Reproductive Sciences
19(9) 939-948

DOI: 10.1177/1933719112438442
http://rs.sagepub.com
SAGE

Maternal Micronutrient Status and Preterm Versus Term Birth for Black and White US Women

Anne L. Dunlop, MD, MPH[1], Robert N. Taylor, MD, PhD[2], Vin Tangpricha, MD, PhD[3], Stephen Fortunato, MD[4], and Ramkumar Menon, PhD[5]

Abstract

Objective. Micronutrient deficiencies are hypothesized to play a role in spontaneous preterm birth (PTB; <37 weeks of gestation) and possibly the racial disparity in rates of PTB between black and white women. Yet relatively few studies have addressed the role of micronutrient deficiencies in spontaneous PTB among black and white women in the United States. The purpose of this study was to investigate whether 25-hydroxy vitamin D (25-OH-D), folate, and omega-6/omega-3 fatty acid status are associated with spontaneous PTB among black and white women in the United States. ***Methods.*** Biospecimens and medical record data for this study were derived from a subsample of the 1547 women enrolled into the Nashville Birth Cohort during 2003-2006. We randomly selected 80 nulliparous and primiparous women for whom stored plasma samples from the delivery admission were available and analyzed the stored plasma for 25-OH-D, folate, and total omega-6/omega-3 fatty acids. We used multivariate logistic regression to assess the odds of spontaneous PTB among women with 25-OH-D <20 ng/mL, folate <5 ug/L, and omega-6/omega-3 >15. ***Results.*** An omega-6/omega-3 ratio >15 was significantly associated with spontaneous PTB for white (adjusted odds ratio [aOR] 4.25, 95% confidence interval [CI] 1.25-14.49) but not black women (aOR 1.90, 95% CI: 0.69-5.40), whereas no significant relationships were observed for folate and 25-OH-D status and PTB for black or white women. ***Conclusion.*** Maternal plasma total omega-6/omega-3 fatty acid ratio >15 at delivery was significantly associated with spontaneous PTB for white, but not black, women.

Keywords

micronutrients, nutritional deficiencies, preterm birth

Introduction

A substantial proportion of cases of spontaneous preterm birth (PTB; <37 weeks of gestation) are attributable to activation of the inflammatory pathway,[1] which is the effector pathway posited to account for the approximately two-fold higher rate of PTB among US black compared with white women.[2,3] Factors hypothesized to affect the inflammatory pathway to PTB include nutritional status (micronutrient deficiencies and weight status), reproductive tract and intrauterine infections, and psychosocial stressors.[4] Relatively limited clinical research has addressed the role of specific nutrients in spontaneous PTB among black and white women in the United States.[4] Existing data demonstrate a black–white racial disparity in vitamin D, folate, and essential fatty acid nutriture among women of reproductive age,[5–8] and emerging data suggest that these nutrients may be linked with PTB, as described below.

Vitamin D

Vitamin D deficiency is hypothesized to underlie the observed seasonality in the rate of PTB in the United States.[9] The placenta expresses vitamin D receptor as well as vitamin D_3 25-hydroxylase, which converts vitamin D into 25-hydroxy vitamin D (25-OH-D; the major circulating form of vitamin D) and vitamin D_3 1-alpha hydroxylase, which converts 25-OH-D to its active form, 1,25-dihydroxyvitamin D (1,25-diOH-D).[10] A significantly higher 1-alpha hydroxylase expression has been found in the placental tissue of pregnant

[1] Department of Family & Preventive Medicine, Emory University School of Medicine, Atlanta, GA, USA
[2] Department of Gynecology–Obstetrics, Emory University School of Medicine, Atlanta, GA, USA
[3] Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA
[4] Department Maternal-Fetal Medicine, Perinatal Research Center, Nashville, TN, USA
[5] Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX, USA

Corresponding Author:
Anne L. Dunlop, Department of Family & Preventive Medicine, Emory University School of Medicine, 1256 Briarcliff Road, Building A, Suite 210-11, Atlanta, GA 30306, USA
Email: amlang@emory.edu

women with preeclampsia or PTB compared to healthy pregnant women, whereas the expression of 25-hydroxylase was significantly reduced resulting in a decrement in the placental ability to synthesize adequate amounts of 1,25-diOH-D.[11] 1,25-Dihydroxyvitamin D is a potent regulator of placental immunity, stimulating antimicrobial responses while suppressing inflammation.[12]

It is theorized that vitamin D insufficiency may increase susceptibility to infection and inflammation and, thereby, increase the risk of PTB. However, to date few trials of vitamin D supplementation have been conducted in pregnant women with adequate power to test effects on birth outcomes and, furthermore, black pregnant women have rarely been studied in vitamin D birth outcomes research.[13] A recently published randomized controlled trial among women with a singleton pregnancy who initiated vitamin D supplementation of 400, 2000, or 4000 IU of vitamin D_3 daily (with their eligibility for each of the study arms determined by their baseline circulating 25-OH-D) from 12 to 16 weeks of gestation until delivery concluded that vitamin D supplementation of 4000 IU daily is safe and more effective than 400 IU or 2000 IU daily in achieving sufficiency in all women and their neonates (defined as a concentration of 80 nmol/L or greater within a month of delivery), regardless of race and that maximal production of 1,25-diOH-D for all racial and ethnic groups occurred for the 400 IU daily group.[14] The gestational age of the pregnancies at delivery, maternal and infant health outcomes, and long-term bone outcomes for the women and their birthed infants are not provided in the report.[14]

Folate

Two US prospective observational studies found that low dietary intake of folate and low serum folate concentrations during the second trimester were associated with a 2-fold greater risk of PTB,[15,16] and another found that supplementation with folic acid (the synthetic form of folate) for 1 year prior to conception was linked with a decreased risk of PTB.[17] Intervention trials of prenatal folic acid supplementation have been conflicting, however.[18–25] Low serum folate is associated with impaired T-cell and neutrophil function and an increased prevalence of bacteriuria in pregnancy.[26–28] In a randomized trial in Nepal, folic acid supplementation decreased markers of inflammation during pregnancy, although this same study found no effect on PTB.[29]

