the third trimester was available from 7576 women while a subsample (n=2313) of these women had plasma folate samples assayed. Participants included in the full sample and those who had plasma folate data had very similar baseline characteristics, except for a higher proportion of non-Hispanic Black women (73% versus 51%) and a slightly higher proportion of PTB (31% versus 27%) in the plasma subsample.

Key Variables

Preeclampsia disorders were assessed from the medical records of study participants and defined as the presence of a diagnosis of gestational hypertension, mild or severe preeclampsia, eclampsia, hemolysis, elevated liver enzymes, and/or low platelets (HELLP) syndrome in the patient chart.

PTB was defined as delivery before 37 completed weeks of gestation. Gestational age was determined from an algorithm based on the last menstrual period and early ultrasound dating (<20 weeks' gestation), as previously published (29).

Multivitamin supplement intake information was obtained from the following questions in the maternal questionnaire: “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 pregnancy), 1st trimester (day 1 - 90 of pregnancy), 2nd trimester (day 91 -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, almost daily. Responses to the two questions were combined for each trimester. Preconception multivitamin intake was dichotomized into “no intake” versus “any intake”. Intake in the 1st to 3rd trimester was dichotomized into consistent intake: “less than three” versus “three or more” times per week. In the US, prenatal and over-the-counter multivitamins usually contain 400 or 800 micrograms of folic acid and are suggested to be taken daily (30).

Plasma folate concentrations were measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd. China) (31) using a Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) (31).

Other covariates included sociodemographic factors such as: 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), maternal age at delivery (<20, 20-34 and 35+years), receipt of public assistance including: WIC (Women Infants and Children), Food Stamps, AFDC (Aid to Families with Dependent Children), Housing assistance or Fuel assistance (yes versus no) and maternal nativity (US born versus non-US born). Behavioral risk factors included alcohol use (never versus any), smoking status (never used, ever used, used in pregnancy) and stress (mother’s report of life or

pregnancy as being 'very' stressful). Biomedical factors included maternal diabetes defined as having either gestational or pregestational diabetes, prepregnancy BMI (kg/m^2 grouped into 4 categories: underweight ($<18.5kg/m^2$), normal weight ($18.5\text{-}24.9kg/m^2$), overweight ($25\text{-}29.9kg/m^2$), and obesity ($>30kg/m^2$), and chronic hypertension (defined as the presence of pre-pregnancy hypertension).

Statistical analysis

Preliminary data analysis was performed in the full sample and plasma folate subsample to compare maternal characteristics by preeclampsia status using chi-squared tests for categorical variables and t-tests for continuous variables. Plasma folate concentrations were assessed as a categorical variable in quartiles and as a continuous variable in nmol/L in two scales: i) unit nmol/L increase and ii) interquartile increase, to assess dose-response relationship. Simple and multiple logistic regressions were used to explore the relationship between self-reported multivitamin supplement intake and plasma folate concentrations on preeclampsia and PTB. Multiple regressions to explore the relationship between folate and preeclampsia controlled for the following maternal factors: race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, diabetes and chronic hypertension. Additional analyses were conducted for subgroups of PTB, spontaneous and medically indicated PTB. Mediation analysis was conducted using the Baron and Kenny approach (32, 33), where mediation is demonstrated when the following conditions are met: (a) the main independent variable is significantly associated with the main dependent variable; (b) the independent variable is significantly related to the mediator variable; and [3] the mediator variable is significantly associated with the dependent variable when the independent variable is controlled for. Thus, the total effect is the sum of the direct effect between the independent and dependent

variable and indirect effect that occurs via the mediator variable. This can be represented as: $\boldsymbol{c = c' + ab}$ *where c* is the total effect, *c'* is the direct effect and *ab* is the indirect effect which is the product of the two path coefficients from the independent to the mediator and from the mediator to the dependent variable, as displayed in Supplemental Figure 1.

The proportion of the total effect that is mediated is ***ab/c***. Our mediation analysis demonstrated the total and direct effect of folate status on PTB, the indirect effect and the proportion of the folate-PTB relationship mediated through preeclampsia. In Stata, the *binary_mediation* command was used to perform the mediation analysis because the mediating variable - preeclampsia disorder - was dichotomous (33). In addition, the *bootstrap* command was used to obtain standard errors and confidence intervals of direct and indirect effects while the *medeff* command was used to provide standard errors and confidence intervals for the proportion of the total effect mediated by preeclampsia (34). All analyses were conducted using STATA version 14 (College Station, TX: StataCorp LP). All p-values in the analyses were two-sided and Type I errors were set at 0.05.

Results

Preeclampsia was present in 13% percent of women in both the total multivitamin supplement group and plasma folate subsample. In the full sample, 27% had PTB compared to 31% in the plasma folate subsample. In the third trimester, 82% of women took multivitamin supplements three or more times a week. Plasma folate concentrations at delivery ranged from 6.63 to 185.5 nmol/L with a median of 30.5nmol/L. The distribution of the sample by key characteristics and preeclampsia status in the total multivitamin supplement sample as well as the plasma folate subsample is presented in Table 1. In the full sample, women with preeclampsia were more likely to be non-Hispanic Black, older, nulliparous, obese, diabetic and have chronic hypertension.

Table 2 displays the independent associations between multivitamin supplement intake and preeclampsia. Multivitamin supplement intake in the preconception period as well as first trimester was not significantly associated with preeclampsia. Supplement intake of three or more times a week in the 2nd and 3rd trimester was associated with reduced odds of preeclampsia (aOR=0.82, 95% CI: 0.68, 0.98 and aOR=0.77, 95% CI: 0.65, 0.93 respectively).

Plasma folate concentrations also demonstrated a similar association with preeclampsia. Specifically, each interquartile increase in plasma folate reduced the odds of preeclampsia (aOR=0.80, 95% CI: 0.68, 0.95). There were consistent associations between both measures of folate (multivitamin supplement intake and plasma folate concentrations) with preeclampsia among non-Hispanic Black women only (Supplemental Table 1). In addition, the association of multivitamin supplement intake with preeclampsia was stronger among multiparous compared to nulliparous women (Table 3). Specifically, among multipara, multivitamin supplement intake 3 or more times a week in the third trimester was associated with 29% reduced odds of PTB

(aOR=0.71, 95% CI: 0.56, 0.90). While the odds of PTB was also lower among nulliparous women, this was not statistically significant (aOR= 0.85, 95% CI: 0.64, 1.12).

Figure 1 shows the direct, indirect and total effects of multivitamin supplement intake on PTB as well as the proportion of the total effect mediated by preeclampsia. Multivitamin supplement intake of three or more times a week in the third trimester was associated with reduced odds of PTB (direct effect=aOR: 0.74, 95% CI: 0.65, 0.84). The indirect effect of multivitamin supplement intake leading to reduced odds of preeclampsia and subsequently lower odds of PTB accounted for 10% of the total effect (total effect=aOR: 0.72, 95% CI: 0.64, 0.82). Furthermore, preeclampsia mediated 62% of the relationship between multivitamin supplement intake and medically indicated PTB but none of the multivitamin supplement intake-spontaneous PTB relationship.

Results specific to plasma folate concentrations are presented in Supplemental Figure 2. Similarly, preeclampsia accounted for 28% of the plasma folate-PTB relationship, 45% of the plasma folate- medically indicated PTB but none of the plasma folate-spontaneous PTB relationship.

Discussion

To our knowledge, this is the first study to investigate the inter-relationship between maternal folate status, preeclampsia and PTB outcomes within a low-income urban minority population in the US. We used mediation analysis to investigate the indirect effect of folate on PTB and its subtypes via preeclampsia. This approach is gaining attention in prevention and treatment research, in which interventions are designed to change outcomes of interest by targeting mediating variables hypothesized to be causally related to the outcome (35). This is particularly relevant, given the need for interventions to address the unacceptably high rate of PTB and preeclampsia, especially among African American women (2, 3, 36).

This study is strengthened by the use of both conventional measures of folate status, maternal self-reported multivitamin supplement intake during preconception and in each trimester of pregnancy, and an objectively measured folate biomarker in maternal plasma samples obtained within a few days of delivery.

Our study demonstrated that consistent multivitamin supplement intake of three or more times a week in the 3rd trimester and higher plasma folate concentrations at delivery were associated with reduced odds of preeclampsia.

This work extends our recent report on the relationship between maternal folate status and PTB in our study population (37). Here, we added to current knowledge by investigating the mechanistic pathway of preeclampsia in the relationship between maternal folate status and PTB. Our study of the interrelations between maternal folate status, preeclampsia and PTB are comparable to the Bánhidy et al. population- based study in Hungary (21) where the unadjusted odds of PTB was reduced among women with preeclampsia who took folic acid supplements from early pregnancy. However, in that study, these associations were seen only among women

with early onset preeclampsia, not late onset preeclampsia. In contrast to our study findings, a case-control study conducted in India observed reduced folate levels in women with PTB only, not in women with both PTB and preeclampsia (22). It is noteworthy that these other studies had both a different race/ethnicity distribution and folic acid fortification context from our study, which was conducted in the US.

Our study also uniquely explored how maternal folate status and preeclampsia were associated with overall PTB and specific PTB subtypes. Preeclampsia mediated a tenth of the multivitamin supplement intake and PTB relationship and about a quarter of the plasma folate and PTB relationship.

Furthermore, we demonstrated that most of the mediation effect of preeclampsia was seen among those with medically indicated PTB and not spontaneous PTB, a trend that was consistent across both measures of maternal folate status. This is presumably because preeclampsia is a common reason for induction of labor.

Nutrition status has long been considered important in pregnancy outcomes for both the mother and fetus. While folate has been advocated for use in the periconception period to reduce neural tube defects as well as megaloblastic anemia during pregnancy, our findings suggest that folate may be beneficial in preventing preeclampsia and mitigating its associated sequelae, medically indicated PTB.

There are plausible mechanisms for the role of maternal folate status in preeclampsia and PTB. Suboptimal folate levels during gestation can impair cellular growth and replication in the placenta and abnormal placentation has been linked with hypertensive disorders (38). Also, higher folate levels can reduce the oxidative stress which causes the release of free radicals,

oxidized lipids, cytokines and serum soluble vascular endothelial growth factor. These substances are responsible for placental and endothelial dysfunction commonly seen in preeclampsia (39). In addition, lower levels of folate also interfere with maternal erythropoiesis, and growth of the uterus and mammary gland (40).

Low folate levels as well as preeclampsia may be caused by risk factors as well as biological processes that occur before or during pregnancy. Thus, preconception or prenatal interventions remain key in ameliorating the effects of these conditions (41-43), as it is hypothesized that the most critical time window to improve placental function is in the late first or early second trimester (44). Our data lent support that adequate folate status in 3rd trimester may be also important in preventing and mitigating preeclampsia and its consequences.

This study has some limitations. While folate biomarkers were assessed within 72 hours of delivery, although plasma folate concentrations are more objective and not as prone to the recall bias observed with dietary history of supplement intake, we recognize that a single plasma folate measurement cannot be used to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. However, in populations that have stable sources of folate intake, such as in the US where there is mandatory folic acid fortification, plasma folate concentrations are unlikely to fluctuate dramatically. We also acknowledge that plasma folate concentration at delivery is at best a proxy for third trimester folate status. In addition, our study design did not permit for inferences of temporality and causality. Finally, although we have adjusted major known confounders in the analyses, unmeasured or unknown confounding may have been an issue. Due to these limitations, we would like to stress that our findings serve as hypothesis generating rather than as conclusive. There is need for prospective studies to further validate our findings as well as demonstrate causality.

If confirmed, our study findings are of clinical and public health importance as folic acid fortification and supplementation remain relatively low cost, safe and feasible clinical and public health interventions for all women of childbearing age. Current American College of Obstetricians and Gynecologists (ACOG) guidelines recommend delivery after 34 weeks of gestation for severe preeclampsia (45) as well as folic acid supplementation in the preconception period and first trimester for women with prior preeclampsia (20). Our study found that the association between multivitamin supplement intake in the third trimester and preeclampsia was stronger among multipara compared to nulliparous women. If our study findings are validated by prospective studies, such guidelines might need to consider the administration of folic acid among women with preeclampsia beyond the first trimester.

The benefits of adequate folate against preeclampsia in nulliparous women remain to be determined. In fact, in our sample the prevalence of preeclampsia was higher in nulliparous than multiparous mothers. Our data suggest that there was a trend of protective effect of adequate folate against preeclampsia, but it was not statistically significant. Due to delayed childbearing and the rising rates of chronic medical conditions, the prevalence of hypertension and preeclampsia is projected to increase over the next decade (46, 47), including among nulliparous women. Administering folic acid in combination with anti-hypertensive drugs compared with anti-hypertensive drugs alone has been shown to reduce the risk of cardiovascular and stroke events in patients with hypertension(48). There is a great need to further investigate the extent to which folic acid administration can mitigate preeclampsia complications in pregnancy as well as reduce the risk of medically indicated PTB among nulliparous women.

In summary, in this study, we found that maternal multivitamin supplement intake of three times a week or more in the third trimester and higher plasma folate concentrations at delivery

protected against preeclampsia, which partially mediated the association between maternal folate levels and PTB, particularly medically indicated PTB. An understanding of these relationships in low income minority US populations is of great public health importance considering the higher prevalence of folate insufficiency, preeclampsia and PTB among such groups. Furthermore, improving folate status via folic acid supplementation or fortification remains a simple and cost-effective intervention, which may prove particularly useful in preventing both preeclampsia and its associated PTB. Our findings warrant additional investigation, preferably using a prospective study design, and especially in populations most at risk.

