Early Maternal Vitamin D Concentrations in Relation to Gestational Diabetes Mellitus, Mood or Anxiety Disorders, and Preeclampsia

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A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington

2013

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Program Authorized to Offer Degree:
School of Public Health - Epidemiology

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Abstract

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Background: Vitamin D is a pleotropic hormone that is involved in diverse cellular functions. Vitamin D deficiency has been associated with several adverse health complications (including cardiometabolic diseases) in the general population. Previous studies examining associations of maternal vitamin D (Vit-D) with gestational diabetes mellitus (GDM), mood or anxiety disorders (MAD), and preeclampsia (PE) risk reported inconsistent findings. In addition, risk factors that may modify these associations (e.g. pre- or mid-pregnancy body mass index [pp-BMI or mp-BMI]) are unknown.

Methods: In a case-cohort study nested within a prospective cohort, we examined associations of maternal early pregnancy vitamin D with GDM (prospective analyses), MAD (cross-sectional analyses), and PE (prospective analyses). Analytic samples included: 135 GDM cases and a randomly selected sub-cohort of 517; 148 MAD cases and a sub-cohort of 554; and 73 PE cases and 600 sub-cohort members. Liquid chromatography-tandem mass spectroscopy was used to measure maternal serum Vit-D (total 25[OH]D and 25[OH]D_3) in early pregnancy (16 weeks on average). GDM was

diagnosed according to the American Diabetes Association guidelines. MAD cases were identified based on physician-diagnosis and/or self-report using medical records. PE diagnosis was made based on the American College of Obstetricians and Gynecologists guidelines.

Logistic regression models were used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CI). Effect modification was assessed using stratified analyses and interaction terms.

Results: GDM cases had lower mean total 25[OH]D (27.3 vs. 29.3ng/ml) and 25[OH]D_3 (23.9 vs. 26.7ng/ml) concentrations compared with women who did not develop GDM (both p-values<0.05). A 5ng/ml increase in 25[OH]D_3 was associated with a 14% decrease in GDM risk (p-value=0.02). Women in the lowest quartile for 25[OH]D_3 had a 2-fold (95%CI 1.15-3.58) higher risk of GDM compared with women in the highest quartile (p-value for trend<0.05). Mean serum total 25[OH]D and 25[OH]D_3 concentrations were higher among women with MAD compared with women without MAD (31.4 vs.29.2 ng/ml for total 25[OH]D, and, 29.2 vs.26.4 ng/ml for 25[OH]D_3, respectively) (both P-values<0.05). Increases of 5 ng/ml in 25[OH]D or 25[OH]D_3 were associated with 1.14 and 1.16-fold higher in risk of MAD, respectively (both P-values<0.05). Similarly, linear decreases in risk of MAD were observed across decreasing quartiles of total 25[OH]D (trend P-value=0.044) and 25[OH]D_3 (trend P-value=0.067). We observed significant interactions of vitamin D with maternal ppOS or maternal smoking history on risk of MAD (interaction P-values<0.05). Decreased risk in MAD related to vitamin D insufficiency/deficiency was observed only among

participants who were over-weight/obese or were current/former smokers. Early pregnancy mean serum total 25[OH]D and 25[OH]D_3 concentrations were lower among women who subsequently developed PE (28.6 and 25.7 ng/ml, respectively) compared with women who did not (29.2 and 26.6 ng/ml, respectively), although the differences were not statistically significant (both p-values>0.05). Risk estimates indicated consistently higher risk of PE (10-40% higher) among women with vitamin D deficiency or women in the lowest quartile for vitamin D concentrations compared with women who were vitamin D sufficient or were in the upper three quartiles for vitamin D concentrations, respectively. However, none of the risk estimates were statistically significant.

Conclusions: Early pregnancy Vit-D, particularly 25[OH]D_3, is inversely associated with GDM risk. These associations are mediated, at least in part, by mp-BMI. Larger prospective studies are needed to further elucidate these relationships. Contrary to previous reports and expectations, serum total 25[OH]D and 25[OH]D_3 concentrations were directly associated with diagnosed MAD. We also observed potential interactions between maternal pre-pregnancy obesity/overweight status or smoking status and maternal vitamin D on MAD. Future prospective studies that employ standardized and validated measures of mood or anxiety symptoms are needed. In the current study, we did not observe significant associations between early pregnancy vitamin D and risk of PE. Given the inconsistency of findings to date and strong mechanistic evidence for this association, future larger prospective studies are warranted.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
25[OH]D	25-Hydroxyvitamin D
ACOG	American College of Obstetricians And Gynecologists
ADA	American Diabetes Association
aOR	Adjusted OR
BMI	Body Mass Index
BP	Blood Pressure
CES-D	Center for Epidemiologic Studies Depression Scale
CI	Confidence Intervals
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
EOSPE	Early Onset Preeclampsia
FFQ	Food Frequency Questionnaire
GA	Gestational Age
GDM	Gestational Diabetes Mellitus
IOM	The Institute of Medicine
IQR	Interquartile Range
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectroscopy
MAD	Mood or Anxiety Disorders
MDD	Major Depressive Disorders
NT	Normotensive
OR	Odds Ratios
PE	Preeclampsia
pp-OS	Pre-Pregnancy Overweight/Obese Status
RAS	Renin-Angiotensin-Aldosterone
RIA	Radioimmunoassay
SBP	Systolic Blood Pressure
SD	Standard Deviation
SPE	Severe Preeclampsia
T2DM	Type 2 Diabetes Mellitus
VDR	Vitamin D Receptors
Vit-D	Vitamin D

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ACKNOWLEDGEMENTS

I first worked with my mentor, Dr. Michelle Williams, in 2006 as a Master's level student in her Minority International Research Training Program. Throughout the years, she has been my advocate and role model and I will always be thankful for her support. I also thank Dr. Daniel Enquobahrie, who is a wonderful demonstration of professional excellence and family balance. Special thanks to Drs. Ann Vander Stoep and Nancy Grote for their valuable contributions to my dissertation work and my career. I am grateful to have worked with the staff of the Center for Perinatal Studies at Swedish Medical Center and for their skillful technical assistance on this project.