Essential Fatty Acids

Essential fatty acids contribute to the membrane phospholipid pool and regulate the production of inflammatory mediators, including prostaglandins and proinflammatory cytokines.[30] Danish cohort studies have found an association between fish consumption, a major dietary source of essential fatty acids, and length of pregnancy.[31,32] A case–control study found that women with PTB had higher serum levels of arachidonic acid (20:4 omega-6) and decosapentaneoic acid (a marker of omega-3 deficiency) than did women with term birth.[33] A recent prospective cohort of 523 healthy pregnant women found that total fasting plasma free fatty acid concentrations in the highest tertile at 30 weeks of gestation experienced a 2-fold increased risk of spontaneous PTB (adjusted odds ratio [aOR] 2.35, 95% confidence interval [CI]: 1.05-5.28), and that this effect was independent of maternal pre-pregnancy obesity and tobacco use.[34] A Cochrane review of 6 randomized trials of marine oil supplementation in northern European parturients found a lower risk of birth <34 weeks of gestation among pregnant women randomized to marine oil supplementation.[33–35] A meta-analysis of 6 randomized trials of marine oil supplementation among European women who had experienced a previous PTB found that women receiving marine oil supplementation experienced a significant reduction in the recurrence of PTB (OR 0.54; 95% CI: 0.30-0.98) in comparison to women who received olive oil supplementation.[36] In contrast, a randomized controlled trial of omega-3 supplementation (beginning from 16-22 weeks' gestation) involving US women with a prior spontaneous PTB who were also receiving weekly intramuscular 17-hydroxyprogesterone caproate found no reduction in recurrent PTB among those receiving omega-3 supplementation.[37] A potential explanation for the differing results between the European[36] and US[37] studies could be that the marine oil supplement used in the European studies can be a significant source of vitamin D[38], whereas the omega-3 supplement (eicosapentenoic acid and docosahexanoic acid) used in the US study would not have vitamin D.

The purpose of this study was to explore whether maternal serum concentrations of 25-OH-D, folate, and the ratio of omega-6 to omega-3 fatty acids measured during the delivery admission were associated with PTB among black and white women.

Materials and Methods

Design Overview

This study involved the biochemical assessment of concentrations of nutrients in stored plasma specimens from women enrolled in the Nashville Birth Cohort at Centennial Women's Hospital, Nashville, Tennessee, which is a tertiary care hospital that receives referrals and transfers of high-risk patients from an area that encompasses a 100-mile radius around Nashville.[39] The samples used for this study were collected as part of an ongoing genetic study, approved by the Western Institutional Review Board, of racial disparities in PTB. All patient participants provided written informed consent at the time of enrollment, and the consent form sought permission to store biosamples for future testing and research. The EDTA plasma samples collected during each woman's delivery admission were evaluated for associations among plasma micronutrient concentrations, PTB versus term birth, race, and body mass index (BMI). The Emory University Institutional Review Board also approved the study protocol.

Sample

Biospecimens and medical record data for this study were derived from a subsample of the 1547 women enrolled into the Nashville Birth Cohort during 2003-2006. For our study sample, we used a computerized random number generator to randomly select 80 women (40 non-Hispanic black and 40 non-Hispanic white) with spontaneous PTB and 80 with term birth (N = 160) from among those participants who were nulli- or primparous upon enrollment; primparas could not have experienced a prior PTB. We choose to study 160 total women (equally divided between those who were black and white) as this was a pilot investigation with sufficient funding to perform the biochemical assessments on this limited number of participants in order to yield important preliminary data to guide the design of future studies. Due to the availability of a limited quantity of stored plasma, biochemical assessment of vitamin D status was performed on the entire study sample (N = 160), while biochemical assessments of essential fatty acid and folate status could only be performed for 155 and 114 participants, respectively.

Criteria for enrollment into the Nashville Birth Cohort included being a woman ≥18 years at the time of the delivery admission who was pregnant with a singleton infant without anomalies. Women with multiple gestations, preeclampsia, placenta-previa, fetal anomalies, medical/surgical complications of pregnancy, and drug or alcohol abuse were excluded. Preeclampsia was defined as a blood pressure on 2 separate readings taken at least 4 hours apart of 140/90 or more *and* ≥300 mg of protein in a 24-hour urine sample (proteinuria). Participants who had any surgical procedures during pregnancy or who were treated for preterm labor or for suspected intra-amniotic infection and delivered at term were excluded.

Cases of PTB were defined as women who delivered between 22-0/7 and 36-6/7 weeks of gestation after a spontaneous onset of labor (defined as the presence of regular uterine contractions at a minimum frequency of 2 contractions per 10 minutes); medically indicated cases of PTB were excluded as were those with preterm premature rupture of membranes diagnosed by positive Amnisure test, fern test, or amniotic fluid pooling. Controls were defined as women with term labor and delivery (≥37-0/7 weeks of gestation), who had intact membranes and no pregnancy-related complications.

Clinical Data

Data abstracted from the medical records of women enrolled into the Nashville Birth Cohort were used for this study, including the following:

Gestational age. The gestational age at delivery was ascertained by review of the prenatal records. Both the last menstrual period (LMP) and ultrasound before the 20th week of gestation were used to assign gestational age. If the LMP and ultrasound results were discordant, the ultrasound result was used, according to the accepted clinical criteria.

Obstetrical history and pregnancy outcome. Information related to the number of prior pregnancies and births and the outcomes of pregnancies and births was ascertained via review of the prenatal record (which included an obstetrical interview conducted at the first prenatal visit). Using the gestational age of the index pregnancy (determined as above), the deliveries were dichotomized as preterm (22-0/7 through 36-6/7 weeks of gestation) or term (≥37 weeks of gestation). Medical record data were used to ascertain preterm premature rupture of membranes prior to the birth, and whether the preterm labor was induced for medical indications.