Tables and figures

Table 5-1: Characteristics of study population by preeclampsia status

Maternal Characteristics	Multivitamin supplement sample (N=7576)			Plasma folate sub sample (N=2313)		
	No preeclampsia	Preeclampsia	p	No preeclampsia	Preeclampsia	p
Total	**6629 (87)**	**947 (13)**		**2017 (87)**	**296 (13)**	
Race/ethnicity			<0.001			0.220
Non-Hispanic Black[a]	3308 (50)	539 (57)		1459 (72)	229 (77)	
Non-Hispanic White	825 (12)	95 (10)		84 (4)	8 (3)	
Hispanic	1911 (29)	239 (25)		392 (20)	46 (16)	
Other	552 (8)	62 (7)		84 (4)	13 (4)	
Missing	33 (1)	12 (1)		0 (0)	0 (0)	
Age in years			<0.001			0.001
<20	716 (11)	87 (9)		203 (10)	23 (8)	
20-34	4861 (73)	638 (67)		1468 (73)	197 (66)	
35+	1019 (15)	210 (22)		346 (17)	76 (26)	
Missing	33 (1)	12 (1)		0 (0)	0 (0)	
Nativity (US born)			0.218			0.343
Not born in US	3980 (60)	541 (57)		1184 (59)	163 (55)	
Born in US	2569 (39)	395 (42)		807 (40)	127 (43)	
Missing	80 (1)	11 (1)		26 (1)	6 (2)	
Education			0.034			<0.001
Less than high school	2047 (31)	256 (27)		580 (29)	58 (20)	
High School/GED	2197 (33)	344 (36)		714 (35)	140 (47)	
Some College and above	2321 (35)	333 (35)		714 (35)	98 (33)	
Missing	64 (1)	14 (2)		9 (1)	3 (0)	
Marital Status			0.770			0.801
Married	2391 (36)	345 (36)		683 (34)	106 (36)	
Unmarried	4099 (62)	579 (61)		1319 (65)	188 (63)	
Missing	139 (2)	23 (2)		15 (1)	2 (1)	
Receipt of public assistance [b]			0.015			0.276
No	1033 (16)	177 (19)		273 (14)	47(16)	
Yes	5596 (84)	770 (81)		1744 (86)	249 (84)	
Parity			0.001			0.734
Multiparous	3815 (58)	484 (51)		1198 (59)	160 (54)	
Nulliparous	2799 (42)	460 (49)		816 (41)	135 (46)	
Missing	15 (0)	3 (0)		3 (0)	1 (0)	

Cigarette smoking			<0.001			0.029
Never	5202 (79)	781 (82)		1632 (81)	234 (79)	
Ever	477 (7)	74 (8)		151 (7)	36 (12)	
Continued in pregnancy	871 (13)	76 (8)		220 (11)	24 (8)	
Missing	79 (1)	16 (2)		14 (1)	2 (1)	
Alcohol consumption			0.038			0.849
No	5827 (88)	813 (86)		1812 (90)	263 (89)	
Yes	601 (9)	91 (10)		165 (8)	26 (9)	
Missing	201 (3)	43 (4)		40 (2)	7 (2)	
Stress[c]			0.022			0.192
No	5235 (79)	713 (75)		1586 (79)	219 (74)	
Yes	1363 (21)	231 (24)		424 (21)	76 (26)	
Missing	31 (0)	3 (0)		7 (0)	1 (0)	
Body Mass Index categories			<0.001			<0.001
Underweight	304 (5)	20 (2)		84 (4)	5 (2)	
Normal	3051 (46)	302 (32)		883 (44)	87 (29)	
Overweight/obese	2791 (42)	549 (58)		944 (47)	187 (63)	
Missing	483 (7)	76 (8)		106 (5)	17 (6)	
Diabetes Mellitus			<0.001			<0.001
No	6184 (93)	828 (87)		1877 (93)	253 (85)	
Gestational Diabetes Mellitus	274 (4)	70 (7)		78 (4)	24 (8)	
Diabetes Mellitus	112 (2)	42 (4)		48 (2)	17 (6)	
Missing	59 (1)	7 (1)		14 (1)	2 (1)	
Chronic Hypertension			<0.001			<0.001
No	6366 (97)	764 (81)		1919 (96)	226 (77)	
Yes	224 (3)	176 (19)		88 (4)	69 (23)	
Preterm Birth			<0.001			<0.001
No	5047 (79)	460 (49)		1483 (74)	110 (37)	
Yes	1582 (24)	487 (51)		534 (26)	186 (63)	
Spontaneous	1280 (19)	73 (8)	<0.001	428 (21)	25 (8)	<0.001
Medically indicated	302 (5)	414 (44)	<0.001	106 (5)	161 (54)	<0.001

[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] Defined as preeclampsia, gestational hypertension and hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome

Table 5-2: Association of multivitamin supplement and plasma folate with preeclampsia

Multivitamin supplement intake	**Preeclampsia (n=947)**		
	n (%) cases	**OR (95% CI)**	**aOR[a] (95% CI)**
Preconception			
None (ref)	868 (12.3)	1.00	1.00
Any[b]	79 (14.7)	1.23 (0.96, 1.57)	1.14 (0.87, 1.49)
1st trimester			
<3x a week (ref)	207 (12.8)	1.00	1.00
≥3x a week	740 (12.4)	0.97 (0.82, 1.14)	0.88 (0.74, 1.05)
2nd trimester			
<3x a week (ref)	175 (13.9)	1.00	1.00
≥3x a week	772 (12.2)	0.86 (0.72, 1.03)	**0.82 (0.68, 0.98)**
3rd trimester			
<3x a week (ref)	198 (14.6)	1.00	1.00
≥3x a week	749 (12.0)	**0.80 (0.67, 0.95)**	**0.77 (0.65, 0.93)**
Plasma Folate (ng/ml)	**Preeclampsia (n=296)**		
Continuous plasma folate (nmol/L)			
Each unit nmol/l increase	296	**0.99 (0.98, 1.00)**	**0.99 (0.98, 1.00)**
Each interquartile increase	296	**0.77 (0.66, 0.90)**	**0.80 (0.68, 0.95)**
Quartiles of plasma folate (nmol/L)			
Lowest quartile (6.64-20.35; reference)	**93 (16.1)**	1.00	1.00
Second quartile (20.36- 30.52)	**79 (13.7)**	0.83 (0.60, 1.14)	0.77 (0.54, 1.09)
Third quartile (30.53- 44.02)	**65 (11.3)**	**0.66 (0.47, 0.93)**	**0.61 (0.43, 0.88)**
Highest quartile Q4 (44.03- 185.51)	**59 (10.2)**	**0.59 (0.42, 0.84)**	**0.62 (0.43, 0.90)**

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; n: number of cases; n/a: not applicable; OR: odds ratio; ref: reference; SD: standard deviation.

[a] Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, diabetes and chronic hypertension.

[b] A very low percentage of women took folic acid supplements during preconception.

Table 5-3: Association of multivitamin supplement with preeclampsia among multiparous versus nulliparous women.

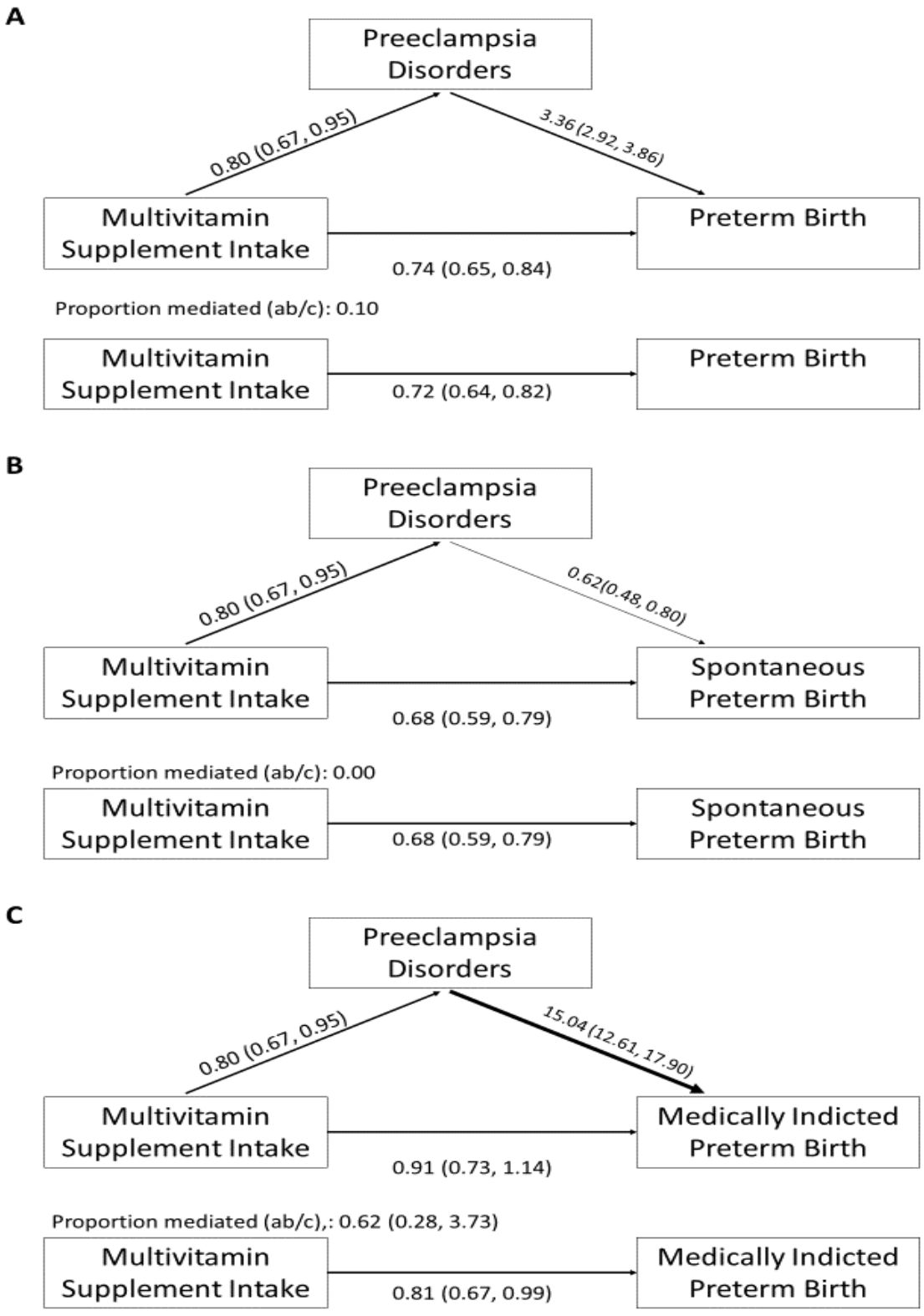
Multivitamin supplement intake	MULTIPAROUS (n=4,299)		NULLIPAROUS (n=3,259)	
	n (%) cases	aOR[a] (95% CI)	n (%) cases	aOR[a] (95% CI)
	Preeclampsia (n=484)		Preeclampsia (n=460)	
Preconception				
None (ref)	445 (11.2)	1.00	420 (13.8)	1.00
Any[b]	39 (12.6)	1.07 (0.74, 1.56)	40 (17.8)	1.25 (0.84, 1.84)
1st trimester				
<3x a week (ref)	121 (11.6)	1.00	86 (15.0)	1.00
≥3x a week	363 (11.2)	**0.85 (0.67, 1.07)**	374 (13.9)	0.90 (0.69, 1.18)
2nd trimester				
<3x a week (ref)	104 (12.7)	1.00	71 (16.2)	1.00
≥3x a week	380 (10.9)	0.79 (0.61, 1.00)	389 (13.8)	0.84 (0.63, 1.13)
3rd trimester				
<3x a week (ref)	120 (13.8)	1.00	78 (16.2)	1.00
≥3x a week	364 (10.6)	**0.71 (0.56, 0.90)**	382 (13.8)	0.85 (0.64, 1.12)

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; n: number of cases; OR: odds ratio; ref: reference

[a] Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, diabetes and chronic hypertension.

[b] A very low percentage of women took folic acid supplements during preconception.

Figure 5-1: Interrelationship between multivitamin supplement intake, preeclampsia and overall PTB (A), spontaneous PTB (B) and medically indicated PTB (C)



SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 5-1: Association of multivitamin supplement and plasma folate with preeclampsia among non-Hispanic Blacks only

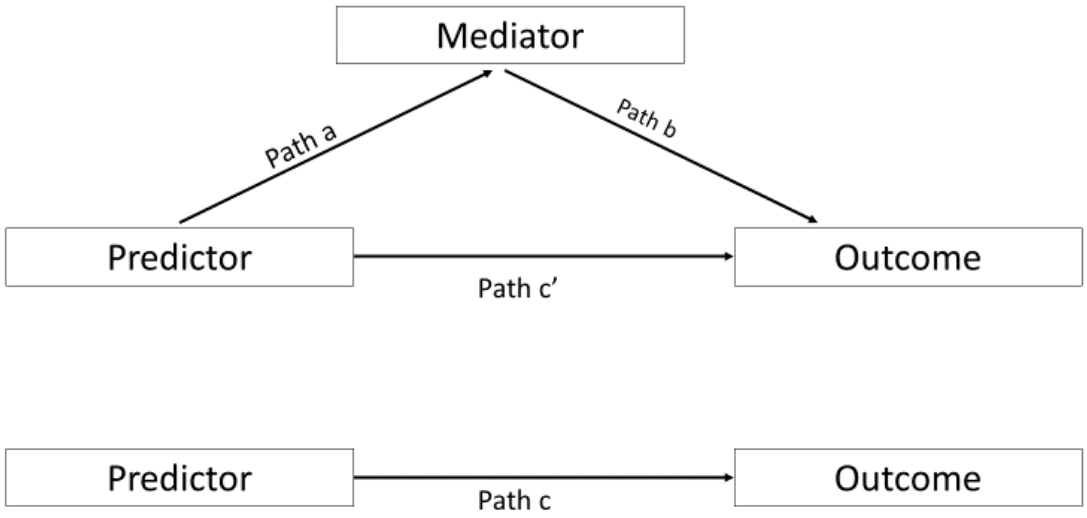
Multivitamin supplement intake	**n (%) cases**	**OR (95% CI)**	**aOR[a] (95% CI)**
	Preeclampsia (n=539)		
Preconception			
None (ref)	495 (13.8)	1.00	1.00
Any[b]	44 (16.7)	1.25 (0.89, 1.75)	1.28 (0.90, 1.84)
1st trimester			
<3x a week (ref)	134 (14.6)	1.00	1.00
≥3x a week	405 (13.8)	0.94 (0.76, 1.16)	0.82 (0.66, 1.03)
2nd trimester			
<3x a week (ref)	111 (15.8)	1.00	1.00
≥3x a week	428 (13.6)	0.84 (0.67, 1.05)	**0.75 (0.59, 0.96)**
3rd trimester			
<3x a week (ref)	128 (16.9)	1.00	1.00
≥3x a week	411 (13.3)	**0.76 (0.61, 0.94)**	**0.71 (0.56, 0.89)**
Plasma Folate (ng/ml)	**Preeclampsia (n=229)**		
Continuous plasma folate (nmol/L)			
Each unit nmol/L increase	229 (100)	**0.98 (0.98, 0.99)**	**0.99 (0.98, 0.99)**
Each interquartile increase	229 (100)	**0.70 (0.57, 0.84)**	**0.80 (0.68, 0.95)**
Quartiles of plasma folate (nmol/L)			
Lowest quartile (6.64-20.35; ref)	83 (17.9)	1.00	1.00
2nd quartile (20.36- 30.52)	59 (14.4)	**0.77 (0.54, 1.11)**	**0.65 (0.44, 0.97)**
3rd quartile (30.53- 44.02)	48 (11.9)	**0.62 (0.42, 0.91)**	**0.57 (0.38, 0.86)**
Highest quartile Q4 (44.03- 185.51)	39 (9.5)	**0.48 (0.32, 0.72)**	**0.49 (0.31, 0.75)**

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; n: number of cases; n/a: not applicable; OR: odds ratio; ref: reference; SD: standard deviation.