I have been blessed with wonderful parents, Wanda and Clifton Arnold, Jr. - their love, support, and encouragement never waiver.

DEDICATION

To my son Caleb, you are surrounded by love, laughter, support, and a family committed to helping you grow into a happy, healthy, confident, and kind young man. Mommy loves you!

CHAPTER 1

Overview of Dissertation Research

Overview of Dissertation Research

Vitamin D

Vitamin D insufficiency and/or deficiency have been associated with Type 2 diabetes mellitus (T2DM), mood or anxiety disorders, and other cardiovascular complications in the general population. While sufficient evidence points to higher frequencies of vitamin D insufficiency/deficiency during pregnancy, up to 50% by some accounts [1], information concerning its association with mood or anxiety disorders (MAD), gestational diabetes mellitus (GDM), and preeclampsia (PE) is sparse.

Vitamin D is a pleiotropic hormone that influences cell growth, cellular differentiation and optimal function of many organs and tissues throughout the body including neurons, immune cells, pancreatic beta cells, and endothelial cells [2, 3]. It is a steroid hormone that is derived primarily from synthesis in the skin through exposure to ultraviolet B radiation, and secondarily through the ingestion of vitamin D rich foods (e.g. fatty fish like salmon and fortified foods like milk) or nutritional supplements. Vitamin D undergoes hydroxylation in the maternal liver or placenta to form 25[OH]D. The 25[OH]D metabolite has a half-life of approximately 3 weeks providing a biologically inactive but stable supply of vitamin D, qualities which make it an ideal indicator of vitamin D status [4,5].

Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus, or glucose intolerance first identified during pregnancy, occurs in 3-8% of pregnancies [8] and is a risk factor for TDM2 in both mothers and infants later in life [6, 7]. Although low vitamin D level has been identified as a potential risk factor of GDM, previous studies reported inconsistent results. Of the

5 studies we identified, 3 studies reported significant negative associations between 25[OH]D levels and GDM risk [9, 10, 11], while the others did not [12,13].

Mood or Anxiety Disorders (MAD)

Depression (or mood disorders) affects 15% to 30% of pregnant women [14, 15, 16]. Self-reported maternal symptoms of depression during pregnancy have been associated with preeclampsia [17, 18], low birth weight, inconsolability and excessive crying in infants [19]. Risk factors for MAD include: history of depression, lack of social support, poverty, family violence, and substance abuse [20]. Evidence from human and animal studies suggest plausible biological mechanisms for an association of depression with low vitamin D [21], and only one study examining the association between maternal 25[OH]D and MAD [22].

Preeclampsia (PE)

Preeclampsia, a pregnancy-related syndrome characterized by hypertension and proteinuria after 20 weeks gestation [23-25]. Women who develop preeclampsia have an increased risk of pulmonary edema, hepatic and/or renal failure, coagulation difficulties, cerebral hemorrhage, seizure, blindness, and death [23, 25-29]. Preeclampsia complicates 5-8% of pregnancies [23-25]. Earlier studies of vitamin D and PE reported reduced maternal serum 25[OH]D in women with PE, however 25[OH]D was measured after the diagnosis of preeclampsia [30, 31]. Although recent studies measured 25[OH]D before preeclampsia was clinically evident (and included larger numbers of participants), the results are inconsistent.

Significance and Innovation

Several aspects of this project can be highlighted to indicate its significance and innovation. Vitamin D deficiency is a prevalent and potentially preventable risk factor that has potentially far-reaching consequences

on the course and outcomes of pregnancy. Evaluated outcomes, GDM, PE, and MAD, are associated with significant maternal and offspring morbidity and mortality. These studies were conducted among a well-characterized cohort of pregnant women and address several limitations of previous studies. Blood samples were collected in early pregnancy, prior to onset or measurement of GDM or PE. We used state-of-the-art liquid chromatography-tandem mass spectroscopy methods (LC-MS/MS) to measure maternal serum total 25[OH]D, $25[OH]D_2$ and $25[OH]D_3$ concentrations. We measured relevant maternal characteristics (such as pre-pregnancy body mass index (BMI), and physical activity) to assess confounding and/or effect modification by these covariates. Finally, our studies included a large number of cases, compared to most previous studies, and utilized an efficient case-cohort design.

In summary, vitamin D insufficiency/deficiency, MAD, GDM, and PE are significant public health problems insofar as they impact the health of the mother, the growing fetus and that of the child later in life, even into adulthood. Current strategies for improving vitamin D status in the general population create an opportunity to reduce morbidity and mortality from conditions that have been associated with low vitamin D including hypertension and T2DM. However, lack of information on associations of vitamin D with regard to pregnancy related complications has limited similar preventive efforts during pregnancy and throughout the life course of both mothers and children. As researchers and clinicians continue to debate the recommended daily allowance and supplementation recommendations for pregnant women, particularly those diagnosed with vitamin D deficiency, studies such as ours will provide evidence that can inform and shape the continuing debate.