Maternal race/ethnicity. Maternal race was self-identified based on the race/ethnicity of their own parents and grandparents. Those who identified as being of Hispanic ethnicity, as well as those of mixed race, and those who were unsure of the race/ethnicity of their parents and grandparents were excluded from this study. Those who identified each of their parents and grandparents as black were regarded as black for this study, and similarly for whites as we have reported previously.[39]

Body mass index. Pre-pregnancy BMI was calculated from measured height at the first prenatal visit and patients' report of their pre-pregnancy weight at the first prenatal visit. The BMI was categorized according to accepted definitions (obesity ≥30 kg/m^2, overweight 25-29.99 kg/m^2, healthy weight 18.5-24.99 kg/m^2, and underweight <18.5 kg/m^2).

Other established risk factors for PTB. Information on other known risk factors for PTB were also obtained via review of the medical record: maternal age, reproductive and urinary tract infections, and behavioral risks (tobacco, alcohol, and drug use).

Biological Specimens

Biological sample acquisition occurred at the time of the delivery admission for all consented participants. Nonfasting venous blood drawn routinely as part of the delivery admission was obtained and an extra aliquot of EDTA plasma was centrifuged and stored at −80°C for up to 5 years. Thawed EDTA plasma was analyzed for the following nutrients and measurements were categorized as described below. All assays were performed by technicians without knowledge of case–control status.

Vitamin D. Plasma 25-OH-D concentration, the major circulating form of vitamin D, was assayed using a commercially available ELISA kit (Immunodiagnostic Systems, United Kingdom), the range of detection which is 2 to 120 ng/mL. Analyses were performed in the Endocrinology laboratory of Emory School of Medicine, which is nationally accredited for performing this assay. Vitamin D deficiency was defined as a measured concentration of 25-OH-D <20 ng/mL.[40]

Folate. Plasma folate concentration was measured by competitive-binding chemiluminescent radioimmunoassay

using competitive displacement of 125-I folic acid from intrinsic factor and folate binding proteins immobilized on microcrystalline cellulose. Analyses were performed by Emory Healthcare laboratory, an accredited clinical and research laboratory using a Beckman Coulter DX1800 (Beckman Coultern, Inc, Fullerton, California). Folate deficiency was defined as a measured serum concentration <5 ug/L.[41]

Omega-6 and Omega-3 fatty acids. Fatty acid analyses were performed in the Lipoprotein Analysis Laboratory, Wake Forest University School of Medicine. Briefly, the total lipid fraction was extracted from 100 uL of plasma, and the phospholipid fraction was isolated by liquid chromatography on silica gel plates. Fatty acid methyl esters were prepared by transesterification involving saponification with 0.5 NaOH in methanol and methylation using 14% boron trifluoride-methanol $(BF)_3$ in methanol followed by extraction in hexane. The fatty acid methyl esters were separated via capillary column gas chromatography and identified by flame ionization. Retention times were determined with mixed fatty acid methyl ester standards from NuChek Prep (Elysian, Minnesota) and quantification by comparison with the peak areas of the internal standards provided absolute concentrations. Polyunsaturated fatty acids were expressed as a ratio of total omega-6 to omega-3 fatty acids. Presently, there is not a defined cut point for an excessive omega-6/omega-3 fatty acid ratio; in this study, the highest tertile was a ratio of omega-6/omega-3 >15, and this was used to categorize the data.

Data Analysis

We compared demographic and clinical characteristics of women with preterm and term deliveries using Student *t* test for continuous measures and Pearson chi-square or Fisher exact test for categorical measures. We stratified our analysis based upon maternal race because of the body of research that demonstrates variability in risk exposures by race.

We compared the mean concentrations of plasma 25-OH-D and folate and the mean ratio of total omega-6/omega-3 fatty acids for women with PTB versus term birth, stratifying by maternal race and obesity, using Student *t* test. Next, we assessed the correlation among the nutrient measures of interest, in the presence and absence of maternal obesity, using Pearson correlation methods. Due to potential confounding by correlation among the nutrients, we further evaluated the linear relationship between the nutrient measures of interest and gestational age in weeks in separate multivariate linear regression models that included variables for maternal age, race, body mass index, income, health care payor, and marital status.

We compared the proportion of women with PTB versus term birth who had plasma 25-OH-D <20 ng/mL, folate <5 ug/L, and total omega-6/omega-3 fatty acid ratio >15 using Pearson chi-square test. We stratified the comparison of birth outcomes × black white race, obese versus nonobese status and nutrient status, assessing for biological interaction using the Breslow-Day test statistic. We used multivariate logistic regression to assess the independent and interactive effects of the nutrient measures in separate models that included covariates noted to be linked with PTB in previous research, including age, race, obesity, health care payor, marital status, smoking status, and bacterial vaginosis during the pregnancy for black and white women, separately and combined. All hypothesis testing and reported probability values were 2 tailed.

Table 1. Demographic Characteristics of Women With Preterm and Term Births

Characteristic	Preterm Birth (n = 80)	Term Birth (n = 80)
Maternal age—mean years (SD)		
All	25.5 (6.2)	26.4 (6.0)
Black	23.5 (5.2)	24.9 (5.6)[a]
White	27.5 (6.4)	28 (6.0)[a]
Education—mean years (SD)		
All	13.6 (2.7)	14.0 (2.4)
Black	13.4 (2.8)	13.6 (2.2)
White	13.7 (2.7)	14.5 (2.6)
BMI—kg/m^2 (SD)		
All	25.9 (6.8)	26.3 (6.8)
Black	26.5 (7.1)	27.6 (8.1)
White	25.3 (6.6)	25.0 (5.2)
Teenager—n (%)		
All	14 (17.5%)	9 (11.3%)
Black	9 (22.5%)	6 (15.0%)
White	5 (12.5%)	3 (7.5%)
Less than high school—n (%)		
All	18 (22.5%)[b]	7 (8.8%)[b]
Black	11 (27.5%)	5 (12.5%)
White	7 (17.5%)	2 (5%)
Single marital status—n (%)		
All	43 (53.8%)[b]	26 (32.5%)[b]
Black	28 (70.0%)[a,b]	19 (47.5%)[a,b]
White	15 (37.5%)[a]	7 (17.5%)[a]
Medicaid—n (%)		
All	44 (55%)	38 (47.5%)
Black	27 (67.5%)[a]	27 (67.5%)[a]
White	17 (42.5%)[a]	11 (27.5%)[a]
BMI $\geq$ 30 kg/m^2—n (%)		
All	21 (27.3%)	23 (28.8%)
Black	11 (29.7%)	14 (35%)
White	10 (25%)	9 (22.5%)