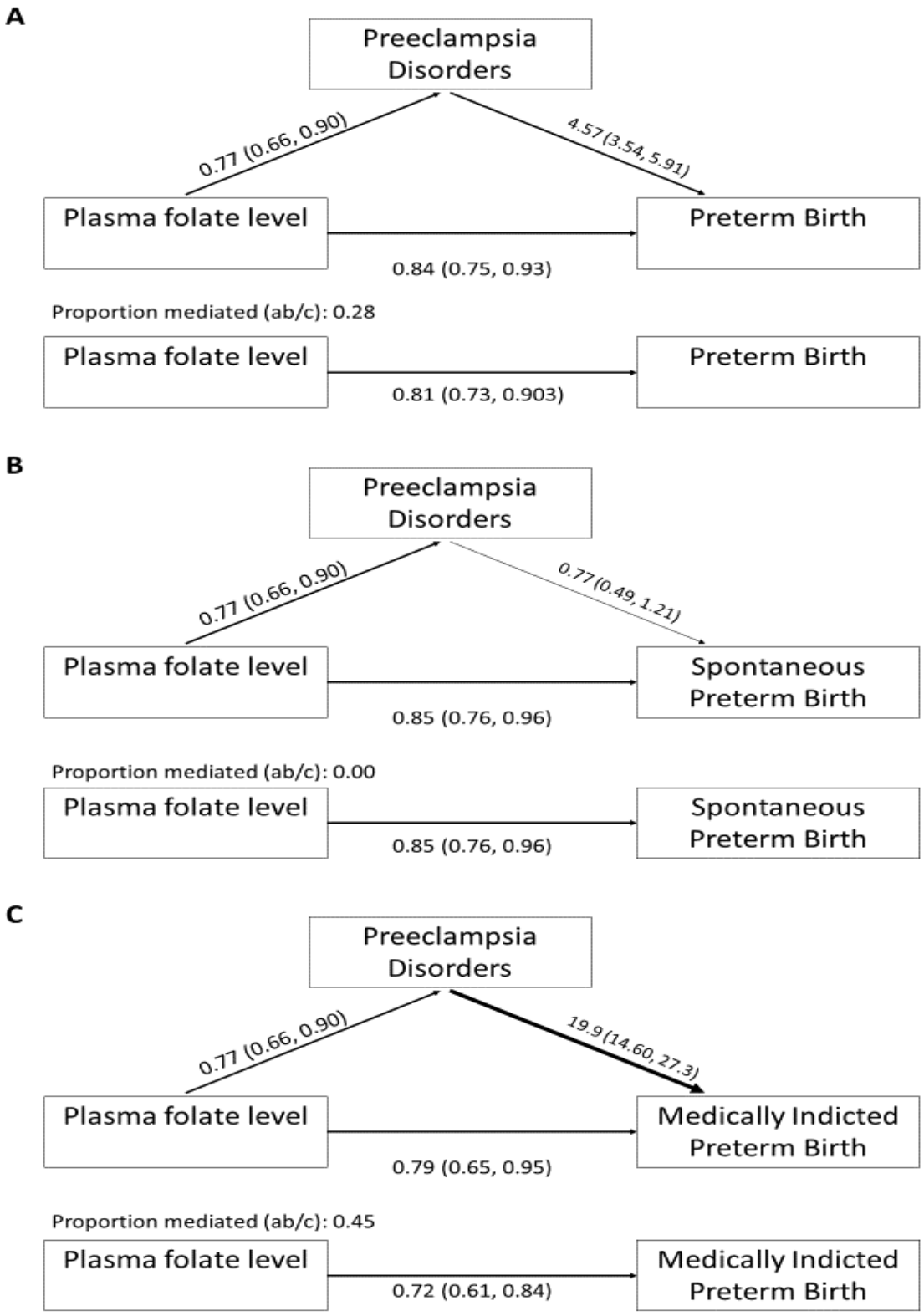
[a] Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use, stress, BMI, preeclampsia, diabetes and chronic hypertension.

[b] A very low percentage of women took folic acid supplements during preconception.

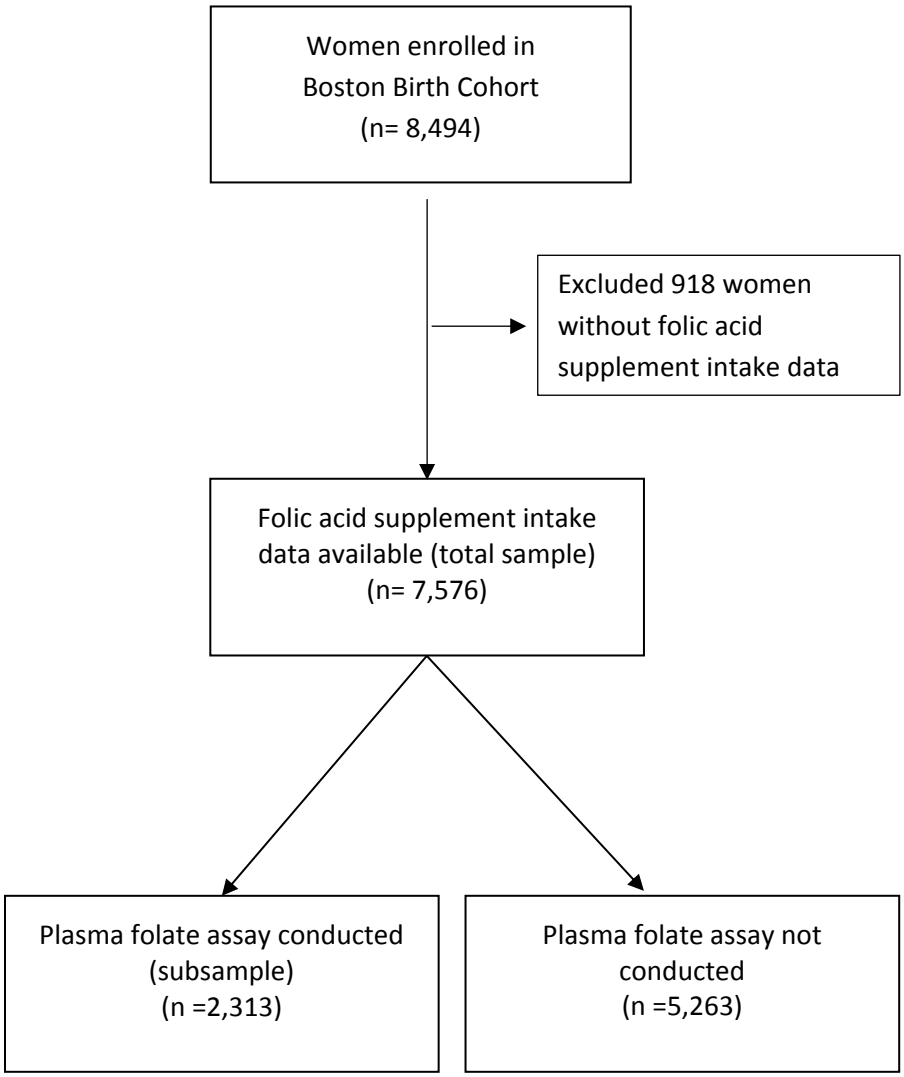
Supplemental Figure 5-1: Schematic of mediation analysis showing total, direct and indirect effects.



Supplemental Figure 5-2: Interrelationship between plasma folate and overall PTB (A), spontaneous PTB (B) and medically indicated PTB (C)



Supplemental Figure 5-3: Study Participants Flow Chart



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Chapter Six: Manuscript III

Inter-relationships of Folate, Intrauterine Infection/Inflammation, and Spontaneous Preterm Birth: New Insight from the Boston Birth Cohort

This paper is to be submitted to British Journal of Obstetrics and Gynecology (BJOG)

Abstract

Objective: To investigate the inter-relationships of maternal folate status, intrauterine infection/inflammation (IUI) and preterm birth (PTB) among a high-risk US population

Design: Birth cohort study

Setting and sample: The Boston Birth Cohort, a predominantly low-income minority sample of mothers and babies recruited at Boston Medical Center.

Methods: Maternal folate status was assessed using self-reported multivitamin supplement intake in pregnancy and plasma folate concentrations at delivery. Multivariate logistic regressions were used to explore interrelations between maternal folate status, IUI and PTB. Adjusted logit regressions assessed the probability of PTB by maternal plasma folate levels, stratified by IUI status.

Main outcome measures: IUI (intrapartum fever or placental pathology evidence) and PTB (birth <37 completed weeks of gestation).

Results: Both multivitamin supplement intake and plasma folate concentrations were associated with reduced odds of IUI (aOR=0.74, 95% CI: 0.63, 0.87; aOR=0.85, 95% CI: 0.73, 0.98, respectively). Multivitamin intake ameliorated the relationship between IUI and PTB, particularly spontaneous PTB. Adjusted logit regressions showed that regardless of IUI status, the probability of PTB and spontaneous PTB decreased as plasma folate concentrations increased.

Conclusions: Our study findings suggest that folate may be beneficial to mitigate IUI and its associated spontaneous PTB.

Keywords: Maternal folate status, intrauterine infection/inflammation, preterm birth, US population, Boston Birth Cohort

Introduction

Folate, also known as vitamin B_9, is a naturally occurring water-soluble B vitamin involved in many cellular pathways and biological reactions needed for growth and normal immune function (1). Reduced folate levels have been linked with a dysfunction of cell-mediated immunity by reducing the capacity of CD8(+) cells to proliferate in response to activation (2), and dysfunctional humoral immunity by reducing antibody responses to several antigens (1). The phagocytic and bactericidal properties of polymorph nuclear white blood cells are reduced in people with folate deficiency, increasing their susceptibility to overt and subclinical infections (3).

Folate sufficiency is particularly important in pregnancy during which deficient levels have been linked to adverse pregnancy outcomes such as anemia, preeclampsia and birth defects (4). Of interest is the association between maternal folate levels and preterm birth (PTB) – delivery before 37 completed weeks of gestation. Studies including our own have demonstrated a link between reduced maternal blood folate concentration or folic acid supplementation and PTB (5-8). One postulated mechanism in which a reduced folate level may be associated with PTB is via inflammation, since folate has anti-inflammatory properties (9). PTB, which is associated with abnormal inflammatory responses, may be triggered by a lower genital tract infection or intrauterine infection (3, 10). The term intrauterine infection/inflammation (IUI), proposed by the National Institute of Child Health and Human Development as a replacement for chorioamnionitis, refers to a heterogeneous setting of conditions characterized by intrauterine infection and/or inflammation or both that occur in pregnancy (11). The diagnosis of IUI is based on the presence of clinical and histopathologic features in addition to maternal fever (12). The literature suggests that IUI is a primary cause of over a quarter of PTB cases (13). One demonstrated pathway is via spontaneous PTB or preterm premature rupture of membranes (14).

While studies have demonstrated the link between lower plasma folate concentrations in early pregnancy and at delivery and lower genital tract infections such as bacterial vaginosis (15), the association between folate and IUI is not well investigated. A US based study found that the link between maternal folate and lower genital tract infection represents a possible pathway for the relationship between maternal folate status and PTB (5, 16). The study analyzed plasma folate and cervical fluid cytokine assays in a low income, predominantly Black population. Each of the standard deviation (SD) increases in serum total folate and serum 5-methyltetrahydrofolate (5MeTHF) concentrations was associated with significant reductions in the risk of spontaneous PTB ($P < 0.05$), while serum 5MeTHF concentrations were also associated with cervical cytokine concentrations, suggesting a possible mechanistic link between folate and PTB risk.

Other studies in South Korea and Nepal have explored the relationship between maternal folate status and C-reactive protein (CRP) levels in pregnancy and resulting PTB. In the South Korea study, serum folate concentration, dietary recall and serum CRP were collected at 12 and 28 weeks of gestation. Serum folate was inversely correlated with serum CRP levels while CRP was positively associated with PTB (17). This is in contrast with the study in Nepal which demonstrated that though CRP was associated with PTB, multiple micronutrient supplementation was not related to CRP (18).

Given the scant literature specific to the US, this paper aims to systematically investigate the interrelationship of maternal folate, IUI and PTB among a high-risk US population. We hypothesize that higher maternal folate status, assessed using complementary measures of reported multivitamin supplement intake and plasma folate concentrations, i) is negatively associated with IUI and ii) ameliorates the relationship between IUI and PTB.

Methods

Materials and Methods

The study sample consisted of a subgroup of mother–infant dyads recruited from 1998 to date as part of the Boston Birth Cohort (BBC), an ongoing prospective cohort enrolled at the Boston Medical Center (BMC). BMC serves a predominantly low-income urban minority population. The BBC has been enriched for PTBs and other adverse pregnancy complications by recruiting at a ratio of approximately one PTB for every two term births. Multiple births as well as newborns with major birth defects were excluded from the BBC study. Patient recruitment and data collection methods are detailed elsewhere (19). Briefly, enrollment took place within 72 hours after birth, and informed written consent was obtained from all study participants. Epidemiologic data was collected via a face to face maternal questionnaire interview while clinical data was collected from maternal and newborn medical records using a standardized abstraction form. Of the 8,570 eligible women enrolled in the BBC (approximately 85% response rate of those approached), we excluded 918 women without multivitamin supplement intake information and 526 women without IUI information.

The final sample for analyses included 7050 women with information on multivitamin supplement intake, IUI, as well as birth outcomes. Of those, a subsample of women (n=2241) had plasma folate levels measured in blood samples collected within a few days after delivery (**Supplemental Figure 1** displays the study flow chart). This subsample included women who consented to enroll their children in a follow up study at BMC (20). Women in the full multivitamin supplement intake sample and plasma folate subsample had very similar baseline characteristics, except for higher proportion of African Americans and slightly higher proportion of PTB in the plasma folate subsample (Table 1).

