HHS Public Access

Author manuscript

BJOG. Author manuscript; available in PMC 2021 January 29.

Published in final edited form as:

BJOG. 2017 November ; 124(12): 1874–1882. doi:10.1111/1471-0528.14372.

Vitamin D, pre-eclampsia, and preterm birth among pregnancies at high risk for pre-eclampsia: an analysis of data from a low-dose aspirin trial

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Abstract

Objective—To examine the relation between maternal vitamin D status and risk of pre-eclampsia and preterm birth in women at high risk for pre-eclampsia.

Design—Analysis of prospectively collected data and blood samples from a trial of prenatal low-dose aspirin.

Setting—Thirteen sites across the USA.

Population—Women at high risk for pre-eclampsia.

Methods—We measured 25-hydroxyvitamin D [25(OH)D] concentrations in stored maternal serum samples drawn at 12–26 weeks' gestation (n = 822). We used mixed effects models to

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Contribution to authorship

LB planned the study; AG, KB, and LB contributed to data analysis; all authors contributed to data interpretation; AG and LB wrote the paper; HS, KB, and SC reviewed and revised the paper for important intellectual content. SC was the principal investigator of the original aspirin trial.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Details of ethics approval

Our study was designated as 'exempt' from ethical approval by the University of Pittsburgh School of Public Health Institutional Review board because we used deidentified data.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

examine the association between 25(OH)D and risk of pre-eclampsia and preterm birth, controlling for confounders including prepregnancy BMI and race.

Main outcome measures—Pre-eclampsia and preterm birth.

Results—Twelve percent of women were vitamin D deficient [25 (OH)D <30 nmol/l]. Women with 25(OH)D <30 versus ≥75 nmol/l had a 2.4-fold (95% CI 1.0–5.6) higher risk of early-onset pre-eclampsia (<35 weeks' gestation) after confounder adjustment. Women with 25(OH)D <50 nmol/l had a 1.8-fold (95% CI 1.0–3.2) increased risk of preterm birth at <35 weeks compared with women who had 25(OH)D ≥75 nmol/l, which was driven by indicated preterm births at <35 weeks' gestation [25 (OH)D <50 versus ≥75 nmol/l adjusted RR 2.5 (95% CI 1.1–5.8)]. There was no association between vitamin D status and pre-eclampsia or preterm birth at <37 weeks.

Conclusion—Maternal vitamin D status in the second trimester was inversely associated with risk of early-onset pre-eclampsia and preterm birth at <35 weeks in women at high risk for pre-eclampsia.

Tweetable abstract

Vitamin D is inversely related to risk of pre-eclampsia and preterm birth at <35 weeks in high-risk pregnancies.

Keywords

High-risk; pre-eclampsia; preterm birth; vitamin D

Introduction

Hypertensive disorders develop in 5–10% of pregnancies,[1] with pre-eclampsia typically occurring in 2–8% of pregnancies worldwide. Pre-eclampsia is among the leading causes of morbidity in offspring[2] and poses immediate and long-term health risks in the mother.[3] Preterm birth, the largest single obstetrical contributor to neonatal deaths in the USA,[4] occurred in 10% of live births in the USA in 2014.[5] The US rate of preterm birth remains higher than that in high-income countries overall[6] and there are few interventions proven to prevent preterm births.[7]

Vitamin D deficiency has emerged as a public health concern in recent decades[8] and has been linked to many adverse pregnancy outcomes.[9] Maternal vitamin D status has been associated with both preterm birth and pre-eclampsia in general obstetric populations, albeit with some mixed results.[10-12] A recent Cochrane review of prenatal vitamin D supplementation found that vitamin D compared with no intervention or placebo reduced the risk of preterm birth [risk ratio (RR) 0.36; 95% CI 0.14–0.93] and suggested a lower risk of pre-eclampsia (RR 0.52; 95% CI 0.25–1.05).[13] Although maternal vitamin D is becoming promising as an intervention, most studies of vitamin D and adverse pregnancy outcomes are in a general obstetric population. It is not known whether vitamin D may have benefits among women with high-risk pregnancies, where pre-existing conditions could overwhelm any effects of vitamin D. Data on maternal vitamin D status and these outcomes in pregnancies at high risk for adverse outcomes is sparse, studies are small, and participants