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CHAPTER 2

Early Pregnancy Maternal Vitamin D Concentrations and Risk of Gestational Diabetes Mellitus

ABSTRACT

Background: Previous studies examining associations of maternal vitamin D (Vit-D) with gestational diabetes mellitus (GDM) risk reported inconsistent findings. Whether pre- or mid-pregnancy body mass index (pp-BMI or mp-BMI) modify or mediate these associations is unknown.

Methods: In a case-cohort study (135 GDM cases and 517 randomly selected sub-cohort), nested within a prospective cohort, we examined associations of Vit-D with GDM risk. Liquid chromatography-tandem mass spectroscopy was used to measure maternal serum Vit-D (total 25[OH]D and 25[OH]D_3) in early pregnancy (16 weeks on average). GDM was diagnosed according to the American Diabetes Association guidelines. Logistic regression models were used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CI).

Results: GDM cases had lower mean total 25[OH]D (27.3 vs. 29.3ng/ml) and 25[OH]D_3 (23.9 vs. 26.7ng/ml) concentrations compared with women who did not develop GDM (both p-values<0.05). Overall, 25[OH]D_3, but not total 25[OH]D, was significantly associated with GDM risk. A 5ng/ml increase in 25[OH]D_3 was associated with a 14% decrease in GDM risk (p-value=0.02). Women in the lowest quartile for 25[OH]D_3 had a 2-fold (95%CI 1.15-3.58) higher risk of GDM compared with women in the highest quartile (p-value for trend<0.05). We did not observe interactions between pp-BMI and Vit-D on GDM risk (interaction p-value>0.05). Mp-BMI accounted for 18% of the effect of 25[OH]D_3 on GDM risk.

Conclusion: Early pregnancy Vit-D, particularly 25[OH]D_3, is inversely associated with GDM risk. These associations are mediated, at least in part, by mp-BMI. Larger prospective studies are needed to further elucidate these relationships.

INTRODUCTION

In 2011, the Endocrine Society's Community of Clinical Practice released guidelines for the evaluation, treatment, and prevention of vitamin D deficiency that included controversial recommendations for daily allowance of vitamin D that exceed levels indicated in prior guidelines [1, 2]. The Institute of Medicine (IOM), which itself had released vitamin D supplementation guidelines earlier that year [2], contends that the new guideline from the Endocrine Society does not provide adequate evidence for the assertion that higher serum levels of vitamin D (25[OH]D) (≥ 30 ng/mL) would be beneficial for at-risk populations (e.g. pregnant women). In 2012, the Cochrane Collaboration released a review of 6 randomized controlled trials examining effects of vitamin D supplementation during pregnancy. Their review showed vitamin D supplementation during pregnancy improved women's vitamin D levels, but did not prevent pregnancy or fetal complications [3]. Despite the well-known cardiovascular and metabolic effects of vitamin D, this finding highlights the need for studies to evaluate the role of vitamin D in pregnancy and pregnancy complications.

Research on vitamin D and gestational diabetes mellitus (GDM) is motivated by over a decade of observational studies which demonstrated a consistent and strong association between vitamin D deficiency and Type 2 diabetes mellitus [4, 5], a condition that has similar pathogenesis and risk factors to GDM [6-8]. Of the 12 published studies that examined vitamin D and GDM, six were published in 2012. Seven studies reported significant inverse associations between vitamin D concentrations and GDM risk [9-15], while five did not [16-20]. Some of the reasons that were put forth for observed inconsistencies included timing of blood collection (e.g. post disease

diagnosis), accuracy of vitamin D (and metabolite) measurements, and inadequate assessment of potential confounders and/or effect modifiers (e.g. pre-pregnancy body mass index).

METHODS

Study setting

This case-cohort study [21-23] was conducted among participants of the Omega Study [24], a prospective cohort study (1996-2008) of 4,000 pregnant women designed to examine risk factors of pregnancy complications.

Study population

Study participants were identified among women attending prenatal care clinics at Swedish Medical Center in Seattle, Washington and Tacoma General Hospital in Tacoma, Washington. Eligibility criteria for the Omega study included initiation of prenatal care before completion of 20 weeks of gestation, being 18 years of age or older, speaking and reading English, planning to carry the pregnancy to term, and planning to deliver at one of the study institutions. An overview of participant selection, exclusion, and *a priori* set restriction criteria for the current study is summarized in **Figure 2.1**. A set of randomly chosen sub-cohort (N=703) and GDM cases (N=195) were identified for the study. After exclusion of participants with unknown non-case status (n=18), gestational age at delivery <24 weeks (9), missing 25[OH]D measurements (n=7), preeclampsia (n=41), mood and/or anxiety disorder (n=54), chronic hypertension (n=27), renal disease (n=5), thyroid condition (n=70), liver disease (n=7), rheumatoid arthritis (n=5), and lupus (n=3), a total of 135 GDM cases and 517 controls remained for analyses. All study participants provided informed consent. Study procedures were approved by the Institutional Review Boards of Swedish Medical Center and Tacoma General Hospital.