Key Variables

Multivitamin supplement intake was assessed from responses to two questions from the maternal questionnaire: "Did you take prenatal vitamins prescribed by your doctor?" and "Did you take any over-the-counter multivitamins?" as published elsewhere (21). Follow up questions assessed the frequency of multivitamin supplement use during specific time periods: prepregnancy (6 months prepregnancy), 1st trimester (day one – day 90 of pregnancy), 2nd trimester (day 91 to 180), 3rd trimester (day 181 to birth). Response options for each time period included: none, 1 time per week, 2 times per week, 3-5 times per week, almost daily. Preconception multivitamin intake was categorized into: "no intake" versus "any intake". Intake in the 1st to 3rd trimester was categorized as: "less than three" versus "three or more times" per week. Typically, prenatal prescribed and over-the-counter multivitamins usually contain 400 or 800 micrograms of folic acid and are suggested to be taken daily in the US (22).

Plasma folate concentrations were assessed from maternal plasma samples using chemiluminescent immunoassay with diagnostic kits using a Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) (23).

Measurement and definition of IUI has been published elsewhere (24). Briefly, placental samples were obtained at the time of delivery for pathology review. Using algorithms consistent with the guidelines of the College of American Pathologists (25, 26), pathologists reported the presence of inflammation in the umbilical cord, amnion, chorionic plate, decidua and chorion. IUI was defined as the presence of any of the following criteria: intrapartum maternal fever >38^{o}C or placental histopathology consistent with IUI including villitis, deciduitis, chorioamnionitis, chorionitis, subchorionitis, funisitis and free membranitis.

PTB was defined as delivery at <37 weeks of gestation, based on an algorithm of the last menstrual period and early ultrasound dating (<20 weeks' gestation) as previously published (19).

Other covariates included in the analysis include maternal age (<20, 20-34 and 35+ years), race/ethnicity (African American, Non-Hispanic white, Hispanic and Other), marital status (unmarried versus married), education (≤Elementary, High School or ≥College), nativity (US born versus foreign born), parity (nulliparous versus multiparous), receipt of public assistance (yes or no), perceived stress (mother's report of life or pregnancy as being 'very' stressful), smoking status (never used, ever used, used in pregnancy), alcohol use in pregnancy (never versus any).

Statistical analysis

Exploratory data analysis was performed on the full sample as well as the plasma folate subsample.

Chi-square- and t- tests were used to compare maternal characteristics by the presence or absence of IUI. Histogram plots were used to display the distribution of plasma folate concentrations by IUI status. Multivariate logistic regressions were used to explore associations between both measures of maternal folate status and IUI adjusting for potential confounding covariates: maternal race, age, nativity, education, marital status, receipt of public assistance, parity, smoking status, alcohol use and stress. Sensitivity analysis was also conducted using multivariate logistic regressions among non-Hispanic Black women only. To further explore the combined effect of folate and IUI on PTB, multivitamin supplement intake and IUI status were cross-classified into a single variable with four categories: i) IUI with low multivitamin supplement intake, ii) IUI with high multivitamin supplement intake, iii) No IUI with low supplement intake and iv) No IUI with high supplement intake. The odds of PTB and PTB subtypes was assessed across these four categories with the highest risk group, IUI and low multivitamin supplement intake, as the reference group. In the subsample with plasma folate levels, adjusted logit regressions were used to graph the probability of PTB by plasma folate level, stratified by women with and without IUI.

Results

In the final sample, most women were unmarried, high-school educated, and on public assistance. Non-Hispanic Black women made up 52% of the full sample while 16% had IUI and 28% had a PTB. Table 1 shows the difference in maternal characteristics by IUI status among the full sample as well as plasma folate subsample while Supplemental Table 1 compares maternal characteristics of women with and without IUI data. As seen in Table 1, for both the full sample as well as plasma subsample, demographic characteristics associated with IUI included nativity and marital status. Specifically, women with IUI were more likely to be born in the US and be unmarried. In addition, women with IUI were more likely to have experienced overall PTB as well as spontaneous PTB.

Table 2 displays crude and adjusted associations between maternal folate status (multivitamin supplement intake and plasma folate concentration) and IUI. Multivitamin supplement intake in the preconception period was not significantly associated with IUI. Intake of three or more times a week in the 1st trimester (aOR=0.78, 95% CI: 0.67, 0.91), 2nd trimester (aOR=0.79, 95% CI: 0.67, 0.93) and 3rd trimester (aOR=0.74, 95% CI: 0.63, 0.87) was associated with reduced odds of IUI. Plasma folate concentration also demonstrated an inverse association with IUI. Specifically, each interquartile increase in plasma folate reduced the odds of IUI by about 15% (aOR=0.85, 95% CI: 0.73, 0.98). Compared with plasma folate concentration in the lowest quartile, the highest quartile was associated with a 38% reduction in the odds of IUI (aOR= 0.62, 95% CI: 0.44, 0.88). The sensitivity analysis presented in Supplemental Table 2 shows that the associations between multivitamin supplement intake or plasma folate concentrations with IUI persisted among all non-Hispanic Black women specifically. Supplemental Tables 3-5 show a similar pattern for multivitamin supplement intake during the first and second trimesters as well as throughout pregnancy.

As presented in Table 3, there was a significant relationship between both multivitamin supplement intake and IUI with PTB and spontaneous PTB but not with medically indicated PTB. Women with IUI had increased odds of PTB (aOR=1.75, 95% CI: 1.53, 2.01). This was primarily due to higher odds of spontaneous PTB (aOR=2.33, 95% CI: 2.00, 2.70). In addition, multivitamin supplement intake of three time or more a week in the third trimester was associated with reduced PTB odds (aOR=0.76, 95% CI: (0.66, 0.86) as well as reduced odds of spontaneous PTB (aOR= 0.71, 95% CI: 0.61, 0.83). Joint analysis of IUI and multivitamin supplement intake showed that multivitamin intake attenuated the relationship between IUI and PTB. Compared to the reference group with IUI and low multivitamin supplement intake, women with IUI but higher levels of multivitamin intake had lower odds of PTB than the reference group (aOR=0.70; 95% CI: 0.53, 0.94). Moreover, the odds of PTB were lowest among women without IUI but with high multivitamin supplement intake (aOR=0.42; 95% CI: 0.32, 0.54). A stronger association was seen with spontaneous PTB but not with medically indicated PTB; compared to the reference group, women without IUI and high multivitamin supplement intake had the lowest odds of spontaneous PTB (aOR: 0.29, 95% CI: 0.22, 0.38).

The interrelationships between plasma folate concentrations, IUI and PTB (as well as spontaneous PTB) are presented graphically in Figure 1. The probability of PTB and spontaneous PTB across plasma folate concentrations are stratified by IUI status. Regardless of IUI status, the probability of PTB and spontaneous PTB decrease as plasma folate concentrations increase. Also, regardless of folate status, the probability of PTB and spontaneous PTB was higher among women with IUI compared to those without IUI. In other words, low folate levels and IUI additively increase the risk of overall PTB and spontaneous PTB.

Discussion

To the best of our knowledge, this is the first study to investigate the interrelationships of maternal folate status, IUI, and PTB in a predominantly urban, low-income, minority US population. In our study population, IUI was positively associated with PTB as well as spontaneous PTB, corroborating existing literature that IUI can lead to PTB via activation of pathological inflammatory processes resulting in preterm labor, preterm premature rupture of membranes (ROM) and dilation of the cervix (27, 28). Normal term labor also involves multiple physiological mechanisms that initiate inflammatory mechanisms, which lead to membrane rupture, cervical ripening and onset of coordinated uterine contractions timed with fetal maturation. While preterm labor frequently involves early activation of the same inflammatory mechanisms seen in normal labor due to pathological causes, the scale and nature of the inflammatory response may be abnormal (29). Pathological inflammatory insults occurring during pregnancy, such as oxidative stress or infection can precipitate PTB, often involve common pathways (29). IUI remains not only an important cause of early PTB, it is associated with the delivery of infants at greatest risk of death and disability (30).

Importantly, we found a protective role of higher maternal folate status against IUI across the two complementary measures of folate status. In addition, this association was also seen among a subgroup of non-Hispanic Black women. This is biologically plausible given the anti-inflammatory properties of folate through its inverse relationship with homocysteine (31). Moreover, the presence of inflammation promotes nitric oxide synthesis, which in turn produces hyperhomocysteinemia and oxidative stress and in turn more inflammation (32). Hyperhomocysteinemia has been demonstrated as a risk factor for many inflammatory conditions such as cardiovascular disease, stroke, renal failure and cancer (33, 34). Further research is needed to explore the role of homocysteine in the folate- IUI relationship.

Even more important, our study demonstrated that higher folate status can mitigate the positive association between IUI and PTB (particularly spontaneous PTB). The lowest odds of spontaneous PTB was observed in mothers with high folate status and no IUI; their odds of spontaneous PTB was more than 70% lower than that of mothers with both risk factors, low folate status and IUI. This finding was also consistent across two measures of maternal folate status: reported multivitamin supplement intake and measured plasma folate concentrations. While the mechanistic pathway by which folate could mitigate the IUI- PTB relationship is still unclear, our findings are supported by animal models where pretreatment of mice with folic acid prevented maternal lipopolysaccharide-induced preterm delivery (35). Additional research is needed using prospective studies to corroborate and elucidate our findings.

This study had some limitations. Plasma folate levels were assessed within 72 hours of delivery and although they are more objective and less prone to the recall bias observed with dietary history/supplement intake, we recognize that a single plasma folate measurement cannot be used to differentiate between a transitory decrease in dietary folate intake and a chronic deficiency state. We used plasma folate concentration at delivery as a proxy for third trimester folate level as it reflects short term folate status (36, 37). In addition, multivitamin supplement intake was based on self-report, which is subject to recall bias. Due to the high correlations between multivitamin supplement intake across all trimesters (rho: 0.58- 0.85, $p<0.001$), further adjustments for intake in other trimesters were not conducted when exploring the associations in each trimester. This was an observational study, and by its nature cannot enable causal inference (38). While no randomized controlled trial has been conducted in the US and is unlikely given the advantageous effect of folate on pregnancy outcomes, our study findings need to be confirmed in prospective longitudinal studies. Our study population included a predominantly minority urban population. While our findings may have implications for populations in

other countries with low folate intake, caution is needed in extrapolating these findings to populations with different demographic and clinical characteristics and contexts.

If further confirmed, our findings have important clinical and public health implications as folic acid supplementation and fortification are proven interventions to improve maternal folate status. This is particularly relevant for members of low income and minority populations in the US who typically are found to have insufficient folate status (39).

Tables and figures

Table 6-1: Characteristics of the study sample by IUI status in the full multivitamin supplement intake sample and plasma folate subsample

Maternal Characteristics	Multivitamin supplement intake (N=7050)			Plasma folate subsample. (N=2241)		
	IUI absent (N=5928)	IUI present (N=1122)	P value	IUI absent (N=1921)	IUI present (N=320)	P value
	N (%)	N (%)		N (%)	N (%)	
Race/ethnicity			0.029			0.298
Non-Hispanic Black [a]	3028 (51)	625 (56)		1390 (72)	247 (77)	
Non-Hispanic White	721 (12)	134 (12)		77 (4)	12 (4)	
Hispanic	1704 (29)	282 (25)		374 (20)	52 (16)	
Other	475 (8)	81 (7)		80 (4)	9 (3)	
Age in years			0.001			0.076
<20	600 (10)	147 (13)		184 (10)	37 (12)	
20-34	4321 (73)	820 (73)		1371 (71)	238 (74)	
35+	1007 (17)	155 (14)		366 (19)	45 (14)	
Nativity (US born)			0.002			0.034
Not born in US	3576 (60)	627 (56)		1142 (59)	169 (53)	
Born in US	2279 (38)	488 (44)		754 (39)	149 (47)	
Missing	73 (1)	7 (1)		25 (1)	2 (1)	
Education			0.488			0.928
Less than high school	1813 (31)	352 (31)		533 (28)	86 (27)	
High School/GED	1985 (34)	382 (34)		703 (37)	123 (38)	
Some College and above	2108 (36)	381 (34)		677 (35)	110 34)	
Missing	22 (0)	7 (1)		8 (0)	1 (0)	
Marital Status			0.003			0.032
Married	2186 (37)	357 (32)		672 (35)	90 (28)	
Unmarried	3660 (62)	743 (66)		1234 (64)	229 (72)	
Missing	82 (1)	22 (2)		15 (1)	1 (0)	
Receipt of public assistance [b]			0.890			0.156
No	936 (16)	179 (16)		261 (14)	53 (17)	
Yes	4992 (84)	943 (84)		1660 (86)	267 (83)	
Parity			<0.001			<0.001
Multiparous	3482 (59)	498 (44)		1157 (60)	153 (48)	
Nulliparous	2432 (71)	622 (55)		760 (40)	167 (52)	
Missing	14 (0)	2 (0)		4 (0)	0 (0)	
Cigarette smoking			0.186			0.430
Never	4711 (80)	861 (77)		1555 (81)	250 (78)	
Ever	434 (7)	90 (8)		153 (8)	29 (9)	
Continuous in pregnancy	742 (13)	164 (15)		198 (10)	40 (13)	
Missing	41 (1)	7 (1)		15 (1)	1 (0)	
Alcohol consumption			0.009			0.120
No	5248 (89)	965 (86)		1738 (91)	278 (87)	

Yes	546 (9)	116 (10)		151 (8)	36 (11)	
Missing	134 (2)	41 (4)		32 (2)	6 (2)	
Stress[c]			0.445			0.514
No	4644 (78)	871 (78)		1494 (78)	253 (79)	
Yes	1262 (21)	244 (22)		420 (22)	67 (21)	
Missing	22 (0)	7 (1)		7 (0)	0 (0)	
BMI categories			0.907			0.609
Underweight	249 (4)	50 (5)		75 (4)	13 (4)	
Normal	2638 (45)	509 (45)		803 (42)	138 (43)	
Overweight/Obese	2646 (45)	491 (44)		946 (49)	148 (46)	
Missing	395 (7)	72 (6)		97 (5)	21 (7)	
Diabetes Mellitus			0.456			0.667
No	5483 (93)	1039 (93)		1767 (92)	296 (93)	
GDM	281 (5)	45 (4)		89 (5)	11 (3)	
DM	119 (2)	29 (3)		54 (3)	10 (3)	
Missing	45 (1)	9 (1)		11 (1)	3 (1)	
Preterm birth			<0.001			<0.001
No	4371 (74)	690 (62)		1361 (71)	169 (53)	
Yes	1557 (26)	432 (39)		560 (29)	151 (47)	
Spontaneous PTB	944 (16)	348 (31)	<0.001	319 (17)	129 (40)	0.001
Medically induced PTB	613 (10)	84 (8)	0.003	241 (13)	22 (7)	0.004

[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.