are relatively homogeneous,[14-16] limiting both the ability to draw conclusions across racial/ethnic groups and latitude, and to examine subgroups of pre-eclampsia and preterm birth. Thus, we conducted a secondary analysis in a multi-site trial across the USA in women who were at high risk of pre-eclampsia. Our aim was to examine the relation between maternal vitamin D status and risk of pre-eclampsia and preterm birth in these high-risk pregnancies.

Methods

The High-Risk Aspirin Study (1991–95) was a randomised, double-blind, placebo-controlled trial to determine whether aspirin therapy reduces the risk of pre-eclampsia in women at high risk for the disease (n = 2539).[17] The study was carried out at 13 medical centres in the USA by the Maternal Fetal Medicine Units (MFMU) network (https://mfmu.bsc.gwu.edu/). Women were eligible if they met one of four high-risk criteria: pre-gestational insulin-treated diabetes mellitus, chronic hypertension, multifetal gestation or pre-eclampsia in a previous pregnancy. Detailed eligibility criteria within each risk group and exclusion criteria have been described.[17] Women were enroled at 13–26 weeks' gestation, randomly assigned to receive daily 60 mg of aspirin or a placebo tablet, and tracked to the end of the pregnancy. At the screening visit and subsequent visits, researchers measured urinary protein (by dipstick) and blood pressure; further details have been published.[17] Following delivery, medical records were abstracted to collect detailed data on delivery events.

Women who enroled in the trial were invited to participate in an ancillary study designed to document aspirin compliance.[18] Women were asked to provide a non-fasting blood sample at baseline and twice later in pregnancy. Serum samples were stored at −70°C. The ancillary study began after the main trial, and approximately 52% of women with singleton gestations in the trial provided blood that was banked and could be retrieved (n = 951).

For the present study, we included women in the ancillary study with serum obtained at ≤26 weeks' gestation (i.e. baseline) and excluded multifetal gestations to provide a more homogeneous study population (n = 839). We excluded women with a pregnancy outcome at <20 weeks' gestation (because they were not at risk of outcomes evaluated; n = 9) or missing BMI (n = 8), leaving a final analytic sample of 822 women. Outcomes investigated were hypertensive disorders of pregnancy [pre-eclampsia, early-onset pre-eclampsia (at <35 weeks' gestation), chronic hypertension with superimposed pre-eclampsia, and gestational hypertension] and preterm birth (overall, indicated, and spontaneous preterm at <37 and <35 weeks' gestation; Table S1).

Pre-eclampsia was defined using diagnostic criteria established by the MFMU Network (Table 1 and Table S1).[17] Three physicians independently reviewed records and agreed unanimously to diagnoses. Gestational hypertension was defined by the MFMU Network as onset of hypertension during pregnancy for women who were normotensive before pregnancy. Pre-eclampsia and gestational hypertension closely align with American Congress of Obstetricians and Gynecologists (ACOG) categories 1 and 4, respectively, for hypertensive disorders of pregnancy.[19] For the present study, we defined 'early-onset' as pre-eclampsia at <35 weeks (there were too few cases to define 'early-onset pre-eclampsia'

at <34 weeks' gestation). We classified chronic hypertension with superimposed pre-eclampsia to correspond to ACOG category 3.[19]

Gestational age was determined in the main trial by integrating clinical and laboratory information (last menstrual period, hormonal tests, and ultrasound).[17] We defined preterm birth as live birth at 24 to <37 weeks' gestation (Table S1). We then classified <35 weeks' gestational as a cut-point for moderate preterm birth, as there were too few cases to evaluate preterm birth at earlier gestational cut-points.[20] We defined spontaneous preterm birth as preterm delivery after spontaneous preterm labor or spontaneous pre-labour rupture of membranes. Indicated preterm births were all preterm births that were not spontaneous.