Abbreviation: SD, standard deviation.
[a] Indicates a significant difference for comparison between black and white women ($\alpha = .05$ level).
[b] Indicates a significant difference for comparison between women with preterm and term deliveries ($\alpha = .05$ level).

Results

Demographic and clinical characteristics of black and white women with PTB and term birth are given in Table 1. The mean gestational age for PTB and term birth to enrolled women was 33.9 ($\pm$ 2.8) weeks and 39.1 ($\pm$ 1.1) weeks,

Table 2. Comparison of Mean Nutrient Levels for Preterm and Term Births

Variable	Preterm Birth (n = 80)	Term Birth (n = 80)	*P* Value Preterm vs Term
Folate (ug/L)—mean (SD)			
All	9.9 (4.8)	11.2 (4.4)	.160
Black	9.7 (5.2)	10.9 (4.4)	.410
White	10.3 (4.2)	11.3 (4.5)	.365
25-OH-D (ng/mL)—mean (SD)			
All	22.8 (11.0)	24.8 (12.5)	.295
Black	18.2 (5.9)[a]	18.8 (8.1)[a]	.741
White	27.4 (13.0)[a]	30.8 (13.3)[a]	.252
Omega-6:Omega-3 ratio—mean (SD)			
All	14.6 (3.2)[b]	12.9 (3.1)[b]	.001
Black	15.0 (2.7)	13.7 (3.0)[a]	.057
White	14.2 (3.7)[b]	12.0 (3.0)[a]	.006

Abbreviation: SD, standard deviation.
[a] Indicates a significant difference in mean nutrient concentration between black and white women ($\alpha = .05$ level).
[b] Indicates a significant difference in mean nutrient concentration between women with preterm and term deliveries ($\alpha = .05$ level).

Table 3. Mean Nutrient Levels for Obese (BMI $\geq$ 30 kg/m^2) and Nonobese (BMI < 30 kg/m^2) Women

Variable	Pre-Pregnancy BMI $\geq$ 30 kg/m^2 (n = 44)	Pre-Pregnancy BMI < 30 kg/m^2 (n = 113)	*P* Value
Folate (ug/L)—mean (SD)			
All	11.49 (4.83)	10.29 (4.43)	.214
Black	11.74 (4.85)	9.42 (4.65)	.110
White	11.19 (4.97)	10.84 (4.25)	.794
25-OH-D (ng/mL)—mean (SD)			
All	21.23 (9.11)	24.92 (12.61)	.079
Black	18.48 (9.10)	18.50 (5.70)	.991
White	24.84 (7.97)	30.39 (14.25)	.060
Omega-6/Omega-3 ratio—mean (SD)			
All	13.81 (3.49)	13.35 (2.77)	.447
Black	14.56 (2.97)	13.74 (2.96)	.261
White	13.18 (3.77)	12.78 (2.43)	.682

Abbreviations: BMI, body mass index; SD, standard deviation.

Table 4. Pearson Correlation Coefficients (*P* Value) for Plasma Folate, 25-OH-D, and Omega-6 to Omega-3 Ratio for Women at Delivery

Nutrient Concentrations	Folate	Omega-6/Omega-3
Folate		
Overall		–0.278 (0.003)
Obese (BMI $\geq$ 30 kg/m^2)		–0.223 (0.228)
Nonobese (BMI < 30 kg/m^2)		–0.285 (0.010)
25-OH-D		
Overall	0.120 (0.205)	–0.167 (0.038)
Obese (BMI $\geq$ 30 kg/m^2)	0.188 (0.311)	–0.311 (0.045)
Nonobese (BMI < 30 kg/m^2	0.109 (0.336)	–0.145 (0.129)

Abbreviations: 25-OH-D, 25-hydroxy vitamin D; BMI, body mass index; SD, standard deviation.

respectively ($P < .001$). The mean gestational age of births to black and white women was not different. Women with PTB were significantly more likely to be currently single (53.8% vs 32.5%; $P < .001$) and significantly less likely to have a high school education (22.5% vs 8.8%; $P = .028$). The mean maternal age was significantly lower for black compared to white women for both PTB and term birth ($P = .003$ and $P = .018$, respectively). The proportion of women who were single or with a Medicaid payor source was significantly higher for black versus white women with PTB ($P = .004$ and $P = .001$, respectively) and term birth ($P = .025$ and $P = .001$), respectively.

The mean concentrations of plasma 25-OH-D, folate, and total omega-6/omega-3 fatty acid ratio according to birth outcome and maternal race are given in Table 2. There was no difference in the mean concentration of 25-OH-D for black and white women (combined or separately) with PTB compared with term birth. The mean concentration of 25-OH-D was significantly lower for black women with PTB and term birth compared to white women with PTB and term birth ($P < .001$ for both comparisons). There was not a significant difference in the mean concentration of folate for women with PTB versus term birth. The mean total omega-6/omega-3 fatty acid ratio was higher among black and white women (combined) with PTB compared with term birth and for white women with PTB compared with term birth, whereas no significant difference was found for black women. The mean omega-6/omega-3 fatty acid ratio was significantly greater among black women compared to white women with term births ($P = .015$), whereas there was not a significant difference between black and white women with PTB ($P = .310$).