Table 6-2: Relationship between maternal folate status and intrauterine infection/inflammation (N=7050)

Maternal Folate Status	n (%) IUI cases	OR (95% CI)	aOR[a] (95% CI)
Multivitamin supplement intake (n=7050)	**IUI (n=1122)**		
Preconception			
None (reference)	1039 (15.8)	1.00	1.00
Any	83 (17.9)	1.16 (0.91, 1.49)	1.21 (0.94, 1.56)
1st trimester			
<3x a week (reference)	277 (18.5)	1.00	1.00
≥3x a week	845 (15.2)	**0.79 (0.68, 0.92)**	**0.78 (0.67, 0.91)**
2nd trimester			
<3x a week (reference)	217 (18.4)	1.00	1.00
≥3x a week	905 (15.4)	**0.81 (0.68, 0.95)**	**0.79 (0.67, 0.93)**
3rd trimester			
<3x a week (reference)	243 (19.1)	1.00	1.00
≥3x a week	879 (15.2)	**0.76 (0.65, 0.89)**	**0.74 (0.63, 0.87)**
Plasma Folate in nmol/L (n=2241)	**IUI (n=320)**		
Continuous			
Each unit nmol/L increase	320 (100)	**0.99 (0.99, 1.00)**	**0.99 (0.99, 1.00)**
Each interquartile increase	320 (100)	**0.85 (0.74, 0.98)**	**0.85 (0.73, 0.98)**
Categorical: quartiles (nmol/L)			
Lowest quartile: 6.6 to 19.4 (reference)	95 (16.7)	1.00	1.00
Second quartile: 19.4- 30.0	86 (15.3)	0.90 (0.66, 1.24)	0.89 (0.64, 1.23)
Third quartile:30.0-43.8	76 (13.8)	0.80 (0.58, 1.11)	0.78 (0.56, 1.09)
Highest quartile: 43.8-185.5	63 (11.3)	**0.64 (0.45, 0.90)**	**0.62 (0.44, 0.88)**

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation; n: number of cases; n/a: not applicable.

a Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use and stress.

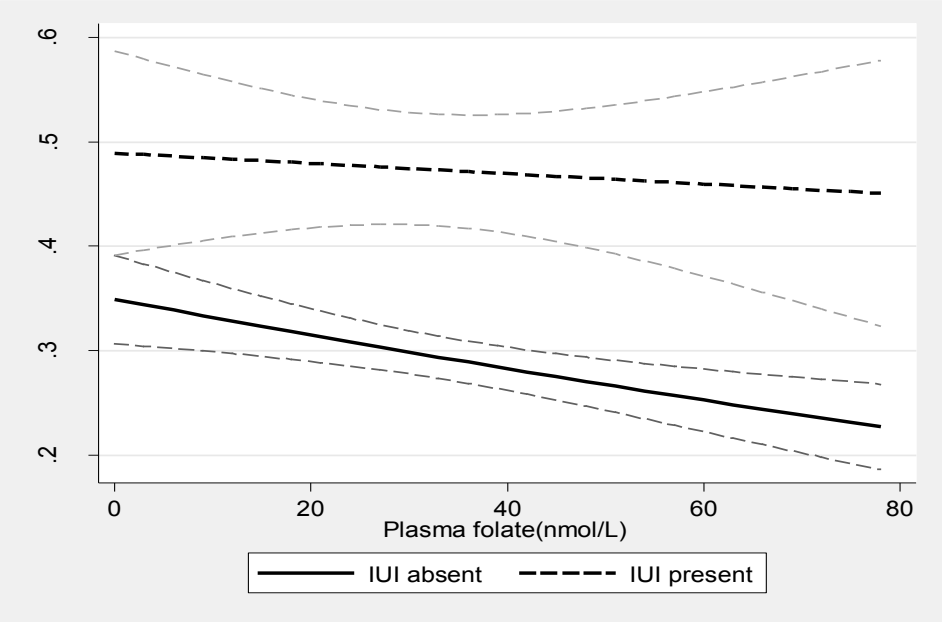
Table 6-3: Individual and joint association of intrauterine infection/inflammation (IUI) with multivitamin supplement intake in the third trimester on preterm birth (overall and subtypes) (N=7050)

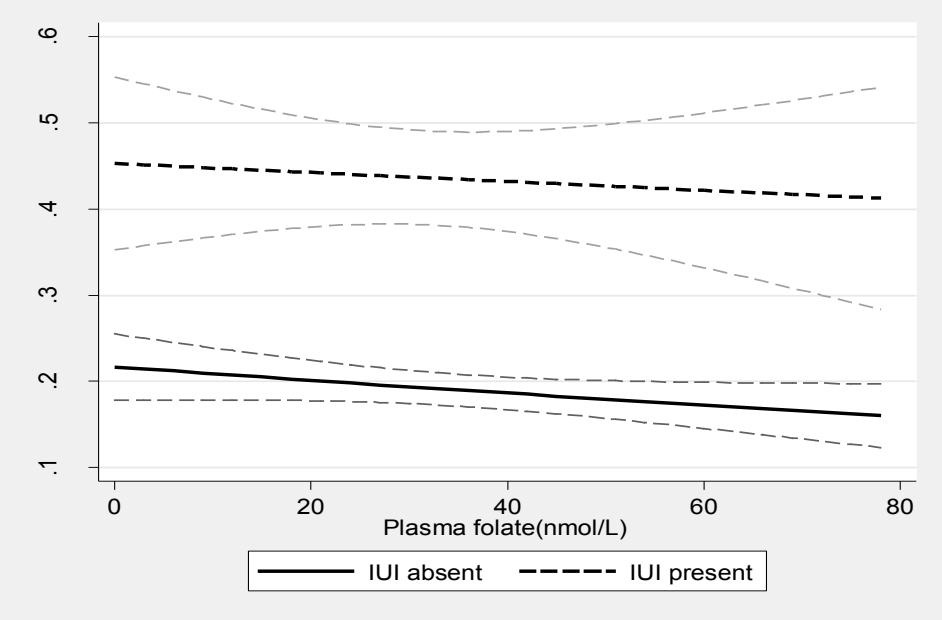
Characteristic		PTB (n=1989)		sPTB (n=1292)		mPTB (n=697)	
		n (%) cases	aOR[a] (95% CI)	n (%) cases	aOR[a] (95% CI)	n (%) cases	aOR[a] (95% CI)
Intrauterine infection/inflammation (IUI)							
No (ref)		1557 (26)	1.00	944 (18)	1.00	613 (12)	1.00
Yes		432 (39)	1.75 (1.53, 2.01)	348 (34)	2.33 (2.00, 2.70)	84 (11)	0.85 (0.66, 1.09)
Multivitamin supplement intake (MVI) in third trimester							
< 3x a week (ref)		431 (34)	1.00	296 (26)	1.00	135 (14)	1.00
≥3x a week		1558 (27)	0.76 (0.66, 0.86)	996 (19)	0.71 (0.61, 0.83)	562 (12)	0.86 (0.70, 1.06)
Joint Analysis of IUI with multivitamin supplement intake in third trimester							
IUI	MVI						
Yes	< 3x/week (ref)	114 (47)	1.00	100 (44)	1.00	14 (10)	1.00
Yes	≥ 3x/week	318 (36)	0.70 (0.53, 0.94)	248 (31)	0.62 (0.46, 0.84)	70 (11)	1.25 (0.68, 2.31)
No	< 3x/week	317 (31)	0.53 (0.40, 0.70)	196 (22)	0.37 (0.27, 0.50)	121 (15)	1.67 (0.92, 3.02)
No	≥ 3x/week	1240 (25)	0.42 (0.32, 0.54)	748 (17)	0.29 (0.22, 0.38)	492 (12)	1.36 (0.77, 2.40)

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation; n: number of cases; n/a: not applicable.

a Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use and stress.

Figure 6-1: Adjusted probability of preterm (top panel) and spontaneous preterm birth (bottom panel) by plasma folate concentrations stratified by intrauterine infection/inflammation status





Notes: IUII-intrauterine infection/inflammation; Probability adjusted for potential confounding variables- maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use and stress.

Supplemental Table 6-1: Comparison of characteristics of women in the study sample with women with missing IUI data

Characteristics	IUI data present (N=7050)		IUI data missing (N=526)		Total (N=7576)	
	No.	%	No.	%	No.	%
Race/ethnicity						
Non-Hispanic Black [a]	3653	51.8	194	36.9	3847	50.8
Non-Hispanic White	855	12.1	65	12.4	920	12.1
Hispanic	1986	28.2	164	31.2	2150	28.4
Other	556	7.9	58	11	614	8.1
Missing	0	0	45	8.6	45	0.6
Age in years						
<20	747	10.6	56	10.6	803	10.6
20-34	5141	72.9	358	68.1	5499	72.6
35+	1162	16.5	67	12.7	1229	16.2
Missing	0	0	45	8.6	45	0.6
Nativity (US born)						
Not born in US	4203	59.6	318	60.5	4521	59.7
Born in US	2767	39.2	197	37.5	2964	39.1
Missing	80	1.1	11	2.1	91	1.2
Education						
Less than high school	2165	30.7	138	26.2	2303	30.4
High School/GED	2367	33.6	174	33.1	2541	33.5
Some College and above	2489	35.3	165	31.4	2654	35
Missing	29	0.4	49	9.3	78	1
Marital Status						
Married	2543	36.1	193	36.7	2736	36.1
Unmarried	4403	62.5	275	52.3	4678	61.7
Missing	104	1.5	58	11	162	2.1
Receipt of public assistance [b]						
No	1115	15.8	95	18.1	1210	16
Yes	5935	84.2	431	81.9	6366	84
Parity						
Multiparous	3980	56.5	154	29.3	2064	27.2
Nulliparous	3054	43.3	370	70.3	5494	72.5
Missing	16	0.2	2	0.4	18	0.2
Cigarette smoking						
Never	5572	79	411	78.1	5983	79
Ever	524	7.4	27	5.1	551	7.3
Continuous in pregnancy	906	12.9	41	7.8	947	12.5
Missing	48	0.7	47	8.9	95	1.3
Alcohol consumption						
No	6213	88.1	427	81.2	6640	87.6
Yes	662	9.4	30	5.7	692	9.1
Missing						
Stress[c]						
No	5515	78.2	433	82.3	5948	78.5
Yes	1506	21.4	88	16.7	1594	21

Missing	29	0.4	5	1	34	0.4
BMI categories						
Underweight	299	4.2	25	4.8	324	4.3
Normal	3147	44.6	206	39.2	3353	44.3
Overweight/Obese	3137	44.5	203	38.6	3340	44.1
Missing	467	6.6	92	17.5	559	7.4
Diabetes Mellitus						
No	6522	92.5	490	93.2	7012	92.6
Gestational DM	326	4.6	18	3.4	344	4.5
DM	148	2.1	6	1.1	154	2
Missing	54	0.8	12	2.3	66	0.9
Preterm birth						
No	5061	71.8	446	84.8	5507	72.7
Yes	1989	28.2	80	15.2	2069	27.3
Spontaneous PTB	1292	20.3	61	11.6	1353	17.9
Medically induced PTB	697	12.1	19	3.6	716	9.5

Abbreviation: IUI: DM: Diabetes Mellitus; GED: General Education Diploma; Intrauterine Infection/Inflammation; PTB: Preterm Birth; US: United States

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

[b] public assistance is defined as receipt of any of the following: including: 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 of pregnancy being very stressful.

Supplemental Table 6-2: Relationship between IUI and multivitamin supplement intake in the third trimester (N=3653) and plasma folate concentration at delivery (n=1630) among non-Hispanic Blacks only.

Maternal Folate Status	**n (%) cases**	**OR (95% CI)**	**aOR[a] (95% CI)**
Multivitamin supplement intake (N=3653)		**IUI (n=625)**	
Preconception			
None (ref)	581 (17.0)	1.00 (reference)	1.00 (reference)
Any	44 (18.3)	1.09 (0.78, 1.53)	1.17 (0.83, 1.66)
1st trimester			
<3x a week (ref)	171 (19.8)	1.00 (reference)	1.00 (reference)
≥3x a week	454 (16.3)	**0.79 (0.65, 0.96)**	**0.72 (0.59, 0.89)**
2nd trimester			
<3x a week (ref)	135 (20.1)	1.00 (reference)	1.00 (reference)
≥3x a week	490 (16.4)	**0.78 (0.63, 0.97)**	**0.72 (0.58, 0.90)**
3rd trimester			
<3x a week (ref)	155 (21.4)	1.00 (reference)	1.00 (reference)
≥3x a week	470 (16.1)	**0.70 (0.58, 0.86)**	**0.65 (0.53, 0.81)**
Plasma Folate in nmol/L (n=1630)		**IUI (n=247)**	
Continuous			
Each unit nmol/L increase	247 (100)	**0.99 (0.99, 1.00)**	**0.99 (0.99, 1.00)**
Each interquartile increase	247 (100)	**0.82 (0.70, 0.97)**	**0.82 (0.69, 0.97)**
Categorical: quartiles (nmol/L)			
Lowest quartile: 6.6 to 19.4 (reference)	78 (17.1)	1.00 (reference)	1.00 (reference)
Second quartile: 19.4- 30.0	70 (17.5)	1.03 (0.73, 1.47)	1.01 (0.70, 1.45)
Third quartile:30.0-43.8	55 (14.3)	0.81 (0.56, 1.18)	0.75 (0.51, 1.10)
Highest quartile: 43.8-185.5	44 (11.1)	**0.61 (0.41, 0.90)**	**0.58 (0.39, 0.87)**

Abbreviation: aOR: adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation; n: number of cases.