GDM diagnosis

As part of perinatal screening procedures, all pregnant women attending perinatal clinics affiliated with our institutes are screened between weeks 24-28 using a 50g 1-hour oral glucose challenge test. Women who failed this screening (glucose ≥7.8mmol/L (140mg/dl)) underwent a 100g oral glucose tolerance test 1-2 weeks after the first failed screening test. GDM was defined according to the recommendations of the American Diabetes Association (ADA) [25]. Women were diagnosed with GDM if two or more of the following exceeded ADA criteria after a 100g oral glucose tolerance test: fasting ≥ 5.3 mmol/L (95 mg/dl); 1-hour ≥ 10.0 mmol/L (180 mg/dl); 2-hour ≥ 8.6 mmol/L (155 mg/dl); 3-hour ≥ 7.8 mmol/L (140mg/dl).

Data Collection

At the time of enrollment, study personnel collected maternal blood specimens. All blood specimens were processed within 30 minutes, and aliquots were stored at -80°C until vitamin D was measured. Soon after blood specimens were collected, participants completed a telephone interview using a structured questionnaire administered in English by a trained interviewer. Questionnaires were used to gather data on socioeconomic status and reproductive and medical histories. Participants also completed and returned a food frequency questionnaire. After delivery, maternal and infant medical records were abstracted for information on the course and outcome of the pregnancy.

Vitamin D Measurement

Maternal early pregnancy 25[OH]D concentrations were assessed by measuring the following vitamin D metabolites in maternal serum collected at enrollment (at 16 weeks of

gestation on average): total 25[OH]D (sum of 25[OH]D_3, and 25[OH]$D_{2)}$, 25[OH]D_3, and 25[OH]D_2. Only a few samples had detectable 25[OH]D_2 concentrations. Therefore, subsequent analyses were restricted to total 25[OH]D and 25[OH]D_3. Measurements were conducted using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods at ZRT Laboratories (ZRT, Portland, OR). The inter-assay precision coefficients of variation for 25[OH]D_2 and 25[OH]D_3 were 9% and 6% respectively.

Statistical analyses

We examined distribution of categorical (number and percentage) and continuous (mean and standard deviation) variables among GDM cases and sub-cohort members. Multivariable logistic regression models were used to determine associations between early pregnancy maternal serum 25[OH]D concentrations and GDM using odds ratios (OR) and 95% confidence intervals (CI). In our analyses we modeled exposure variables (both total 25[OH]D and 25[OH]D_3) in three different ways: 1) as a continuous variable, 2) as a categorical variable (quartiles), and 3) as categorical variables characterizing vitamin D deficiency status. P-value for trend was computed by assessing linear trend across increasing quartiles. According to previously published criteria [26], vitamin D sufficiency (≥30 ng/ml), insufficiency (20–29 ng/ ml) and deficiency (<20 ng/ml) were used to categorize participants according to their vitamin D concentrations. In addition to *a priori* selected potential confounders, covariates that altered unadjusted odds ratios (ORs) by 10% or more were included in final adjusted models [27]. We fit four separate models, unadjusted (Model 1) and three adjusted models, in these analyses. We adjusted for race/ethnicity (White non-Hispanic, other), maternal age (years), education , marital status, season of blood draw, parity, smoking status, peri-conceptional multivitamin use in the

first adjusted model (Model 2). In the second adjusted model (Model 3), we included all Model 2 adjustment variables along with maternal pre-pregnancy BMI (kg/m^2). In the last adjusted model, Model 4, we included Model 2 adjustment variables along with mid-pregnancy BMI (BMI at 18-22 weeks of gestation). Models 3 and 4 were aimed at evaluating the roles of pre-pregnancy BMI or mid-pregnancy BMI, in associations between maternal early pregnancy vitamin D and GDM risk. To evaluate the role of pre-pregnancy obesity, we conducted stratified analyses [28], to examine the independent and joint effects of total 25[OH]D or $25[OH]D_3$ and pre-pregnancy obesity status on the risk of GDM. P-values of the interaction terms were used to determine statistical significance (if p-value < 0.05) of interactions. We also assessed whether mid-pregnancy BMI mediated associations between maternal vitamin D and GDM risk. We assessed mediation by mid-pregnancy BMI at 18-22 weeks gestation by fitting "seemingly unrelated" regression models as described by Preacher et al [29]. These models were then used to measure effects of maternal vitamin D (either total 25[OH]D or $25[OH]D_3$) that are mediated by mid-pregnancy BMI [29]. We also conducted sensitivity analyses to assess possibility of different relationships among subgroups (e.g. restricted among non-Hispanic Whites). Our findings from these analyses were similar to our findings from our primary analyses reported in this manuscript. All reported CIs were calculated at the 95% level, and all reported P-values are two-tailed. Analyses were performed using STATA 11.0 (STATA, College Station, TX).

RESULTS

Women with GDM were more likely to be older, have a family history of diabetes mellitus or hypertension, have higher BMI (both pre-pregnancy and at 18-22 weeks of gestation), deliver early, and were less likely to be non-Hispanic white as compared with women in the comparison sub-cohort (**Table 2.1**). Early maternal serum total 25[OH]D and 25[OH]D_3 were lower in women with GDM (mean ± SD, GDM cases vs. comparison sub-cohort: 27.3 ± 8.7 vs. 29.3 ± 8.3 ng/ml, P = 0.01 for total 25[OH]D; and, 23.9 ± 9.4 vs. 26.7 ± 8.8 ng/ml, P = 0.002 for 25[OH]D_3). Among GDM cases, 17% of women were vitamin D deficient (total 25[OH]D <20 ng/ml) compared to 10.8% of women in the comparison sub-cohort.