Among women whose pre-pregnancy BMI was $\geq$30 kg/m^2, while the plasma folate concentration and the mean total omega-6/omega-3 ratio were higher for black and white women (combined and separately) compared with those whose BMI was <30 kg/m^2 these differences were not statistically significant (Table 3). Conversely, the mean 25-OH-D concentration was lower for black and white women (combined and separately) whose pre-pregnancy BMI was $\geq$30 kg/m^2 compared with those whose BMI was <30 kg/m^2, although these differences were not statistically significant either. There was a significant association between the presence of bacterial vaginosis during the pregnancy and folate <5 ug/L (42.9% vs 10.3%; $P = .039$).

Correlation analysis revealed statistically significant correlations among the nutrient measures of interest (Table 4). Specifically, there were statistically significant negative correlations between the total omega-6/omega-3 fatty acid ratio and the concentration of folate (-0.278, $P = .003$) and 25-OH-D (-0.167, $P = .038$). When stratifying on pre-pregnancy BMI ($\geq$30 kg/m^2 or <30 kg/m^2), the correlation between total omega-6/omega-3 fatty acid ratio and folate concentration was stronger for nonobese compared with obese women, whereas the correlation between the omega-6/omega-3 fatty acid ratio and 25-OH-D concentration was stronger for obese women.

Table 5. Comparison of Proportion of Women With Preterm and Term Births With Nutrient Deficiencies or Imbalances

Nutrient Level	Preterm Birth n = 80	Term Birth n = 80	cOR (95% CI)	aOR (95% CI)*
Folate < 5 ug/L—n (%)				
All	11 (19.3%)	3 (5.3%)	4.30 (1.13-16.37)	2.81 (0.76-11.96)
Black	8 (25%)	1 (5.3%)	6.00 (0.69-52.38)	3.02 (0.31-29.33)
White	3 (12%)	2 (5.3%)	2.46 (0.38-15.86)	2.28 (0.32-16.50)
25-OH-D < 20 ng/mL—n (%)				
All	38 (47.5%)	31 (38.8%)	1.43 (0.76, 2.68)	1.49 (0.82-3.06)
Black	24 (60%)	25 (62.5%)	0.90 (0.37-2.21)	0.89 (0.32-2.48)
White	14 (35.0%)	6 (15%)	3.05 (1.03-9.02)	3.01 (0.99-9.21)
Omega-6/Omega-3 > 15—n (%)				
All	69 (92%)	67 (83.8%)	2.39 (1.20-4.76)	2.64 (1.21-5.78)
Black	36 (94.7%)	37 (92.5%)	1.67 (0.67-4.15)	1.90 (0.69-5.40)
White	33 (89.2%)	30 (75%)	4.26 (1.35-13.44)	4.25 (1.25-14.49)

Abbreviations: cOR, crude odds ratio; adjusted odds ratio; aOR, adjusted odds ratio; 25-OH-D, 25-hydroxy vitamin D; BMI, body mass index.
[a] The multivariate logistic model included age, BMI, health care payor, marital status (and race for model in which races were combined)

Linear regression analyses revealed no significant linear association between gestational age (in weeks) as the dependent variable and any of the nutrient measures of interest for black or white women considered together or separately, although the relationship approached statistical significance for the omega-6/omega-3 fatty acid ratio (standardized $\beta = -.175$, $P = .052$). The standardized β coefficients for the concentrations of folate and 25-OH-D in the linear modeling of gestational age in weeks were .007 ($P = .948$) and .025 ($P = .804$), respectively.

The proportion of black and white women with PTB versus term birth who were deficient in vitamin D, folate, or who had a total omega-6/omega-3 fatty acid ratio >15, along with the crude and adjusted odds of PTB, are given in Table 5. For folate <5 ng/L, univariate analysis revealed a significant increase in the odds of PTB versus term birth for black and white women combined (crude odds ratio [cOR] = 4.30, 95% confidence interval [CI] 1.13-16.37), however, this association was no longer statistically significant upon multivariate analysis (adjusted odds ratio [aOR] = 2.81, 95% CI 0.76-11.96) that accounted for maternal age, BMI, health care payor, marital status, and race.

For 25-OH-D <20 ng/mL, univariate analysis revealed a significant increase in the odds of PTB versus term birth for white women (cOR = 3.05, 95% CI 1.03-9.02) but not black women (Table 5). However, upon multivariate analysis, this association for white women did not remain significant (aOR = 3.0, 95% CI 0.99-9.21).

A total omega-6/omega-3 fatty acid ratio >15 was significantly associated with PTB in both univariate and multivariate analyses for black and white women combined, with little change in the point estimates and 95% confidence limits observed between the overall crude (cOR 2.39, 95% CI 1.20-4.76) and adjusted estimates (aOR 2.64, 95% CI 1.21-5.78), suggesting a small potential for confounding by maternal age, BMI, health care payor, marital status, and race. When examining the relationship between total omega-6/omega-3 fatty acid ratio >15 and PTB versus term birth for black and white women separately, significantly significant associations were observed for white women upon univariate (cOR 4.26, 95% CI 1.35-13.44) and multivariate (aOR 4.25, 95% CI 1.25-14.49) analyses but not for black women. Of note, only a small portion of black women in this study (5.3% of those with PTB and 7.5% of those with term birth) had an omega-6/omega-3 fatty acid ratio that was $\leq$15.

In our multivariate analyses, there was no significant interaction effects among the variables included in the model.