We shipped serum samples (one per woman) to the laboratory of Dr Michael Holick for assay of total 25-hydroxyvitamin D [25(OH)D] [$25(OH)D_2$ + $25(OH)D_3$]. This DEQAS (Vitamin D External Quality Assessment Scheme)-proficient laboratory uses liquid-chromatography-tandem mass spectrometry 25(OH)D assay based on National Institute of Standards and Technology standards.[21] The assay had a coefficient of variation of 6.0%. In the effort to find meaningful concentrations for 25(OH)D related to pregnancy outcomes,[22,23] we used multiple cut-points of 30, 50, and 75 nmol/l (which correspond to 12, 20, and 30 ng/ml, respectively). These cut-points correspond to the Institute of Medicine definitions of risk of deficiency (at <30 nmol/l) and risk of inadequacy (30 to <50 nmol/l) in relation to bone health,[24] and the Endocrine Society definitions of deficiency (<50 nmol/l) and insufficiency (50 to <75 nmol/l).[23]

Inflammation is associated with pre-eclampsia,[25] preterm birth,[26] and vitamin D status.[27] We measured C-reactive protein (CRP) as a marker of inflammation because it is an acute-phase protein for which concentrations rise in response to inflammation. High-sensitivity CRP was measured in a laboratory at the Magee Womens Research Institute using a Bio-Rad protein enzyme immunoassay with a coefficient of variation of less than 10%.

In the parent trial, baseline interviewers collected self-reported prepregnancy weight, predominant race/ethnicity, parity, marital status, smoking habits, age, and years of schooling; height was measured at the first visit. Prepregnancy body mass index was calculated as weight (kg) divided by height (m) squared. Month of blood draw, gestational age at blood draw, and infant sex data were also available.

Statistical analysis

We used mixed-effects Poisson regression models to estimate adjusted risk ratios and their 95% confidence intervals for the association between 25(OH)D and the risk of each outcome. The models were specified with 25(OH)D and covariates as fixed-effects and study site as a random effect (to account for women clustered by study site). Denominators for each outcome were based on pregnancies at risk of the outcome at the start of each gestational period. We tested for interactions in the relation between 25 (OH)D and outcomes by infant sex; however, as no interaction was observed; interaction testing was not presented in the results. We used theory-based causal models (diagrams that help to identify multiple confounders)[28] to identify the following potential confounders: prepregnancy BMI, race/ethnicity, smoking, parity, age, education, marital status, season at blood draw,

gestational age at blood draw, inflammation (CRP), treatment group (aspirin versus placebo), and baseline risk group. Our goal was to develop a parsimonious model, so we built a full model and retained a variable as a confounder if its removal from the full model changed the main effect risk ratio by >10%. Parity (primiparous versus multiparous), marital status (married versus not married), and season of blood draw (winter/spring versus summer/fall) met this criterion. We retained prepregnancy BMI (continuous), maternal race/ethnicity (Black versus non-Black), treatment group (aspirin versus placebo), and baseline risk group (prepregnancy diabetes, chronic hypertension, previous pre-eclampsia) out of convention. For outcomes with a small number of cases with a 25(OH)D concentration of <30 nmol/l, we used <50 nmol/l as the lower category. In sensitivity analyses, we reran models restricting to live births. We also replicated the pre-eclampsia and early-onset pre-eclampsia analyses after removal of women with chronic hypertension in an attempt to mirror more closely the current ACOG classification. We used STATA 13.1 (College Station, TX, USA) for all analyses.

Results

The majority of mothers were non-Hispanic black, parous, 20–29 years old, not married, non-smokers, and high school graduates at enrolment (Table 2). A quarter of women were severely obese. Mean (SD) gestational age at enrolment and blood draw was 19.5 (3.9) weeks (range 12.0–26.9 weeks); CRP was 10.6 (11.9) μg/ml. Males comprised 52% of offspring.