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

Supplemental Table 6-3: Individual and joint association of IUI with multivitamin supplement intake in the 1st trimester on preterm birth (N=7050)

Characteristic		PTB (n=1989)		sPTB (n=1292)		mPTB (n=697)	
		n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)
Intrauterine infection/inflammation (IUI)							
No (ref)		1557 (26)	1.00	944 (18)	1.00	613 (12)	1.00
Yes		432 (39)	1.75 (1.53, 2.01)	348 (34)	2.33 (2.00, 2.70)	84 (11)	0.85 (0.66, 1.09)
Multivitamin supplement intake (MVI) in first trimester							
≥3x a week (ref)		1509 (27)	1.00	959 (19)	1.00	550 (12)	1.00
< 3x a week		480 (32)	1.19 (1.05, 1.35)	333 (25)	1.29 (1.11, 1.49)	147 (13)	1.01 (0.82, 1.23)
Joint Analysis of IUI with multivitamin supplement intake in third trimester							
IUI	MVI						
Yes	< 3x/week (ref)	116 (42)	1.00	100 (38)	1.00	16 (9)	1.00
Yes	≥ 3x/week	316 (37)	0.93 (0.70, 1.23)	248 (32)	0.84 (0.62, 1.13)	68 (11)	1.42 (0.79, 2.53)
No	< 3x/week	364 (30)	0.62 (0.47, 0.81)	233 (21)	0.45 (0.34, 0.61)	131 (14)	1.62 (0.93, 2.82)
No	≥ 3x/week	1193 (25)	0.52 (0.40, 0.67)	711 (17)	0.36 (0.27, 0.47)	482 (12)	1.52 (0.90, 2.59)

Abbreviation: aOR-adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation mPTB: medically indicated PTB; MVI: multivitamin supplement intake; n-number of cases; ref- reference; PTB: preterm birth; sPTB: spontaneous PTB.

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

Supplemental Table 6-4: Individual and joint association of IUI with multivitamin supplement intake in 2nd trimester on Preterm Birth (N=7050)

Characteristic		PTB (n=1989)		sPTB (n=1292)		mPTB (n=697)	
		n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)
Intrauterine infection/inflammation (IUI)							
No (ref)		1557 (26)	1.00	944 (18)	1.00	613 (12)	1.00
Yes		432 (39)	1.75 (1.53, 2.01)	348 (34)	2.33 (2.00, 2.70)	84 (11)	0.85 (0.66, 1.09)
Multivitamin intake (MVI) in second trimester							
≥3x a week (ref)		1604 (27)	1.00	1023 (19)	1.00	581 (12)	1.00
< 3x a week		385 (33)	1.25 (1.09, 1.43)	269 (25)	1.35 (1.15, 1.58)	116 (12)	1.06 (0.85, 1.31)
Joint Analysis of IUI with multivitamin supplement intake in third trimester							
IUI	MVI						
Yes	< 3x/week (reference)	93 (43)	1.00	82 (40)	1.00	11 (8)	1.00
Yes	≥ 3x/week	339 (37)	0.85 (0.63, 1.15)	266 (32)	0.76 (0.55, 1.05)	73 (11)	1.57 (0.80, 3.07)
No	< 3x/week	292 (30)	0.60 (0.44, 0.81)	187 (22)	0.44 (0.31, 0.60)	105 (14)	1.85 (0.96, 3.56)
No	≥ 3x/week	1265 (25)	0.48 (0.36, 0 63)	757 (17)	0.33 (0.25, 0.44)	508 (12)	1.63 (0.87, 3.05)

Abbreviation: aOR-adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation mPTB: medically indicated PTB; MVI: multivitamin supplement intake; n-number of cases; ref- reference; PTB: preterm birth; sPTB: spontaneous PTB.

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

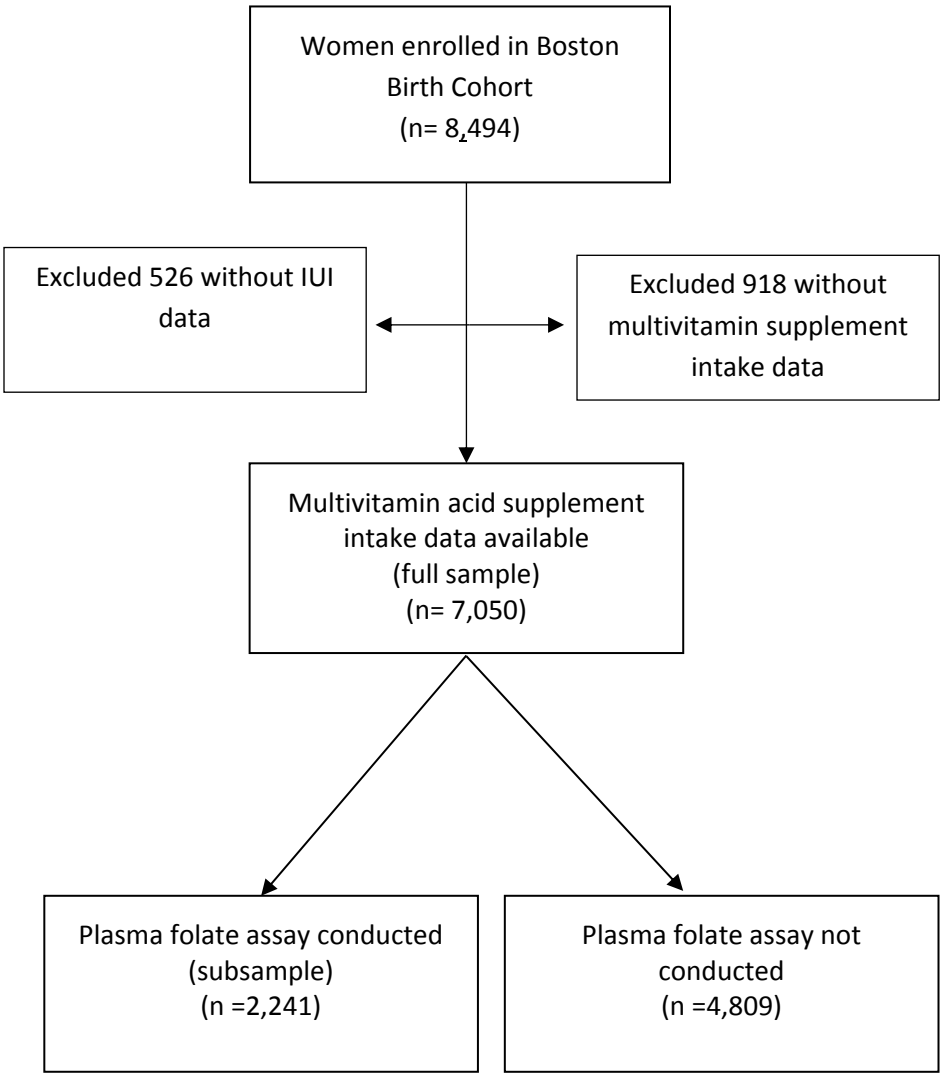
Supplemental Table 6-5: Individual and joint association of IUI with multivitamin supplement intake in overall pregnancy on Preterm Birth (N=7050)

Characteristic		PTB (n=1989)		sPTB (n=1292)		mPTB (n=697)	
		n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)	n (%) cases	aOR (95% CI)
Intrauterine infection/inflammation (IUI)							
No (ref)		1557 (26)	1.00	944 (18)	1.00	613 (12)	1.00
Yes		432 (39)	1.75 (1.53, 2.01)	348 (34)	2.33 (2.00, 2.70)	84 (11)	0.85 (0.66, 1.09)
Multivitamin intake (MVI) in second trimester							
≥3x a week (ref)		1384 (27)	1.00	870 (19)	1.00	514 (12)	1.00
< 3x a week		605 (33)	1.26 (1.12, 1.42)	422 (25)	1.39 (1.21, 1.59)	183 (13)	1.04 (0.86, 1.25)
Joint Analysis of IUI with multivitamin supplement intake in third trimester							
IUI	MVI						
Yes	< 3x/week (reference)	156 (44)	1.00	138 (41)	1.00	18 (8)	1.00
Yes	≥ 3x/week	276 (36)	0.77 (0.59, 1.00)	210 (30)	0.65 (0.50, 0.86)	66 (12)	1.55 (0.89, 2.70)
No	< 3x/week	449 (30)	0.55 (0.43, 0.70)	284 (21)	0.39 (0.30, 0.50)	165 (13)	1.77 (1.05, 2.96)
No	≥ 3x/week	1108 (25)	0.45 (0.36, 0.57)	660 (17)	0.30 (0.24, 0.39)	448 (12)	1.59 (0.96, 2.61)

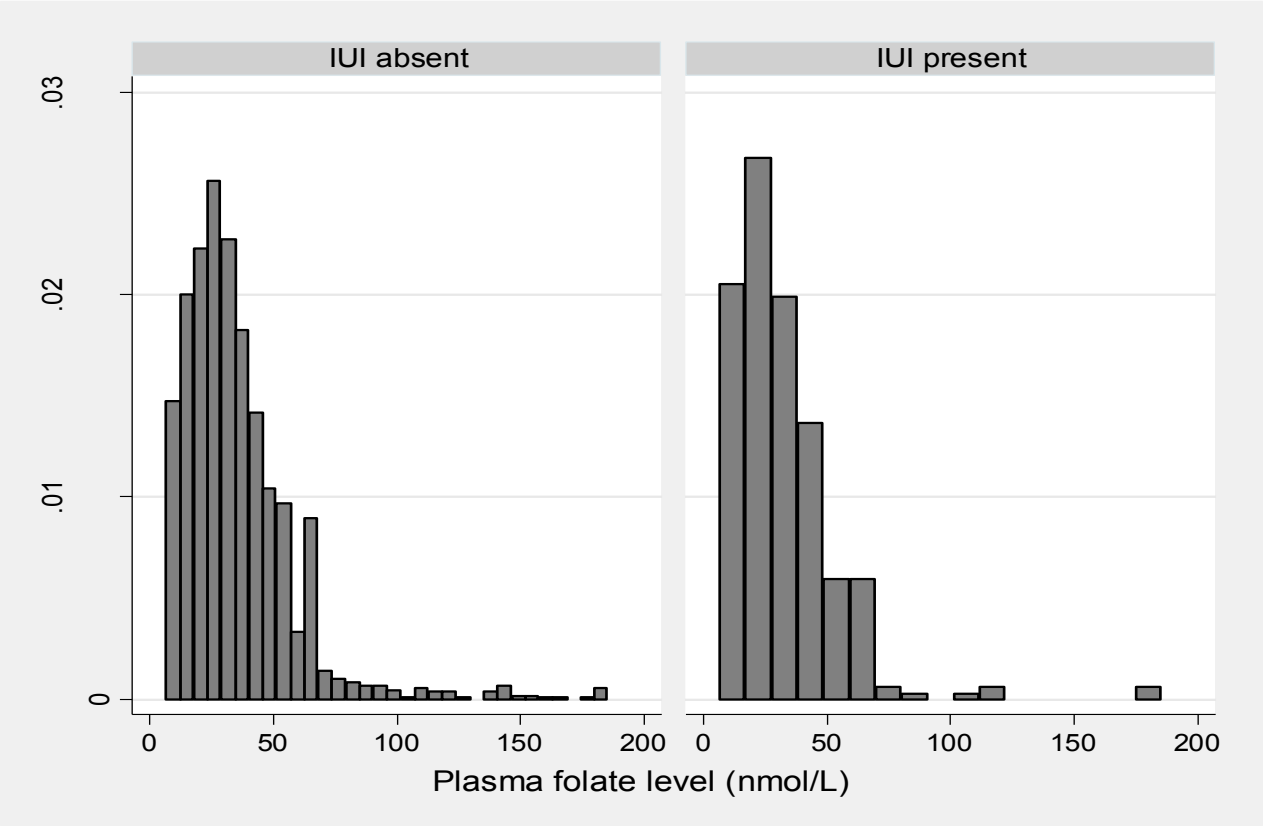
Abbreviation: aOR-adjusted odds ratio; CI: confidence interval; IUI: intrauterine infection/inflammation mPTB: medically indicated PTB; MVI: multivitamin supplement intake; n-number of cases; ref-reference; PTB: preterm birth; sPTB: spontaneous PTB.

a Adjusted for maternal race, age, nativity, education, marital status, receipt of public assistance, parity, tobacco use, alcohol use and stress

Supplemental Figure 6-1: Study flow chart



Supplemental Figure 6-2: Distribution of Plasma Folate concentration by IUI status



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Chapter Seven: Conclusion

Overview

This chapter summarizes this dissertation which aimed to: i) Evaluate the relationship between maternal folate status (using multiple measures and assessed from preconception to delivery) and risk of PTB (overall and subtype of PTB) and ii) Investigate the biologic plausibility of the folate-PTB association by evaluating the role of folate on major pathogenic pathways- preeclampsia and IUI- leading to PTB. The chapter begins with a brief discussion of the main findings, followed by a discussion of strengths and limitations, public health implications and conclusions of the research.

Key Findings

Relationship between maternal folate status and PTB

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.

Interrelationships between maternal folate status, preeclampsia and medically indicated PTB

Intake of multivitamin supplements containing folate, three or more times a week in the 3rd trimester was associated with reduced odds of preeclampsia (aOR=0.77, 95% CI: 0.65, 0.93). Each interquartile increase in plasma folate reduced the odds of preeclampsia by 20% (aOR=0.80, 95% CI: 0.68,0.95). Compared to plasma folate concentrations in the first quartile,

the highest quartile was associated with reduced risk of preeclampsia (aOR=0.62, 95% CI: 0.43, 0.90). Preeclampsia mediated 62% of the multivitamin supplement intake-medically indicated PTB relationship. Preeclampsia did not mediate the relationship between either measure of folate status and spontaneous PTB.

Interrelationships between maternal folate status, IUI and spontaneous PTB

Both multivitamin supplement intake and plasma folate concentrations were associated with reduced odds of IUI (aOR=0.74, 95% CI: 0.63, 0.87; aOR=0.85, 95% CI: 0.73, 0.98, respectively). Joint analysis of IUI and multivitamin supplement intake showed that multivitamin intake attenuated the relationship between IUI and spontaneous PTB. Compared to the reference group with IUI and low multivitamin supplement intake, women without IUI and high multivitamin supplement intake had the lowest odds of spontaneous PTB (aOR: 0.29, 95% CI: 0.22, 0.38). Adjusted logit regressions showed that regardless of IUI status, the probability of PTB and spontaneous PTB decreased as plasma folate concentrations increased.

Strengths and Limitations

This section presents the strengths and limitations of the study.

Strengths: The strengths of this dissertation include the following:

Study sample: The study consisted of a high risk, predominantly low income urban minority US population. Study findings are generalizable to similar low income urban women populations in the US who are more likely to have lower folate intake and bear greater risk of preeclampsia, IUI, and PTB. Thus, findings from this study are directly relevant to the design of relevant research, programmatic or policy interventions to those who need it most.

Multiple measures of folate status: This study employed two complementary measures of folate status- self reported multivitamin supplement intake from preconception to third trimester as well as plasma folate samples taken at delivery. In general, study findings were consistent across measures of folate status, strengthening the validity of study findings.

Large sample size: This study sample size is among the latest ever conducted in the US population, and it is further strengthened by the availability of data on PTB subtypes, preeclampsia and IUI. The plasma folate subsample was one of the largest US sample sizes to date. There were also large samples of pregnancy complications such as preeclampsia and IUI to enable mediation/moderation analysis.