Discussion

In this cross-sectional study, we evaluated the potential association between maternal vitamin D, folate, and total omega-6/omega-3 fatty acid status during the delivery admission and PTB versus term birth. We found that the mean total omega-6/omega-3 fatty acid ratio was significantly greater for black compared with white women and that a total omega-6/omega-3 serum fatty acid ratio >15 was associated with a significantly greater adjusted odds of PTB for black and white women combined and for white women considered separately. We cannot definitively state that there is not an association between total omega-6/omega-3 fatty acid ratio and PTB for black women as the study was underpowered to adequately evaluate this relationship (power = 0.64). While obese women had a higher mean total omega-6/omega-3 fatty acid ratio than did nonobese women, differences were not statistically significant and controlling for obesity did not meaningfully alter the aOR. One previously published study has examined the relationship between essential fatty acid status and PTB, finding that among 37 preterm (mean gestational age of 34 weeks) and 34 term (mean gestational age of 40 weeks) deliveries, the following measures were elevated among those with PTB: maternal red blood cell omega-6/omega-3 fatty acid ratio ($P < .009$) and docosapentaenoic acid (22:5 omega-6), a marker of omega-3 essential fatty acid deficiency ($P < .001$).[33] Conversely, in the same study women with PTB had higher concentrations of total arachidonic acid in red blood cells and in plasma than did term controls (3.8- and 1.6-fold, respectively; $P < .05$).[33]

Our study revealed a statistically significant association between plasma 25-OH-D <20 ng/mL and PTB for white but not black women; however, this association did not remain statistically significant in the adjusted analysis (cOR 3.01, 95% CI: 0.99-9.21). As has been shown in multiple studies, the mean 25-OH-D concentration was significantly lower among black compared with white women, which is likely related to greater skin pigmentation and resulting reduced ultraviolet B light absorption.[40,42] The absence of significant association between vitamin D status and PTB in our study, particularly for black women, could be due to the relatively small number of black women with favorable vitamin D status and, thus, insufficient variation in the exposure variable to enable investigation of its effect upon the outcome of spontaneous PTB. The sample size available for this study yielded a power of 0.61 for assessing the association between 25-OH-D concentration and gestational age. As has been documented previously,[43] we found that the variability in 25-OH-D status for black obese and nonobese women was not as great as was observed for white obese and nonobese women.

We did not find a significant relationship between maternal folate status at delivery and PTB. Although the point estimates for the aOR for both black and white women were high (3.02 and 2.28, respectively), the wide confidence intervals for both uni- and multivariate analyses underscore the limited power (power = 0.59) for assessing this relationship and suggest that unmeasured confounding may be present. Also, considering the recent research that suggests the importance of folate status preconceptionally,[17] it is possible that folate status at the time of delivery may not be a precise predictor with respect to pregnancy outcome.

The present study has limitations. First, this study is of cross-sectional design with the measurement point occurring upon admission to the labor and delivery unit, thereby limiting our ability to evaluate the potential importance of periconception and early pregnancy nutritional status and PTB. A growing body of data suggest that the preconception period may be a crucial period for intervening to influence pregnancy outcomes.[44–46] Related to this, the timing of specimen collection for case and control women was different, with a mean gestational age for PTB and term birth being 33.9 ($\pm$ 2.8) weeks and 39.1 ($\pm$ 1.1) weeks, respectively ($P < .001$). It is possible that differences in the gestational age of cases and controls at the time of sampling may contribute to differences in measured concentrations of nutrients. However, this phenomenon is unlikely to account for the study findings as previous longitudinal research documents that 25-OH-D concentrations do not differ significantly throughout pregnancy[47,48] and there is a continuous increase in the ratio of total omega-6/omega-3 throughout pregnancy (although differences were not statistically different from 34 to 40 weeks of gestation),[49] the latter of which would actually bias study findings related to the relationship between omega-6/omega-3 ratio >15 and PTB toward the null.

Other potential limitations of this study relate to the measurement of particular nutrients. In the case of plasma fatty acids, a limitation is that, as participants were enrolled during their delivery admission with spontaneous onset of labor, we were restricted to the use of nonfasting samples. The use of nonfasting specimens could have influenced our findings, however, a previous study that assessed prenatal dietary intake and nonfasting plasma fatty acids found high correlations between fasting and postprandial plasma lipid measures (ranging from 0.90 to 0.99; $P < .001$).[50]

In the case of ascertainment of vitamin D status, there are also limitations to this study. Due to limited quantities of stored plasma, we were able to measure the major circulating form of vitamin D, 25-OH-D, but not its active form, 1,25-diOH-D; it would be informative to compare the levels of circulating 25-OH-D to levels of 1,25-diOH-D observed in this study to findings of a recent US study examining the safety and efficacy of vitamin D supplementation in pregnancy women.[14] Similarly, as we did not have sufficient sample to measure serum albumin and vitamin D binding protein, we were unable to calculate the free fraction of 25-OH-D. However, as only a very small fraction (0.02%-0.05%) of 25-OH-D remains free and free 25-OH-D concentrations are well maintained, even in participants with liver disease with low binding protein concentrations, suggesting there would be little utility in assessing free 25-OH-D concentrations.[51] Finally, the women in this study had mean 25-OH-D concentrations that were well below the level of 40 ng/mL, the level considered to optimize the conversion of 25-OH-D to 1,25-diOH-D.[14] Future studies of the role of vitamin D status and PTB should ensure adequate enrollment of a group of women with adequate 25-OH-D concentrations to ensure a group of women in the study are able to maximize 1,25-diOH-D production.

In the case of folate measurements, a limitation is that, due to having access to only stored plasma, we were able to measure circulating folate and fatty acids but not intracellular erythrocyte folate and fatty acid concentrations. Red blood cell measures of these nutrients provide a more stable and reliable measure of nutrient status over several months.[52,53] However, previous research demonstrates very high correlations between plasma and red blood cell folate and fatty acids.[54,55]

A link between fatty acid status and PTB is epidemiologically and biologically plausible. The ratio of omega-6/omega-3 fatty acids in the Western diet has increased over recent decades, commensurate with the rising prevalence of PTB. Moreover, the ratio is greater among blacks compared with whites.[7,56] Competition between omega-6 and omega-3 fatty acid precursors occurs in prostaglandin and leukotriene biosynthesis at the level of cyclooxygenase and lipoxygenase enzymes. Specifically, the ingestion of omega-3 fatty acids results in decreased production of potent vasoconstrictors, inflammatory mediators, and platelet aggregators including (1) prostaglandin E_2 metabolites, (2) thromboxane A_2, and (3) leukotriene B_4, accompanied by increased synthesis of more vasodilatory eicosamoids including, (4) thromboxane A_3, (5) prostacyclin PGI_3, and (6) leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent.[57] By contrast, the omega-6 fatty acid arachidonic acid (20:4 omega-6)

plays well-characterized roles in the initiation and progression of labor.[58]

Despite its limitations, we believe this research study is important in that it is among the few studies conducted in the United States to investigate the potential role of micronutrient deficiencies for which there are documented racial disparities in prevalence among women of reproductive age—including folic acid,[13,15,16] vitamin D,[9] and polyunsaturated fatty acids[33,37,59]—in the important problem of spontaneous PTB in the United States.