Maternal serum 25(OH)D <30, 30–49.9, 50–74.9, and ≥75 nmol/l was prevalent in 12, 25, 31, and 32% of women, respectively, with a mean (SD) of 64 (30) nmol/l (range 6–184). Vitamin D status differed by race/ethnicity and the season of blood draw, with non-Hispanic Black women and women with blood draws in the winter and spring having the lowest concentrations (Table 2). Women who were parous at the start of the index pregnancy, unmarried or obese were more likely to have 25(OH) D < 50 nmol/l than were their counterparts. Serum 25 (OH)D varied by latitude because of the varying racial composition of the study sites.

Vitamin D status varied by risk group. Women with previous pre-eclampsia had lower 25(OH)D concentrations at baseline [mean (SD): 59 (30) nmol/l, $n = 293$] compared with women with chronic hypertension [64 (29) nmol/l, $n = 338$] or prepregnancy diabetes [72 (29) nmol/l, $n = 191$]; and those with chronic hypertension had lower concentrations than those with prepregnancy diabetes (all $P < 0.05$).

Pre-eclampsia developed in 24% of pregnancies and early-onset pre-eclampsia (at <35 weeks) occurred in 7% (Table 3). After adjusting for prepregnancy BMI, race, parity, marital status, season of blood draw, treatment group (aspirin versus placebo), baseline risk group, and study site, the risk of early-onset pre-eclampsia among women with 25(OH)D <30 nmol/l was 2.4 times higher than among women with 25(OH)D ≥75 nmol/l (Table 3). After dropping stillbirths ($n = 20$) in sensitivity analysis, results were similar but the 95% confidence interval included the null [adjusted risk ratio (aRR) 2.2, 95% CI 0.9–5.5].

Excluding women with chronic hypertension from the analysis (n = 338) led to the same conclusion, though precision was greatly compromised (aRR 4.65, 95% CI 1.03–20.9).

Preterm birth at <37 weeks and <35 weeks occurred in 27 and 11% of all pregnancies at risk, respectively (Table 4). After confounder adjustment, women with 25 (OH)D <50 nmol/l had a 1.8-fold increased risk of preterm birth at <35 weeks compared with women with 25(OH) D ≥75 nmol/l. Risk was 2.1 times higher (95% CI 1.0–4.4) for a value of <30 nmol/l than ≥75 nmol/l. For medically indicated preterm birth at <35 weeks, women with 25(OH) D <30 nmol/l versus ≥75 nmol/l had a 2.5-fold increased risk (Table 4). Results were essentially identical after dropping stillbirths. Other results by outcome are presented in Tables 3 and 4, and Supporting Information Table S2.

The top medical reasons for induction at <35 weeks (n = 41) were hypertension (n = 9; 22.0%) and pre-eclampsia/gestational hypertension (n = 8; 19.5%); top indications at <37 weeks were pre-eclampsia/gestational hypertension (n = 29; 23.6%), hypertension (n = 26; 21.1%), and diabetes (n = 9; 7.3%). Overall, 39% (n = 35) of preterm births at <35 weeks and 61% (n = 25) of indicated preterm births at <35 weeks were mothers who also had diagnosed onset of pre-eclampsia at <35 weeks' gestation.

Discussion

Main findings

In this geographically and racially diverse population of US pregnant women, second-trimester maternal vitamin D status was inversely associated with risk of early-onset pre-eclampsia (at <35 weeks' gestation) in women at high risk for pre-eclampsia. Further, vitamin D status was associated with risk of preterm birth at <35 weeks' gestation—an association driven by indicated deliveries, many of which were medically necessary due to hypertensive disorders. With relatively few cases for each of these outcomes, we report these findings cautiously.