A 5ng/ml increase in serum total 25[OH]D was associated with a 14% reduction in risk of GDM (OR 0.86 [95% CI: 0.77, 0.97]) (**Table 2.2**). Women with vitamin D deficiency had a 1.97-fold increased risk of GDM compared to women who were sufficient in total 25[OH]D (≥ 30 ng/ml) [95% CI: 1.12, 3.47] in the unadjusted model (**Table 2.2**). Women in the lower three quartiles for total 25[OH]D had higher risk of GDM compared with women in the highest quartile in unadjusted models (p-value for trend=0.013) (**Table 2.3**). However, these associations were attenuated and became statistically insignificant after adjustment for potential confounders (**Tables 2.2 and 2.3**). Additional adjustments for pre-pregnancy BMI and mid-pregnancy BMI further attenuated these relationships. In stratified analyses, we observed no interaction between pre-pregnancy obesity and vitamin D deficiency (based on total 25[OH]D) (P value for interaction= 0.279) (**Table 2.4**).

We repeated these analyses using 25[OH]D_3 as our exposure variable. A 5ng/ml increase in serum total 25[OH]D_3 was associated with a 14% reduction in risk of GDM (Adjusted OR 0.88 [95% CI: 0.76, 0.96]) (**Table 2.5**). Women who were vitamin D deficient based on 25[OH]D_3 measures had a 1.95-fold increased risk of GDM compared to women who were sufficient in 25[OH]D_3 [95% CI: 1.16, 3.29] (**Table 2.5**). Women in the lower three quartiles for total 25[OH]D_3 had higher risk of GDM compared with women in the highest quartile (adjusted p-value for trend=0.010) (**Table 2.6**). Women in the lowest quartile for 25[OH]D_3 had a 2-fold (95%CI 1.15-3.58) higher risk of GDM compared with women in the highest quartile (**Table 2.6**). These associations were slightly attenuated but remained statistically significant or marginal, after adjusting for pre-pregnancy BMI or mid-pregnancy BMI, respectively (**Table 2.6**). In stratified analyses, similar to our findings for total 25[OH]D, we did not find significant interactions between pre-pregnancy obesity and 25[OH]D_3 in relation to GDM risk (p-value for interaction=0.487) (**Table 2.7**). Mid-pregnancy BMI reduced the effect of 25[OH]D_3 on GDM risk (p-value=0.025) by 17.6%

DISCUSSION

In this study, we found that maternal early pregnancy vitamin D, particularly 25[OH]D_3, was inversely associated with risk of GDM. Women who were vitamin D deficient in early pregnancy, based on 25[OH]D_3 concentrations, had a 1.9-fold higher risk of developing GDM after adjustment for potential confounders. We did not find significant interactions between vitamin D and pre-pregnancy obesity on GDM risk. Mid-pregnancy BMI mediated 18% of the effect of 25[OH]D_3 on GDM risk.

Our findings are similar to some previous studies that investigated vitamin D and GDM risk [10, 12, 13, 15] but not others [17, 19, 20]. Previously, our research group reported that total 25[OH]D was associated with a 2.66-fold increased risk of subsequent GDM [95% CI 1.01, 7.02] in a nested-case control study conducted among 57 GDM cases and 114 controls [10]. Similarly, Parlea et al. [13], in a nested case-control study of 116 women with GDM and 219 controls found that women with vitamin D deficiency were 2.21 times more likely to develop GDM compared to those without GDM. In their nested case control study of 200 women with GDM and 200 women with normal glucose tolerance, Wang et al. [15], reported that women with total 25[OH]D levels <25 nmol/L had a 1.59 fold risk of GDM compared to women with higher level and within the GDM. Moreover, a meta-analysis published in 2012 reported that vitamin D deficiency in pregnancy was significantly related to the GDM risk (OR 1.61 [95% CI 1.19, 2.17]) [12]. However, associations of vitamin D with GDM risk were not observed in some studies. A cross-sectional study of 559 women in India found equivalent levels of vitamin D deficiency among women with and without GDM at 30 weeks gestation and similar risk of GDM among women with or without vitamin D deficiency (7% prevalence of GDM in women with and without 25[OH]D

concentrations below 50 nmol/L) [17]. Baker et al. [19], reported that women with vitamin D deficiency did not have a significantly higher risk of GDM compared with women who did not have vitamin D deficiency (OR 0.78 [95% CI 0.22, 2.78]). Similarly, researchers did not observe associations of vitamin D deficiency with risk of GDM in a recently reported study (OR 2.2 [95% CI 0.8, 5.5]), although glucose levels were inversely associated with total 25[OH]D levels [20].

Inconsistency in reported results may be due to, at least in part, timing of vitamin D measurements, differences in assays used to measure vitamin D, inadequate/inconsistent control for confounding, and differences in criteria used to diagnose GDM. For instance, two of the three studies that found no association between vitamin D and GDM risk used 75-g oral glucose tolerance test (OGTT) to determine GDM diagnoses [16, 18]. On the other hand, all studies that reported inverse associations, including the present study, used 100-g OGTT to determine GDM diagnosis [9-11]. The 75-g test has been shown to detect GDM less effectively compared with 100-g OGTT (7.1% diagnosed with GDM using 75-g; 21.4% diagnosed with GDM using 100-g) [30]. This may lead to misclassification and a loss of power in some of these studies.