Conclusion

From this study we conclude that maternal total plasma omega-6/omega-3 fatty acid ratio >15 at delivery is significantly associated with PTB in univariate and multivariate analyses for black and white women (combined) and for white women alone. Due to the cross-sectional nature of the study, it is not clear whether elevated maternal free fatty acids, or an imbalance in omega-6/omega-3 fatty acid ratio, plays a role in the mechanism of PTB or is simply a risk marker for PTB. We cannot draw conclusions about maternal folate and vitamin D status at delivery and PTB due to limited power. It is possible that a larger study sample, or a sample that included black women with more variability in their nutrient status, would have resulted in significant findings. It is also possible that there is a racial difference in susceptibility to PTB related to poor vitamin D and essential fatty acid status. Larger, prospective studies that begin peri-conceptionally or early in pregnancy, and that include black and white women with more variability in their nutrient status, are needed to fully explore these hypotheses.

Acknowledgments

We gratefully acknowledge Ms Cayce Owens, Nashville Perinatal Research Center, for handling of stored specimens; Dr Meena Kumari, Emory University, for performance of 25-hydroxyvitamin D assays; and Dr Martha Wilson, Wake Forest School of Medicine, for performance of fatty acid assays.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported in part by PHS Grant (UL1 RR025008) from the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources. The establishment of the Nashville Birth Cohort and banking of plasma and DNA was funded by the Thrasher Research Fund and data coordination was made possible by a grant to Dr. Menon from the March of Dimes (21-FY08-557). This research was supported in part by PHS Grant (UL1 RR025008, KL2 RR025009 or TL1 RR025010) from the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources.

References

1. Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. *Nutr Rev*. 2007;65(12 pt 2): S194-S202.
2. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L. The role of inflammation and infection in preterm birth. *Semin Reprod Med*. 2007;25(1):21-39.
3. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75-84.
4. Behrman RE, Butler AS. (Eds). *Preterm birth: causes, consequences, and prevention*. Washington, DC: National Academies Press; 2006.
5. Yang QH, Carter HK, Mulinare J, Berry RJ, Friedman JM, Erickson JD. Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001-2002. *Am J Clin Nutr*. 2007;85(5):1409-1416.
6. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*. 2002;76(1):187-192.
7. Jen KL, Brogan K, Washington OG, Flack JM, Artinian NT. Poor nutrient intake and high obese rate in an urban African American population with hypertension. *J Am Coll Nutr*. 2007;26(1):57-65.
8. Dunlop AL, Kramer M, Hogue CJ, Menon R, Ramakrishnan U. Racial disparities in preterm birth: an overview of the potential role of nutrient deficiencies. *Acta Obstetricia et Gynecologica Scandinavica* 2011; 90(12): 1332-41. doi: 10.1111/j.1600-0412.2011.01274.x. [Epub ahead of print] PMID: 21910693.
9. Bodnar LM, Simhan HN. The prevalence of preterm birth and season of conception. *Paed Perinatal Epidemiol*. 2008;22(6):538-545.
10. Hewison M, Burke F, Evans KN, et al. Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. *J Steroid Biochem Mol Biol*. 2007;103(3-5):16-21.
11. Fischer D, Schroer A, Ludders D, et al. Metabolism of vitamin D3 in the placental tissue of normal and preeclampsia complicated pregnancies and premature births. *Clin Exp Obstet Gynecol*. 2007;34(2):80-84.
12. DeLuca H. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr*. 2004;80(6):1689S-1696S.
13. Bodnar LM, Simhan HN. Vitamin D may be a link to black-white disparities in adverse birth outcomes. *Obstet Gynecol Surv*. 2010; 65(4):273-284.
14. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011;26(10):2341-2457.
15. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. *Am J Clin Nutr*. 1996;63(4):520-525.
16. Siega-Riz AM, Savitz DA, Zeisel SH, Thorp JM, Herring A. Second trimester folate status and preterm birth. *Am J Ob Gyn*. 2004; 191(6):1851-1857.