Strengths and limitations

We used the current best method for assessment of vitamin D status in a laboratory internationally certified to do so. Serum had been stored for approximately 20 years but we and others have shown that 25(OH)D is not likely to degrade with long-term storage.[29,30] We were limited by only having one measurement of vitamin D status per woman, at approximately 20 weeks' gestation. It is unknown what timepoint is best for assessing vitamin D status in pregnancy to investigate the relation with these outcomes, and earlier timepoints (including preconception) should be tested based on the presumed early origins of pre-eclampsia[31] and previous vitamin D research.[32]

This study was both racially and geographically diverse. However, the sample size for outcomes at <35 weeks was relatively small and some associations were imprecise. For this reason, we did not make statistical adjustments for multiple testing in spite of assessing several outcomes. With observational studies there is always the potential for unmeasured confounding, and in this study we did not have information on diet, vitamin and mineral supplement intake, sun exposure or skin pigmentation. With the potential to impact both

vitamin D status and risk of pre-eclampsia or preterm, these factors should be measured in future studies. Intake of calcium, unmeasured here, could confound the vitamin D and pre-eclampsia relation, although one previous study suggests it might not.[33] Calcium may be important in studying preterm birth. The 2016 Cochrane meta-analysis found a lower risk of preterm birth from vitamin D supplementation alone but a higher risk when women were supplemented with vitamin D and calcium together.[13]

Interpretation

Our finding of an association between maternal 25(OH)D concentration and early-onset pre-eclampsia, but not pre-eclampsia overall, was not necessarily surprising. There is compelling evidence that supports an effect of maternal vitamin D status on risk of overall pre-eclampsia in general obstetric populations;[13,34-36] however, studies in high-risk pregnancies have not found a link.[14,15] In an observational study of women at high risk for pre-eclampsia in Canada, 221 women had 25(OH)D measured at 10–20 weeks' gestation and were followed across pregnancy.[14] This study found no relation between the mother's vitamin D status and risk of pre-eclampsia, or any other adverse pregnancy outcomes; however, there were only 28 cases of pre-eclampsia. A randomised controlled trial of 60 high-risk women in Iran found no difference in risk of pre-eclampsia between women receiving bi-weekly doses of 50 000 IU and those receiving placebo, but only four women developed pre-eclampsia.[15] Our current study with a larger sample size (196 cases of pre-eclampsia) also found no relation between vitamin D and overall pre-eclampsia.

We could not find any studies of high-risk pregnancies that examined subgroups of hypertensive disorders. Most studies in general obstetric populations that divided pre-eclampsia into groups by severity or timing of onset found an association between maternal vitamin D status and severe or early-onset pre-eclampsia.[11,37-40] It is interesting that our findings were similar, as in our study pre-eclampsia developed in 24% of pregnancies, compared with 2–6% in other studies.

For preterm birth, there is limited but growing evidence of a relation with maternal vitamin D status in general obstetrics populations.[10,13,41] There is inadequate data on high-risk pregnancies—we identified only two comparable studies. Similar to our study, Shand et al.[14] found no association between 25(OH)D measured at 10–20 weeks' gestation and preterm birth at <37 weeks, but preterm subgroups were not examined. In a nested case-control study of women considered high-risk due to a previous preterm birth, no association was observed between midgestation maternal 25(OH)D concentration and preterm birth at <37 or <32 weeks.[16] Although our finding of an association between vitamin D status and preterm birth at <35 weeks appears to be the result of pre-eclampsia-indicated delivery, subgroups of preterm birth should be still be given attention in future vitamin D studies.