Extensive covariables: Data from this study included epidemiologic data from a face to face maternal questionnaire interview, clinical data from abstracted medical records, and biomarkers from maternal and placental samples, which provided opportunities to address the study hypotheses while controlling for potential confounding factors.

Novel statistical methods: This dissertation employed novel statistical methods including mediation analysis and additive as well as multiplicative interaction analysis. This enabled the exploration of intersection of nutritional, biomedical and psychosocial risk factors influencing PTB.

Study Limitations: This study however acknowledges the following limitations.

Maternal folate status: Plasma folate levels were assessed within 72 hours of delivery and though they are objective and not prone to the recall bias observed with dietary history/supplement intake, we recognize that a single plasma folate measurement cannot be used

to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. Plasma folate at delivery can only be used as a proxy for third trimester folate levels as it reflects short term folate status (1, 2). In addition, multivitamin supplement intake was based on self-report, which is subject to recall bias. Also, the determination of folate status from the frequency of supplement intake may be incomplete as 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 multivitamin supplement intake across all trimesters (rho: 0.58- 0.85, $p<0.001$), further adjustments for intake in other trimesters were not conducted when exploring the associations in each trimester.

Causality: This is an observational study, and by its nature cannot enable causal inference (3) as unobserved confounding remains a threat to validity. Randomized Control Trials (RCT) have been lauded as the gold standard of clinical research (4). However, no RCT on the relationships between folate status and PTB, preeclampsia or intrauterine infection 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 prospective longitudinal studies.

Generalizability: Not all participants of the BBC study are included in this dissertation based on the eligibility criteria. It is possible that those who are not included might differ in some way from the study sample. Furthermore, results of this dissertation are only generalizable to similar urban low-income minority populations in the US such as Detroit, Baltimore, New York (5). Study findings may also have implications for populations in the developing countries with low folate intake, but caution is needed in extrapolating our findings to populations with different demographic and clinical characteristics and contexts. This dissertation is also limited by its focus on only two minority populations: Non-Hispanic Blacks and Hispanic women. Other

minority populations such as Asians and Alaska Native/ American Indians are not explored in this study.

Selection Bias: There were some differences in the full and plasma subsample of study participants. Women who consented to be in the subsample were women who intended to receive follow up pediatric care at BMC. The proportion of non-Hispanic Blacks as well as PTB was higher in the plasma subsample compared to full sample of study participants. Other characteristics remained comparable across samples.

Missing Data: A total of 526 women in the full sample did not have data on IUI and thus had to be excluded from the analysis when the outcomes of interest were IUI (hypothesis 2c-d). The sample characteristics of these women were similar to women included in the study.

Study Implications

This study demonstrates the importance of maternal folate nutrition during pregnancy and adverse pregnancy outcomes. The link between maternal folate and PTB is corroborated in this study. In addition, plausible pathways in the folate-PTB relationship are identified. The study findings, if further confirmed, are very important given the need to identify modifiable risk factors for PTB to inform relevant research, policy and programmatic and clinical interventions to mitigate PTB risk.

Research Implications

Causality: In 1965 Hill and others proposed certain aspects of evidence that should be considered when trying to draw conclusions about causality. These include strength of association, consistency, specificity, temporality, biological gradient, plausibility, experimental

evidence, and analogy (6). In this dissertation, the strength of association and biological gradient was partially addressed with the dose-response effects of maternal folate status on key outcomes. Consistency was ensured with the similar results from both multivitamin supplement intake and plasma folate levels. This dissertation also provided biologically plausible mechanistic pathways for relationships that were identified such as folate-PTB, folate-preeclampsia and folate-IUI. However, this research was unable to address specificity, given the multiple etiologies of PTB. Also, temporality remains an issue given that all the measures of maternal folate were collected after delivery. Finally, as the research was unable to manipulate participants into a treatment or control group, experimental evidence and analogy remain an issue. Future longitudinal studies should attempt to address causal criteria that this dissertation was unable to.

Folate status measurement: Additional efforts needed in future longitudinal studies include the use of standardized folic acid supplementation across the study sample. Also, adequate sample sizes of preconceptional folic acid supplement intake is needed to explore the role of preconceptional folate status on key pregnancy outcomes. Folic acid supplementation should also be measured as soon as possible after intake preferably through a longitudinal design in order to avoid recall bias. The use of intake diaries can also help ensure the accuracy of supplementation intake data. Ideally, plasma folate samples should also be collected from all study participants in each trimester and not just at delivery. Alternatively, red cell folate can be collected at fewer intervals compared to plasma folate as red cell folate is a better measure of long term folate status.

Study populations: In addition to research among vulnerable populations, additional studies are needed among low-risk populations such as non-Hispanic Whites and middle/high income women to explore the extent to which relationships between folate and PTB also exist among

such populations. Research among nationally representative samples may help inform the generalizability of the associations between maternal folate status and key pregnancy outcomes.

Analytical methods: In future longitudinal studies, additional analysis that may prove useful in further elucidating the role of folate in mitigating adverse pregnancy outcomes include population attributable risk- for example this method can demonstrate the proportion of PTB cases that are due to lower maternal folate status. Propensity score matching is another analytical method that can also be used to estimate the effect of maternal folate status on key outcomes, accounting for confounding covariates. Structural equation modelling and path analysis can also be used to explore the interrelationships between folate, other maternal characteristics and pregnancy outcomes. For example, the effect of maternal race/ethnicity, parity and age on maternal folate status as well as PTB. This may help clarify to what extent folate status may explain racial disparities in PTB.

Animal studies: Experimental studies involving appropriate animal models can prove useful in describing the temporal events from low folate status to PTB, particularly in the setting of intrauterine infection or preeclampsia. The use of animal models to investigate specific hypotheses related to PTB and to demonstrate the pathophysiological events associated with PTB will aid the development of rational and efficacious treatment and prevention strategies for PTB (7).

Data collection: given the lack of natality data on PTB subtypes- spontaneous versus medically indicated on a national level, there is need for systematic data collection on this. Information on presentation at labor should be included with information at gestational age to ascertain whether deliveries are spontaneous or induced. This will help to fully understand the prevalence and

trends of each PTB subtype. Likewise, information on maternal folate status should be collected routinely. This includes dietary habits and supplement intake from preconception to delivery. National level perinatal research such as Pregnancy Risk Assessment Monitoring System (PRAMS) such include information on sub-types of PTB. Data should also be collected on multivitamin supplement intake in pregnancy.

Behavior change research: behavior change research is intricately tied in with folate status as dietary habits as well as supplementation intake are individual behaviors. There is need for additional efforts to understand the drivers and deterrents of these behaviors. This includes knowledge, attitudes and perceptions towards folate rich foods or folic acid supplementation. For example, women may simply not know how important folic acid supplementation is for birth outcomes. In addition, behavior change theories may be used to explore how to design interventions to improve maternal folate status. For example, the Extended Parallel Processing Model describes how rational considerations (efficacy beliefs) and emotional reactions (fear of a health threat) combine to determine behavioral decisions. Potential questions under the constructs of EPPM include: i) Perceived severity–How serious are the consequences of poor birth outcomes? ii) Perceived susceptibility–How likely is it that you might have a poor birth outcome as a result? iii) Response efficacy–How effective is folic acid supplementation to prevent these outcomes? iv) Self-efficacy–How confident are you that you can successfully take folic acid supplements throughout pregnancy?

EPPM can be used to segment audiences and come up with relevant strategies. For example, among women with high efficacy and high perceived threat, the strategy would be to provide calls to action. Whereas, among women with low efficacy and low perceived threat, the strategy would be to educate about risk and about solutions. In addition, qualitative research using focus

groups among women can be used to elucidate the issues regarding folic acid supplementation at an individual level.

Similar to the need for research among women, behavior change research may be beneficial among clinicians. Research may explore clinician's knowledge and perceptions regard folate supplementation while qualitative interviews with clinicians can help understand barriers to effective counselling and patient communication. Findings from such research can inform the design of relevant clinical interventions such as the use of job aids, training on interpersonal communication.

In summary, key research recommendations include the following:

- Conduct longitudinal studies in which women are enrolled from periconception to delivery
- Use standardized measurement of folic acid supplement intake e.g., mg/day
- Monitor folic acid supplement intake closely preferably weekly
- Conduct behavior change studies among women and clinicians
- Collect data on PTB subtypes and multivitamin supplement intake in national research such as PRAMS.

Policy Implications

This research highlighted several relevant policies as described below.

Folic acid supplementation: In 1991, the Centers for Disease Control and Prevention (CDC) recommended that women with a prior NTD-affected pregnancy should consume 4000 µg of folic acid daily starting at the time they begin planning a pregnancy(8). Shortly after, in 1992, the U.S. Public Health Service recommended that all women of childbearing age consume 400 µg of

folic acid daily through fortification, supplementation, and diet to prevent NTDs (9). In 1998, the Institute of Medicine (IOM) recommended that women capable of becoming pregnant should consume 400 μg of folic acid daily from fortified foods or supplements, or both, in addition to their diet. Also, the Institute of Medicine recommends higher doses of folate are for pregnant (600 mcg DFE) and lactating (500 mcg DFE) mothers to prevent neural tube defects (10). In 2009 and 2017, the U.S. Preventive Services Task Force published updated guidelines that reinforced these recommendations- that all women who are planning or capable of pregnancy take a daily supplement containing 0.4 to 0.8 mg (400 to 800 μg) of folic acid (11).

This dissertation showed that maternal folate status in later pregnancy and not just early pregnancy was associated with PTB. Interestingly the policies do not explicitly tell women to continue taking folic acid supplements throughout pregnancy. Thus, if this dissertation findings are confirmed, policies may need to be updated and encourage that pregnant women take folic acid supplements throughout pregnancy. Furthermore, minority populations are less likely to know about or consume folic acid supplements. This suggests additional efforts are needed to ensure that the policy is dispersed across all populations.

Mandatory folic acid fortification: In the US, mandatory fortification of enriched cereal grain products with folic acid was authorized in 1996 and fully implemented in 1998 (12). The U.S. program adds 140 μg of folic acid per 100 g of enriched cereal grain product and has been estimated to provide 100–200 μg of folic acid per day to women of childbearing age (13). Although mandatory flour fortification programs increase folic acid intake, research has shown that they do not reach all women of reproductive age adequately with Hispanic and non-Hispanic women less likely to consume foods fortified with folic acid (14). In Hispanics, this is presumably because folic acid-enriched cereal grain products are often not a staple in their diet.

In 2016, the FDA approved the addition of folic acid to corn masa flour, an ingredient in foods including tortillas, tacos, tortilla chips and tamales. Foods made from this flour are staple foods of Mexican and some Central and South American diets (15). Additional efforts are needed to ensure that foods typically consumed by non-Hispanic Blacks are also fortified with folic acid.

American College of Obstetricians and Gynecologists recommendations: In addition to recommending the use of 0.4mg of folic acid for at least one month before pregnancy and during pregnancy to prevent neural tube defects, ACOG recommends a nutritious diet and folic acid and iron supplementation for treatment of pregnancy-induced folic acid deficiency (16). This is relevant given the fact that this dissertation highlighted the prevalence of folate insufficiency in pregnancy among a high-risk population. Also, ACOG guidelines recommend delivery after 34 weeks of gestation for severe preeclampsia (17) as well as folic acid supplementation in the preconception period and first trimester for women with prior preeclampsia (18). In this study, multivitamin supplement intake and plasma folate levels were generally adequate or high, as expected in this era of mandatory folic acid fortification of the food supply. However, about a quarter of women had relatively low plasma folate levels (<19.4nmol/L), which was associated with an increased risk of PTB, preeclampsia and IUI. This study also found comparable relationships between folate and preeclampsia among nulliparous and multiparous women. If study findings of the relationship between maternal folate status and preeclampsia, as well as the role of preeclampsia as a mediator in the folate-PTB relationship are validated by prospective studies, such guidelines might need to consider the administration of folic acid among women with preeclampsia beyond the first trimester and among nulliparous women.

In summary, key policy recommendations

- Encourage women to continue folic acid supplements throughout pregnancy.
- Explore folic acid fortification policies that consider cultural differences in diet among minority populations
- Investigate the need to update ACOG recommendations for folic acid supplementation beyond women with prior preeclampsia

Programmatic Implications

Preterm Birth Prevention: This study has implications for PTB prevention among individuals, governmental, non-governmental organizations

Individual: Interventions to prevent PTB should explore opportunities to sensitize the public on the adverse effects of PTB as well as modifiable risk factors for PTB. In addition, women should be able to assess their risk for PTB and be informed of necessary actions to take such as meet with their health care provider. Interventions should also focus on the improving women's knowledge and perceptions regarding improving their folate status through diet and folic acid supplementation Example of health communication interventions include the use of mass-media such as social media, radio, television, flyers, websites and brochures.

Governmental nutritional programs: Efforts should be made to explore opportunities to leverage already existing nutrition programs such as Women Infants and Children (WIC) to incorporate relevant nutritional interventions to improve folate status. For example, recipients of WIC should be informed about the need for an optimal folate status and this may involve the use of posters, flyers and brochures at WIC offices that remind women to eat healthy and use their supplements. This is important as WIC recipients are likely to be

low-income minority women and prenatal WIC participation has been found to be associated with significant improvements pregnancy outcomes among such women (19).

Health departments: PTB must be considered a priority health issue in many areas in the US and this should be operationalized with the formation of coalitions involving multidisciplinary experts in relevant areas such as clinicians, nutritionists, psychologists, etc. The PTB coalition can advocate for funding of interventions to address PTB in their community, explore opportunities to work synergistically with other similar coalitions and champion outreaches among clinics and communities where relevant information is disseminated among audiences.

Non-governmental institutions: There are several opportunities in which non-governmental organizations can be engaged in mitigating PTB. The March of Dimes is a non-governmental organization committed to addressing the unacceptably high PTB rates in the US and globally. In 2017, the March of Dimes convened the Prematurity Campaign Collaborative of 200 leading maternal and child health organizations and experts nationwide. The March of Dimes Prematurity Campaign activities include research and discovery, community engagement, advocacy and education. Opportunities should be made available to organizations such as March of Dimes to continue to gain strides in the fight against PTB in the US and across the world.