In the current study, we took some steps to address limitations of previous studies. First, vitamin D concentrations were measured from samples collected in early pregnancy (<20 weeks gestation), well before the diagnosis of GDM, establishing a relatively clear temporal relationship between vitamin D deficiency and GDM. Second, state-of-the-art LC-MS/MS methods were used to measure maternal vitamin D concentrations, enabling us to measure both total 25[OH]D and 25[OH]D_3 concentrations in serum. Third, our well-characterized study

population allowed for collection of relevant data to assess confounding, effect modification or mediation. For example, we conducted analyses to evaluate effect modification by pre-pregnancy BMI or mediation effects of mid-pregnancy BMI. Fourth, our GDM case definition used current, widely accepted criteria [25]. Finally, our study is one of a few recent studies to include a large number of GDM cases [13, 15].

Relatively few previous studies have considered the different constituents of total vitamin D in relation to disease risk. There are two forms of vitamin D, vitamin D_2 (ergocalciferol), a synthetic form, and vitamin D_3 (cholecalciferol), obtained through diet and exposure of skin to sunlight. Unless otherwise specified, vitamin D refers to the sum total of D_2 and D_3 concentrations. While both forms of vitamin D utilize the same enzymes in their metabolic pathways, the differences in their side chains (vitamin D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24) result in the production of unique metabolites of differing potencies and bioeffectiveness [31]. Active metabolites of vitamin D_2 have a diminished ability to bind to (1) vitamin D binding proteins and (2) vitamin D receptors, compared to metabolites of vitamin D_3 [32-34]. Further, the enzyme 25-hydroxylase converts vitamin D_3 to 25[OH]D_3 (which is an ideal indicator of vitamin D_3 status due to its 3 week half-life [35]) five (5) times as fast as it converts vitamin D_2 to 25[OH]D_2 [36]. A 2004 study by Armas et al. compared the time course of serum 25[OH]D over a 28 day period after a single 50,000 IU dose of either vitamin D_2 or vitamin D_3 administered to 20 healthy men [33]. Both produced a similar rise in serum total 25[OH]D in the first 3 days, however serum total 25[OH]D concentrations of participants who received vitamin D_2 rapidly declined reaching baseline by day 14 and then fell below baseline by day 28. Participants treated with vitamin D_3 had serum

25[OH]D concentrations that continued to rise after day 3, peaking at day 14 and were above baseline at day 28. Moreover, the investigators stated that vitamin D_3 was more than 3 times more potent than vitamin D_2 [33]. These findings and previous reports support the stronger relationship of vitamin D with risk of GDM we observed when using 25[OH]D_3 measures, compared to using total 25[OH]D measures.

Several biological mechanisms have implicated vitamin D insufficiency or deficiency in GDM pathogenesis. First, vitamin D deficiency may contribute to the development of GDM via calcium pool dysregulation in the pancreas. Normally, 1,25[OH] binds to vitamin D receptors (VDR) in pancreatic beta-cells, regulating the balance between extracellular and intracellular beta cell calcium pools in the pancreas [10, 37-39]. Second, vitamin D may contribute to lower GDM risk via its effects on insulin-sensitive tissues. Vitamin D promotes insulin sensitivity by stimulating insulin receptor expression and enhancing insulin responsiveness to glucose [4, 39, 40]. Third, interactions of vitamin D and vitamin D related genetic variations (e.g. VDR) with the insulin like growth factor system influence glucose homeostasis [41]. Fourth, vitamin D may contribute to lower GDM risk via its role in mitigating inflammation and its effects [4]. Several studies have shown that systemic inflammation, through pancreatic beta cell dysfunction and apoptosis, is associated with T2DM [42-44]. Finally, the close relationship of vitamin D deficiency with other common risk factors, such as obesity, may account (in part) for its association with GDM and other related pregnancy complications. In our study, we observed mediation of the effects of vitamin D on GDM risk by mid-pregnancy BMI supporting these hypotheses.

Several limitations of our study deserve mention. First, we had a single measurement of vitamin D which does not include a time integrated measure reflecting vitamin D status over the course of pregnancy. Second, we did not measure other related indicators of biological activities of vitamin D such as vitamin D receptors, vitamin D binding protein, and enzymes related to vitamin D metabolism (such as 25-OHase and 1-OHase). Third, it is possible that a limited number of women may have had undiagnosed pre-pregnancy glucose intolerance or DM at the time of blood collection, which would result in a misclassification. Fourth, there may be residual confounding in our adjusted measures of association as well as confounding from unmeasured variables. Fifth, generalizability in our study is guarded to other populations that have different socio-economic and ethnic composition. However, similarity of findings of reports from studies conducted in different study populations mitigates this concern.

Our study provides modest evidence for associations of maternal early pregnancy $25[OH]D_3$ levels, with subsequent risk of GDM. We also found that this association is mediated, at least in part, by mid-pregnancy BMI. Additional studies are needed to better understand the association between maternal $25[OH]D_3$ deficiency and GDM, the biological pathways involved, and the impact of vitamin D supplementation (in terms of dose and duration) on risk of GDM. If our results are confirmed, possibilities of reducing the risk of GDM through vitamin D supplementation would present a viable opportunity to reduce GDM related morbidity and mortality.