There are plausible mechanisms that could underlie the effect of vitamin D on hypertensive disorders of pregnancy. Calcitriol, the biologically active metabolite of vitamin D, may impact maternal blood pressure by stimulating the production of estradiol in the syncytiotrophoblast[42] or through local actions in vascular smooth muscle cells where vitamin D receptors are present.[43] Pre-eclampsia is arguably a syndrome rather than a single disease and the heterogeneous symptoms across different pregnancies, including lack of

hypertension among some women with HELLP syndrome, make it difficult to understand the aetiology.[31] Research suggests early-onset pre-eclampsia is caused by poor implantation, spiral artery remodelling, and placental development. Angiogenic and anti-angiogenic factors produced by the placenta are released into maternal circulation, and these factors appear to be the most likely candidates for maternal vascular damage leading to early-onset pre-eclampsia. Calcitriol impacts gene transcription of a wide range of proteins, including vascular endothelial growth factor (VEGF), one of the most prominent angiogenic factors.[44,45]

Conclusion

Thus far, very few randomised vitamin D supplementation trials have assessed pre-eclampsia and preterm birth, and although the data are suggestive of an effect, causal evidence is limited. We aimed to investigate whether vitamin D status would be associated with these adverse outcomes among women with high-risk pregnancies, where pre-existing conditions could overwhelm any effects of vitamin D. Our results support a connection between maternal vitamin D status and pre-eclampsia and preterm birth at <35 weeks. These early outcomes are particularly concerning because health risks to the mother and baby are higher compared with the same outcomes occurring later in pregnancy.[46] The current study supports the need for vitamin D research in high-risk pregnancies. Vitamin D supplementation is a safe, practical, public health measure that could be adopted if proven effective. With the enormous burden of poor maternal and child health outcomes resulting from preterm birth and pre-eclampsia, vitamin D should be given continued attention in the search for preventative factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Jill Diesel for her assistance with data preparation.

Funding

The current study was funded by NIH grant R01 HD056999 (PI: Bodnar) and the parent trial was funded by grants from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) (HD27869, HD21410, HD40512, HD34136, HD34208, HD40485, HD27915, HD40544, HD40560, HD27917, HD40500, HD34116, HD40545, HD27860, HD36801) and the National Center for Research Resources (M01 RR00080, UL1 RR024989). Dr Gernand's research is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health under BIRCWH award number K12HD055882, 'Career Development Program in Women's Health Research at Penn State'. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD, NHLBI, NCRR or NIH.

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Table 1.

Diagnostic criteria for preeclampsia, High-Risk Aspirin Study

Hypertension at baseline	Proteinuria at baseline	Pre-eclampsia diagnosis
Absent	Absent	Hypertension (BP 140 mmHg or greater or DBP 90 mmHg or greater on two occasions at least 4 hours apart) and one of the following: Proteinuria (300 mg protein in a 24-hour urine collection, or two dipstick test results of at least 2 + recorded at least 4 hours apart with no evidence of urinary tract infection) Thrombocytopenia (platelet count <100 000/m^3) Pulmonary oedema
Absent	Present	One of the following: thrombocytopenia, serum aspartate aminotransferase concentration ≥70 U/l, or hypertension with severe headaches, epigastric pain, or a sudden increase in proteinuria
Present	Absent	Proteinuria or thrombocytopenia
Present	Present	At least one of the following: thrombocytopenia, serum aspartate aminotransferase concentration ≥70 U/l, or worsening hypertension (two diastolic readings ≥110 mm Hg taken 4 hours apart in the week before delivery) And one of the following: exacerbation of proteinuria, severe headaches or epigastric pain
Any	Any	Eclamptic convulsions or the HELLP syndrome

Table 2.

Characteristics of mothers with singleton gestation and blood draw at enrolment to the trial of low-dose aspirin to prevent preeclampsia in women at risk (1991–96)