- In summary, key programmatic recommendations include the following: Health communication interventions are needed to improve knowledge and perceptions related to PTB, eating healthy and taking folic acid supplements
- Explore opportunities to within existing nutritional programs such as WIC to incorporate interventions providing access to folate rich foods and folic acid supplements

- PTB coalitions should continue to be supported financially and other necessary resources to address PTB rates and risk in vulnerable populations

Clinical Implications

Optimizing maternal folate status: Improving folate status before and during pregnancy would be a low-cost intervention to prevent PTB. With over a third of all US pregnancies being unintended (20), improving pre-pregnancy health is important for all women of reproductive age in order to optimize maternal and child health outcomes on a national level. (21). In keeping with ACOG recommendations, folic acid supplementation should be given to women with low levels in pregnancy.

Screening for folate status in pregnancy: This means that clinicians need to directly ask their patients of childbearing age about their dietary habits as well as folic acid consumption. Also, obstetricians need to monitor their patients' nutritional status and folic acid consumption closely. The indications or guidelines for the collection of biomarkers of folate status versus reliance on self-reported supplement intake needs to be developed or updated. Prenatal assessment commonly conducted by clinicians during the first prenatal visit is a good opportunity to collect information on and assess maternal folate status as well as address indicators of lower maternal folate status such as poor diet or low supplement intake. This would involve an additional set of checklists on the form for supplement intake and dietary habits.

Patient care coordination: There is a great need to not just diagnose patients with low folate status or other risks for PTB but to connect patients to where they can get the help that they need to address identified health issues. Hospitals, service organizations and health departments should work together to institute functional care coordination centers that link women identified

by clinicians to be at risk for PTB or have low folate status with available services to address their needs such as home visiting, nutrition/weight programs, social services. An example is HealthCare Access Maryland, a nonprofit agency that connects residents to public health care coverage and helps them navigate services effectively. Each year, HealthCare Access Maryland employees connect more than 125,000 uninsured and underinsured clients to health insurance, health care, and vital community resources. The programs and services offered by HealthCare Access Maryland are designed to bridge gaps in services to pregnant and postpartum women, and other vulnerable groups. Such programs are especially useful among low income urban minority populations.

Counseling on consistent folic acid supplementation: This study reaffirmed the importance of consistent folate intake throughout pregnancy and not just in the periconception period to mitigate PTB risk (22-24). The study demonstrated minimal difference in PTB mitigation by multivitamin supplement intake of 3-5 times 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, it suggests that the same protective benefit can be derived from a thrice weekly compared to a daily dosing. However, it is crucial that women continue to take their supplements throughout pregnancy and not discontinue use prior to delivery. Clinicians should be trained and given resources to counsel women adequately on optimizing their folate status.

Mitigating effects of preeclampsia or IUI: as a result of delayed childbearing ,the prevalence of hypertension and preeclampsia is projected to increase over the next decade (25, 26). Administering folic acid in combination with anti-hypertensive drugs compared with only anti-hypertensive drugs has been shown to reduce the risk of cardiovascular and stroke events in

patients with hypertension (27). There is a great need to further investigate the extent to which folic acid administration can mitigate preeclampsia complications in pregnancy as well as reduce the need for medically indicated PTB.

In summary, key clinical recommendations include:

- Include dietary habits and use of multivitamin supplements in prenatal assessments
- Clinicians should be trained on how to counsel women on how to optimize folate status
- Efficient patient care coordination is needed to link women with low folate status and other modifiable PTB risk factors to the services that they need

Conclusion

PTB is considered one of the leading health indicators of a nation and is a public health priority in the US because of its associated infant and childhood morbidity and mortality as well its substantial economic burden for the U.S (28, 29). Thus, additional novel and early prevention or intervention approach is needed to further lower PTB rates in the US and in the world. An article by Jain and Gyamfi-Bannerman (30) stated *"with evidence that 95% of cases of spontaneous preterm birth are intractable to current interventions, our best hope in resolving this problem may lie in new, innovative ideas"*.

This dissertation presented a new and innovative approach to understanding and mitigating PTB risk. The research addressed a key gap in maternal health research and added to the literature on the relationship between maternal folate status and PTB as well as the little known mechanistic pathway underlying the association between maternal folate status and PTB. This study examined the relationship between folate and PTB by exploring

multivitamin supplement intake from preconception to third trimester; as well as maternal plasma folate status at delivery. In addition, the study investigated to what degree the observed association between maternal folate and PTB was mediated by preeclampsia or (IUI)- two major pathways for PTB.

The analyses included 7565 mother-newborn dyads and a subsample (n=2313) with plasma folate assay at delivery in Boston Birth Cohort, a predominantly urban, low income minority population. Unadjusted and adjusted logistic regressions revealed an inverse relationship between both multivitamin supplement intake and plasma folate levels with PTB. Throughout pregnancy, consistent intake of 3-5 times or more than 5 times a week was associated with reduced PTB risk. Such beneficial effect was observed in the third trimester. Consistently, compared to the lowest quartile, the highest quartile of plasma folate level was associated with a reduction in PTB risk; and the association was consistent for spontaneous and medically indicated PTB.

Consistent multivitamin supplement intake of three or more times a week in the 3rd trimester as well as higher levels of plasma folate was associated with reduced odds of preeclampsia as well as IUI. Preeclampsia mediated 62% of the multivitamin supplement intake- medically indicated PTB relationship. Both multivitamin supplement intake and plasma folate concentrations were associated with reduced odds of IUI. Multivitamin intake ameliorated the relationship between IUI and PTB, particularly spontaneous PTB. Adjusted logit regressions showed that regardless of IUI status, the probability of PTB and spontaneous PTB decreased as plasma folate concentrations increased.

Thus, this dissertation demonstrated that optimizing maternal folate levels across pregnancy may help reduce the risk of PTB, and this is partly due to the protective effect of

folate against preeclampsia and IUI.

Additional prospective research is needed to corroborate study findings as this research is directly relevant to policy on the recommendation of folate intake and interventions, specifically among vulnerable US- urban low-income minority populations. The results of this dissertation have a potential impact on ongoing research, clinical and programmatic efforts to improve prepregnancy and prenatal nutrition and birth outcomes and may help inform the design and implementation of nutrition-based screening and interventions to prevent PTB and associated short-term and long-term consequences.

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Curriculum Vitae

BOLANLE OLAPEJU MBBS, MSPH, CPH

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FOCAL AREAS

Malaria
Intermittent Presumptive Treatment in pregnancy; Insecticide Treated Net Use, Seasonal Chemoprophylaxis, Rapid Diagnosis and Testing, Social Behavior Change Communication,

Survey Implementation:
-Study Protocols development
-Institutional board review
Training agendas/manuals
-Interactive learner friendly training
-Teleform data entry
-Mobile data collection
-Performance Monitoring
-Meta data analysis

Biostatistics:
Longitudinal-, Multilevel-, Factor-Analysis; Propensity Score Matching; Structural Equations, Path-, Mediation-, Geospatial- and Network Analysis.

Qualitative Data Analysis- coding and interpretation

SOFTWARE PROFICIENCY

Statistics: STATA, R, SPSS, MPLUS, UCINET/ NetDraw

Geographic Information System: ArcGIS, CartoDB, Google Earth

Qualitative data: Atlat.ti, Nvivo

Business Intelligence: REDCAP, PowerBI

Mobile Data Collection: Magpi, Open Data Kit, SurveyCTO, Commcare

Literature Review: Endnote, Mendeley, Refworks

I am an internationally trained physician and certified public health expert with over ten years' experience. I am highly proficient in survey design, training of data collectors, survey implementation, data analysis and results dissemination. I have analyzed DHS/MIS data from various countries and very familiar with the malaria modules, the mobile DHS app and StatCompiler. As a researcher, I am abreast of all aspects of vector control, chemoprevention, testing, diagnosis and treatment of malaria. My background in Sub- Saharan Africa affords me a unique understanding of the challenges and opportunities of survey implementation low resource settings. I am a dynamic leader with excellent verbal and written communication skills. I am also well versed in innovative electronic/mobile data collection technology for survey implementation. I am certified in DHIS2, proficient in GIS and advanced statistical methods. My passion is researching and implementing interventions to improve health outcomes among vulnerable and minority populations.

PROFESSIONAL EXPERIENCE- PUBLIC HEALTH RESEARCH

SENIOR RESEARCH DATA ANALYST

Center for Communication Programs: 02/2016- Date

The Center for Communications programs (CCP) is a renowned heath communication organization with over 60 projects in more than 30 countries and with a yearly expenditure of over $100 million

Provide high level technical oversight to all levels of public health research, monitoring and evaluation activities such as

- Designing the malaria behavior assessment questionnaire
- Analysis of DHS/MIS data
- Conceptualizing research protocols and study tools
- Developing mHealth data collection tools
- Training in-country data collectors
- Report writing and dissemination

Select Projects include: *VectorWorks- Global, Health Communication Capacity Collaborative- Global, Urban Reproductive Health Initiative- Nigeria*

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EDUCATION

Johns Hopkins Bloomberg School of Public Health
PhD Candidate, '18

Johns Hopkins Bloomberg School of Public Health
MSPH Public Health, *'12*

Obafemi Awolowo College of Health Sciences, Nigeria
MBBS, 2008

HONORS/AWARDS

Chenoweth-Pate Award; 2016
HRSA Trainee Fellowship; 2015
Apgar/Bramley/Clifford Award; 2014
Metamorforces Nigeria Award for Public Health

CERTIFICATION

Certified in Public Health (CPH)
USAID Global Health Certificate in Maternal Health
JHSPH Certificate in Maternal and Child Health
Strategic Leadership
CITI certification in human subjects' research

RESEARCH ASSISTANT

JHPIEGO- September - December 2015

Worked with the Immediate Postpartum Implants (IPPI) project- a Gates funded research initiative to increase the understanding, acceptability and feasibility of immediate postpartum implants in Indonesia and Kenya. Duties included:

- Conceptualization of study research design.
- Analysis of Demographic Health Survey (DHS) data
- Development of study research protocol and tools

Johns Hopkins School of Public Health: 2014–2015

Worked as the data entry team leader and research assistant with the Department of Population, Family and Reproductive Health. Specific duties include:

- Conducting literature reviews.
- Advanced quantitative data analysis.
- Developed protocols for data entry.
- Monitored all stages of data entry.
- Manuscript writing

Johns Hopkins Center for Communication Programs: 2012 - 2014

Provided technical support to formative and evaluation research activities in several countries and conducted qualitative and quantitative analysis on national databases. Select projects include:

Urban Reproductive Health Initiative (Nigeria and Kenya)

Provided technical support to the following studies: longitudinal study on contraceptive discontinuation, simulated client study to assess capacity of family planning providers, and baseline and midterm evaluation of the flagship project.

SELECTED PAPERS

Olapeju, B et al. Maternal Folate Status and Preterm Birth in the Boston Birth Cohort (*submitted 2017*)

Makinde, O, **Olapeju, B**, Ogbuoji, O, Babalola, S.(2017). Trends in the Completeness of Birth Registration in Nigeria: 2002 – 2010. *Demographic Research*

Babalola, S., Kusemiju, B., Calhoun, L., Corroon, M., & **Olapeju, B**. (2015). Factors associated with contraceptive ideation among urban men in Nigeria. *IJOG.*

Babalola, S., John, N., **Ajao, B**., & Speizer, I. S. (2015). Ideation and intention to use contraceptives in Kenya and Nigeria. *Demographic Research*.

SELECTED POSTERS

Who Buys Nets? Factors Associated with Mosquito Net Purchase in Sub-Saharan Africa. 2017

Influence of a Mass Media Health Communication Campaign on Family Planning Behaviors and Intentions in Ghana. 2015.

Modern Contraceptive Use among Married Women in **Indonesia** who are Undecided on Their Next Pregnancy. 2015

• Activities included literature reviews, development of study tools and training manuals for research, development of technical reports and dissemination tools, co-authoring of peer-reviewed papers for publication.

Behavior Change Support Project (Ghana)

Provided key technical support on this integrated multi-focus health and behavior change program. Key focus areas included family planning, maternal and child health, nutrition, water and sanitation and malaria.

• Activities included: revisions to study protocol and data collection tools, submission to institutional review boards; development of training agenda for field staff, database cleaning and management, analysis of quantitative endline survey data and development of a final report.

Research to Prevention (R2P) (Botswana)

Present in-country as the Senior Research Coordinator for this HIV/AIDS risk/prevention study.

• Represented CCP on collaborations and meetings with project stakeholders including in-country MOH and PEPFAR staff;

• Assisted with training the local team on time-location sampling procedures, use of mHealth technology, database development and field supervision

• Coordinated field activities for this translational research study on alcohol use, behavior risk and HIV, coaching team leaders in quality control checks and problem solving.

• Used Magpi software to develop all phases of an electronic survey for mobile data collection.

• Conducted capacity strengthening for in-country staff on qualitative and quantitative analysis using Atlas.ti and SPSS respectively.

• Assisted with the generation of codebooks, coding of transcriptions and qualitative data analysis using Atlas.ti

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SELECTED REPORTS

ITN Use Access Ratio Report.2017

Ghana ITN Use Memo.2017

Post-Campaign Survey for Social Marketing of Long Lasting Insecticide-treated Nets in Dakar, Senegal, 2017.

National Knowledge, Attitude and Practices Survey on Ebola Virus Disease in Liberia. 2017

Nigerian Urban Reproductive Health Institute- Simulated Client Study. Baseline Report 2014

Ghana Behavior Change Support Project. Evaluation Report. 2014

Integrated HIV Serological and Behavioral Surveillance among Persons Attending Alcohol Consumption Venues in Gaborone, Botswana. 2013

Nigeria Northern Education Initiative. Baseline Study on Gender Inequalities in Education and Living Conditions of Orphans and Vulnerable Children in Bauchi and Sokoto States. 2012

PROFESSIONAL EXPERIENCE- PUBLIC HEALTH PROGRAMS

PHYSICIAN: 2008 – 2011

Lagos University Teaching Hospital

Worked at the Hematology Center which received funding and support from the International Center for AIDS Care and Treatment Programs (ICAP) and President's Emergency Plan for AIDS Relief (PEPFAR). Duties included:

- Provided clinical care, support and treatment to PLWHA
- Managed the day-to-day operations of HIV clinics
- Implemented community-based capacity-strengthening programs to increase and knowledge of HIV in rural communities.

COMMUNITY HEALTH MOBILIZER/ PROGRAM COORDINATOR

(2002-2007)

Nigerian Conference of Medical and Dental Students

- Developed entertainment-education campaigns focusing on global health issues such as malaria, HIV/AIDS, maternal and child health.
- Organized the provision of basic food and clothing for women and children in the poorest communities
- Provided mental health counselling.
- Supported community health awareness programs on maternal, child and geriatric health.