17. Bukowski R, Malone FD, Porter FT, et al. Preconceptional folate supplementation and the risk of spontaneous preterm birth: A cohort study. *PLoS Med*. 2009;6:e1000061. doi:10.1371/journal.pmed.1000061.
18. Baumslag N, Edelstein T, Metz J. Reduction of incidence of prematurity by folic acid supplementation in pregnancy. *Br Med J*. 1970;1:16-17.
19. Blot I, Papiernik E, Kaltwasser JP, Werner E, Tchernia G. Influence of routine administration of folic acid and iron during pregnancy. *Gynecol Obstet Invest*. 1981;12(6):294-304.
20. Tchernia G, Blot I, Rey A. Maternal folate status, birth weight and gestational age. *Dev Pharmacol Ther*. 1982;4(supp 1): 58-65.
21. Fleming AF, Martin JD, Hahnel R, Westlake AJ. Effects of iron and folic acid antenatal supplements on maternal haematology and fetal wellbeing. *Med J Aust*. 1974;2(12):429-436.
22. Fletcher J, Gurr A, Fellingham FR, Prankerd TA, Brant HA, Menzies DN. The value of folic acid supplements in pregnancy. *J Obstet Gynaecol Br Commonw* 1971;78(9):781-785.
23. Giles PF, Harcourt AG, Whiteside MG. The effect of prescribing folic acid during pregnancy on birth-weight and duration of pregnancy. A double-blind trial. *Med J Aust*. 1971;2(1): 17-21.
24. Christian P, Jiang T, Khatry SK, LeClerq SC, Shrestha SR, West KP. Antenatal supplementation with micronutrients and biocehcmical indicators of status and subclinical infection in rural Nepal. *Am J Clin Nutr*. 2006;83(4):788-794.
25. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. *Am J Clin Nutr*. 2000;71(5 suppl):1295s-1303s.
26. Martin JD, Davis RE, Stenhouse N. Serum folate and vitamin B12 levels in pregnancy with particular reference to uterine bleeding and bacteriuria. *J Obstet Gynaecol Br Commonw*. 1967;74 (5 suppl):697-701.
27. Dhur A, Galan P, Hercberg S. Folate status and the immune system. *Prog Food Nutr Sci*. 1991;15(1-2):43-60.
28. Courtemanche C, Elson-Schwab I, Mashiyama ST, Kerry N, Ames BN. Folate deficiency inhibits the proliferation of primary human CD8+ T lymphocytes in vitro. *J Immunol*. 2004;173(5): 3186-3192.
29. Christian P, Jiang T, Khatry SK, LeClerq SC, Shrestha SR, West KP. Antenatal supplementation with micronutrients and biocehcmical indicators of status and subclinical infection in rural Nepal. *Am J Clin Nutr*. 2006;83(4):788-794.
30. Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc Natl Acad Sci*. 2003;100(4):1751-1756.
31. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *Br Med J*. 2002;324(7335):1-5.
32. Olsen SF, Hansen HS, Secher NJ, Jensen B, Sandström B. Gestation length and birth weight in relation to intake of marine n-3 fatty acids. *Br J Nutr*. 1995;73(3):397-404.
33. Reece MS, McGregor JA, Allen KG, Harris MA. Maternal and perinatal long-chain fatty acids: possible roles in preterm birth. *Am J Obstet Gynecol*. 1997;176(4):907-914.
34. Chen X, Scholl T. Association of elevated, free fatty acids during late pregnancy with preterm delivery. *Obstet Gynecol*. 112(2 pt 1): 297-303.
35. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *Cochrane Database Syst Rev*. 2006;3:CD003402.
36. Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in high risk pregnancies. *Br J Obstet Gynaecol*. 2000;107(3):382-395.
37. Harper M, Thom E, Klebanoff MA, et al. for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Omega-3 fatty acid supplementation to prevent recurrent preterm birth: a randomized controlled trial. *Obstet Gynecol.* 2010;115(2 pt 1):234-242.
38. Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: National Academy Press, 2010.
39. Menon R, Pearce B, Velez DR, et al. Racial disparity in pathophysiologic pathways of preterm birth based on genetic variants. *Repro Biol Endocrinol*. 2009;7:62. Doi: 10.1186/1477-7827-7-62.
40. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin insufficiency in an adult normal population. *Osteoporos Int*. 1997; 7(5):439-443.
41. McNulty H. Folate requirements for health in different population groups. *Br J Biomed Sci*. 1995;52(2):110-119.
42. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr*. 2007;137(2):447-452.
43. Looker AC. Body fat and vitamin D status in black versus white women. *J Clin Endocrinol Metab*. 2005;90(2):2635-2640.
44. King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. *J Nutr*. 2003;133(5 suppl 2):1732S-1736S.
45. Lu M, Halfon N. Racial and ethnic disparities in birth outcomes: a life-course perspective. *Matern Child Health J*. 2003;7(1):13-30.
46. Gardiner PP, Nelson L, Shellhass CS, Dunlop AL. The clinical content of preconception care: diet, supplements, and vitamins. *Am J Ob Gyn*. 2008;199;6(suppl 2):S345-S356.
47. Dent CD, Gupta MM. Plasma 25-hydroxy vitamin-D levels during pregnancy in Caucasians and in vegetarian and non-vegetarian Asians. *Lancet*. 1975;306(7944):1057-1060.
48. Ardawi MS, Nasrat HA, BA'Aqueel HS. Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study. *Eur J Endocrinol*. 1997;137(4):402-409.
49. Al MD, vanHouwelinsen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am J Clin Nutr*. 2000;71(1 suppl):285S-291S.
50. Williams MA, Frederick IO, Qui C, et al. Maternal erythrocyte omega-3 and omega-6 fatty acids, and plasma lipid concentrations, are associated with habitual dietary fish consumption in early pregnancy. *Clin Biochem*. 2006;39(11):1063-1070.

51. Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. *J Clin Invest*. 1984; 74(6):1966-1971.
52. Willett W. *Nutritional Epidemiology*. 2nd ed. Oxford: Oxford University Press; 1998:415. ISBN 0-19-512297-6.
53. Gibson RS. *Principles of Nutritional Assessment*. New York, NY: Oxford University Press; 1990.
54. Balin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol*. 2005;162(4):373-381.
55. Weinstein SJ, Ziegler RG, Frongillo EA, et al. Low serum and red blood cell folate are moderate, but non-significantly, associated with invasive cervical cancer. *J Nutr*. 2001;131(7): 2040-2048.
56. Eaton SB, Eaton SB III, Sinclair AJ, Cordain L, Mann NJ. Dietary intake of long-chain polyunsaturated fatty acids during the Paleolithic. *World Rev Nutr Diet*. 1998;83:12-23.
57. Wathes DC, Robert D, Abayasekara E, Aitken RJ. Polyunsaturated fatty acids in male and female reproduction. *Biol Reprod*. 2007;77(2):190-201.
58. Ogburn PL Jr, Johnson SB, Williams PP, Holman RT. Levels of free fatty acids and arachidonic acid in pregnancy and labor. *J Lab Clin Med*. 1980;95(6):943-949.
59. Smuts CM, Huang M, Mundy D, Plasse T, Major S, Carlson SE. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet Gynecol*. 2003;101(3):469-479.