	Total ***n*** **= 822**	**25(OH)D ≥50 nmol/l** ***n*** **= 519**	**25(OH)D <50 nmol/l** ***n*** **= 303**	***P*-value**
		n (%)		
Race				
Non-Hispanic White	249 (30.3)	210 (40.5)	39 (12.9)	<0.01
Non-Hispanic Black	516 (62.8)	262 (50.5)	254 (83.8)	
Other *	57 (6.9)	47 (9.1)	10 (3.3)	
Maternal age **				
<20 years	100 (12.3)	63 (12.3)	37 (12.3)	0.07
20–29 years	458 (56.1)	274 (53.3)	184 (60.9)	
≥30 years	258 (31.6)	177 (34.4)	81 (26.8)	
Parity				
Nulliparous	184 (22.4)	132 (25.4)	52 (17.2)	<0.01
Parous	638 (77.6)	387 (74.6)	251 (82.8)	
Marital status				
Married	301 (36.6)	220 (42.4)	81 (26.7)	<0.01
Not married	521 (63.4)	299 (57.6)	222 (73.3)	
Smoking at study entry				
Smoker	138 (16.8)	89 (17.2)	49 (16.7)	0.72
Non-smoker	684 (83.2)	430 (82.9)	254 (83.8)	
Prepregnancy BMI (kg/m^2)				
Normal & underweight ***, <25	265 (32.2)	203 (39.1)	62 (20.5)	<0.01
Overweight, 25–29.9	195 (23.7)	120 (23.1)	75 (24.8)	
Obese, 30–34.9	160 (19.5)	92 (17.7)	68 (22.4)	
Severely obese, ≥35	202 (24.6)	104 (20.0)	98 (32.3)	
Education **				
<High school graduate	222 (27.0)	137 (26.5)	85 (28.1)	0.07
High school graduate	374 (45.6)	225 (43.4)	149 (49.2)	

	Total *n* = 822	25(OH)D ≥50 nmol/l *n* = 519	25(OH)D <50 nmol/l *n* = 303	*P*-value
		n (%)		
≥Some college or higher	225 (27.4)	156 (30.1)	69 (22.8)	
Season of blood draw				
Winter (December–February)	188 (22.9)	99 (19.1)	89 (29.4)	<0.01
Spring (March–May)	210 (25.6)	124 (23.9)	86 (28.4)	
Summer (June–August)	199 (24.2)	144 (27.8)	55 (18.2)	
Fall (September–November)	225 (27.4)	152 (29.3)	73 (24.1)	
Latitude of study site				
≥37° North	315 (38.3)	220 (42.4)	95 (31.4)	<0.01
35–36° North	295 (35.9)	170 (32.8)	125 (41.3)	
<35° North	212 (25.8)	129 (24.9)	83 (27.4)	

* *n* = 53 Hispanic white, *n* = 1 Hispanic black, *n* = 2 American Indian/Alaskan.

** 6 missing values for age; 1 missing value for education.

*** 19 women were underweight (<18.5 kg/m^2).

Table 3.

Maternal vitamin D status at 13–26 weeks' gestation and risk of hypertensive disorders of pregnancy in women at high risk for pre-eclampsia

Serum 25(OH)D categories	*n*	No. cases	Risk per 100 births	Unadjusted risk ratio	95% CI	Adjusted risk ratio*	95% CI
Pre-eclampsia	822	196	0.24				
<30 nmol/l	95	21	0.22	0.98	0.63–1.51	1.13	0.66–1.92
30–49 nmol/l	208	55	0.26	1.17	0.85–1.60	1.15	0.77–1.72
50–74 nmol/l	254	60	0.24	1.04	0.76–1.43	1.09	0.75–1.59
≥75 nmol/l	265	60	0.23	Reference		Reference	
Early-onset pre-eclampsia (<35 weeks)	822	61	0.07				
<30 nmol/l	95	11	0.12	1.92	0.92–3.98	2.40	1.04–5.56
30–49 nmol/l	208	15	0.07	1.19	0.60–2.36	1.29	0.60–2.74
50–74 nmol/l	254	19	0.07	1.24	0.65–2.36	1.43	0.72–2.85
≥75 nmol/l	265	16	0.06	Reference		Reference	
Chronic hypertension with superimposed pre-eclampsia**	338	91	0.27				
<50 nmol/l	128	37	0.29	1.13	0.74–1.71	1.16	0.67–2.01
50–74 nmol/l	101	26	0.26	1.00	0.63–1.59	1.03	0.58–1.83
≥75 nmol/l	109	28	0.26	Reference		Reference	
Gestational hypertension***	379	65	0.17				
<50 nmol/l	136	20	0.15	0.76	0.44–1.31	0.72	0.35–1.46
50–74 nmol/l	119	21	0.18	0.91	0.54–1.55	0.94	0.51–1.75
≥75 nmol/l	124	24	0.19	Reference		Reference	

25(OH)D, 25-hydroxyvitamin D.