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Figure 2.1: Sampling and analytic groups, GDM study

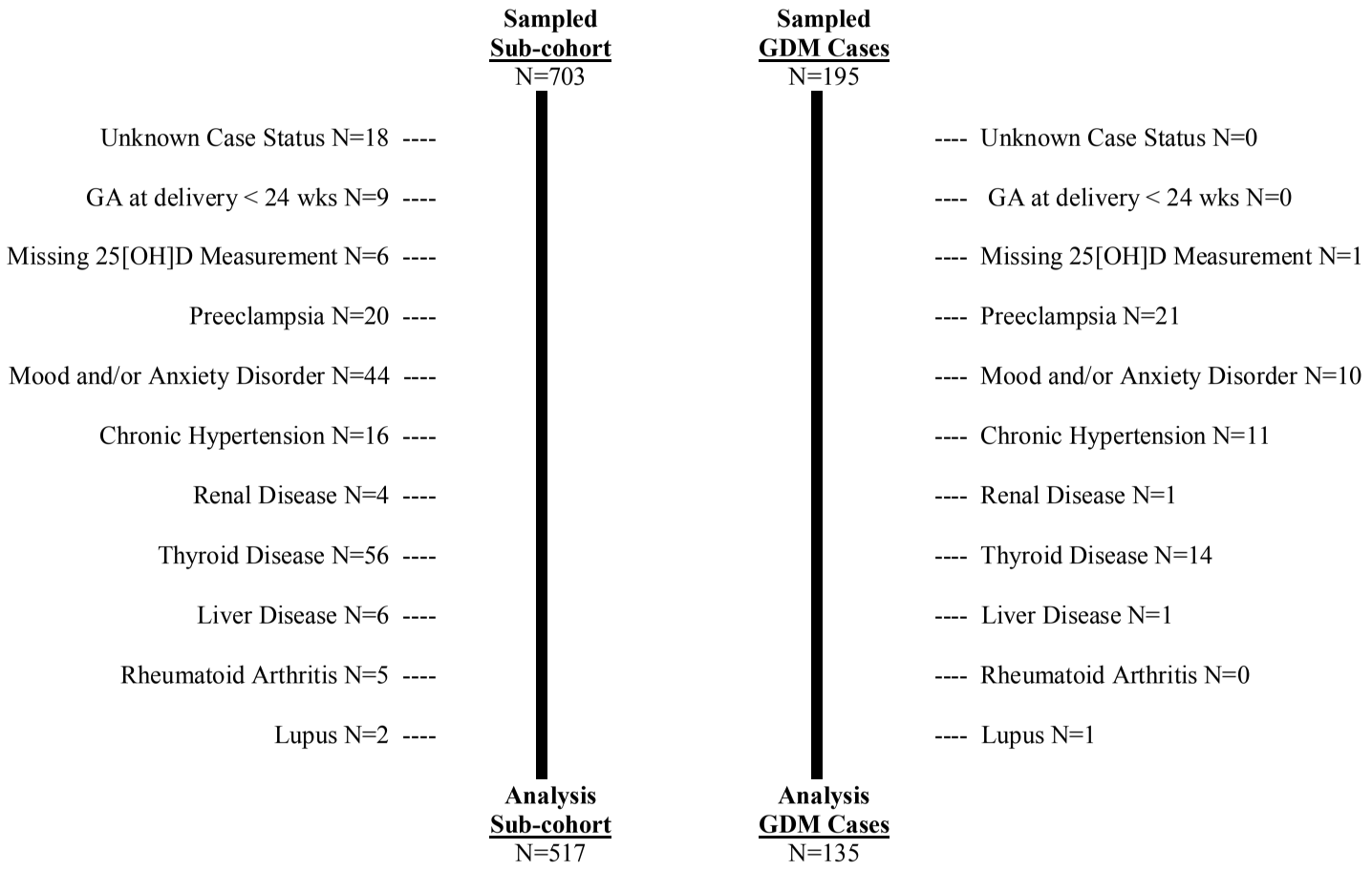

Table 2.1: Characteristics of study participants according to gestational diabetes mellitus (GDM) case-cohort status

Characteristics	**GDM Cases (N=135)** n (%)	**Sub-Cohort (N= 517)** n (%)	**P-value[§]**
Maternal Age (years)	33.5 ± 4.6	32.6 ± 4.4	**0.03**
<35	79 (58.5)	345 (66.7)	0.08
≥35	56 (41.5)	172 (33.3)	
Marital Status			
Married	116 (85.9)	447 (86.5)	0.87
Household Income			
≥$70,000	98 (72.6)	360 (69.6)	0.75
Education			
Post High School	122 (90.4)	474 (91.7)	0.86
Payment Status			
Insurance	127 (94.1)	464 (89.8)	0.25
Maternal Race/Ethnicity			
White/Non-Hispanic	96 (71.1)	441 (85.3)	**<0.001**
African American	1 (0.7)	11 (2.1)	
Asian	26 (19.3)	38 (7.3)	
Other	11 (8.2)	27 (5.2)	
Primigravida	45 (33.3)	200 (38.7)	0.25
Nulliparous	79 (58.5)	320 (61.9)	0.47
Leisure Time Physical Activity	98 (72.6)	393 (76.0)	0.70
Prenatal Vitamin Use	124 (91.9)	477 (92.3)	0.96
Infant Birth weight (grams)	3479 ±584	3433 ± 670	0.47
GA at Delivery (weeks)	38.3 ± 1.9	38.7 ± 2.1	0.03
Singleton Pregnancy	130 (96.3)	496 (95.9)	0.85
Lowfat Diary (servings/week)	13.6 ± 11.9	15.4 ± 11.3	0.14
Regular Diary (servings/ week)	6.1 ± 6.4	6.7 ± 6.5	0.32
Calcium (mg/day)	1139 ± 645	1249 ± 617	0.08
≥1,000 mg/day	60 (44.4)	284 (54.9)	0.09
Smoking History			
Never	96 (71.1)	350 (67.7)	0.53
Former	21 (15.6)	108 (20.9)	