*Mixed-effects Poisson regression model controlling for prepregnancy BMI, race, parity, marital status, season of blood draw, treatment group (aspirin versus placebo), and baseline risk group as fixed effects and study site as a random effect. Additional adjustment for C-reactive protein concentration, maternal age, maternal education, or infant sex had no meaningful impact on results.

**Women who were chronically hypertensive before pregnancy and developed pre-eclampsia (ACOG category 3).

***Women who were normotensive before pregnancy and did not develop pre-eclampsia (ACOG category 4).

Table 4.

Maternal vitamin D status at 13–26 weeks' gestation and risk of preterm birth in women at high risk for pre-eclampsia

Serum 25(OH)D categories	*n**	No. cases	Risk per 100 births	Unadjusted risk ratio	95% CI	Adjusted risk ratio**	95% CI
Preterm birth (<37 weeks)	804	215	0.27				
<30 nmol/l	92	21	0.23	0.82	0.54–1.25	1.06	0.63–1.78
30–49 nmol/l	203	50	0.25	0.88	0.65–1.20	1.12	0.75–1.66
50–74 nmol/l	251	72	0.29	1.03	0.78–1.36	1.18	0.84–1.65
≥75 nmol/l	258	72	0.28	Reference		Reference	
Indicated preterm birth (<37 weeks)	712	123	0.17				
<50 nmol/l	264	40	0.15	0.79	0.54–1.17	1.29	0.80–2.09
50–74 nmol/l	218	39	0.18	0.94	0.63–1.38	1.24	0.79–1.95
≥75 nmol/l	230	44	0.19	Reference		Reference	
Spontaneous preterm birth (<37 weeks)	681	92	0.14				
At <50 nmol/l	255	31	0.12	0.93	0.58–1.50	0.97	0.54–1.73
50–74 nmol/l	212	33	0.16	1.19	0.75–1.90	1.23	0.73–2.07
≥75 nmol/l	214	28	0.13	Reference		Reference	
Preterm birth (<35 weeks)	805	89	0.11				
<50 nmol/l	295	37	0.13	1.48	0.90–2.44	1.79	1.00–3.22
50–74 nmol/l	251	30	0.12	1.41	0.83–2.37	1.57	0.89–2.76
≥75 nmol/l	259	22	0.08	Reference		Reference	
Indicated preterm birth (<35 weeks)	757	41	0.05				
<50 nmol/l	276	18	0.07	1.47	0.71–3.05	2.48	1.07–5.76
50–74 nmol/l	233	12	0.05	1.16	0.52–2.58	1.51	0.65–3.47
≥75 nmol/l	248	11	0.04	Reference		Reference	
Spontaneous preterm birth (<35 weeks)	764	48	0.06				
<50 nmol/l	277	19	0.07	1.55	0.75–3.19	1.48	0.65–3.39
50–74 nmol/l	239	18	0.08	1.70	0.82–3.52	1.61	0.74–3.52
≥75 nmol/l	248	11	0.04	Reference		Reference	

25(OH)D, 25-hydroxyvitamin D.

*Total number is higher for preterm <35 weeks' than preterm <37 weeks' gestation because all births (including stillbirths) at ≥35 weeks' gestation are included.

*** Mixed-effects Poisson regression model controlling for prepregnancy BMI, race, parity, marital status, season of blood draw, treatment group (aspirin versus placebo), and baseline risk group as fixed effects and study site as a random effect. Additional adjustment for C-reactive protein concentration, maternal age, maternal education or infant sex had no meaningful impact on results.