Current	10 (7.4)	30 (5.8)	
Family History of Diabetes Mellitus	46 (34.1)	65 (12.6)	**<0.001**
Family History of Hypertension	76 (56.3)	226 (43.7)	**0.009**
Pre-pregnancy BMI (kg/m^2)	25.9 ± 6.7	23.1 ± 4.7	**<0.001**
18-22 Weeks Gestation BMI (kg/m^2)	28.4 ± 6.5	25.6 ± 4.4	**<0.001**
GA at Blood Draw (weeks)	15.2 ± 2.9	15.3 ± 2.8	0.75
Season of Blood Draw			
Spring (March-May)	36 (26.7)	145 (28.1)	0.99
Summer (Jun-Aug)	37 (27.4)	135 (26.1)	
Autumn (Sept- Nov)	35 (25.9)	135 (26.1)	
Winter (Dec-Feb)	27 (20.0)	102 (19.7)	
Total Maternal Serum 25[OH]D (ng/ml)	27.3 ± 8.7	29.3 ± 8.3	**0.01**
Maternal Serum 25[OH]D$_3$ (ng/ml)	23.9 ± 9.4	26.7 ± 8.8	**0.002**
Maternal Serum 25[OH]D$_2$ (ng/ml)	3.6 ± 5.0	2.9 ± 4.4	0.13

§ P-values comparing GDM cases to sub-cohort
Mean ± SD (standard deviation)
Abbreviations: **BMI**, body mass index; **GA**, gestational age

Table 2.2: Odds ratios (OR) and 95% Confidence Intervals (CI) for gestational diabetes mellitus (GDM) according to maternal serum total 25[OH]D concentrations (ng/ml) in early pregnancy

	GDM Cases (N=135)	**Sub-Cohort (N=517)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**	**Adjusted++ OR (95%CI)**	**Adjusted+++ OR (95%CI)**
TOTAL 25[OH]D (categorical)						
Sufficient (≥30)	50 (37.0)	234 (45.3)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Insufficient (20-29)	61 (45.2)	226 (43.7)	1.26 (0.83-1.92)	1.27 (0.82-1.95)	1.12 (0.72-1.74)	1.04 (0.66-1.63)
Deficient (<20)	24 (17.8)	57 (11.0)	1.97 (1.12-3.47)	1.63 (0.89-2.97)	1.38 (0.74-2.57)	1.30 (0.69-2.44)
P for trend			**0.023**	0.099	0.321	0.481
TOTAL 25[OH]D (continuous)						
Per 5 ng/ml increase			**0.86 (0.77-0.97)**	0.90 (0.80-1.03)	0.95 (0.83-1.08)	0.96 (0.84-1.10)

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes
++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, Pre-pregnancy BMI
+++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, BMI at 18-22 weeks pregnancy

Table 2.3: Odds Ratios (OR) and 95% Confidence Intervals (CI) for gestational diabetes mellitus (GDM) according to maternal serum total 25[OH]D quartiles measured in early pregnancy

	GDM Cases (N=135)	**Sub-Cohort (N=517)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**	**Adjusted++ OR (95%CI)**	**Adjusted+++ OR (95%CI)**
TOTAL 25[OH]D (ng/ml)						
1st Quartile (≥34.9)	22 (16.3)	129 (25.0)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd Quartile (29.1-34.8)	33 (24.4)	129 (25.0)	1.50 (0.83-2.71)	1.32 (0.71-2.43)	1.29 (0.70-2.39)	1.29 (0.69-2.39)
3rd Quartile (23.5-29.0)	34 (25.2)	130 (25.0)	1.53 (0.85-2.76)	1.39 (0.76-2.56)	1.29 (0.70-2.38)	1.20 (0.64-2.23)
4th Quartile (<23.5)	46 (34.1)	129 (25.0)	**2.09 (1.19-3.67)**	1.65 (0.92-2.98)	1.43 (0.78-2.62)	1.37 (0.74-2.52)
P for trend	---	---	**0.013**	0.104	0.284	0.388

§ Quartiles based on distribution of the comparison sub-cohort
+ Adjusted for season of blood draw maternal age, race/ethnicity, family history of diabetes
++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, Pre-pregnancy BMI
+++ Adjusted for season of blood draw maternal age, race/ethnicity, family history of diabetes, BMI at 18-22 weeks pregnancy

Table 2.4: Interaction of overweight/obesity status and current/former smoking status with maternal serum total 25[OH]D on gestational diabetes mellitus (GDM)

	GDM		Stratified model		Joint model	
Characteristics	**Yes (N=135)**	**No (N=517)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	36 (26.7)	191 (36.9)	Referent	Referent	Referent	Referent
No, BMI <25	40 (29.6)	206 (39.9)	1.02	(0.61-1.70)	1.02	(0.61-1.70)
Yes, BMI ≥25	14 (10.4)	43 (8.3)	Referent	Referent	1.68	(0.81-3.46)
No, BMI ≥25	45 (33.3)	77 (14.9)	1.96	(0.90-4.27)	**2.81**	**(1.64-4.83)**
P-value for interaction						0.279

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes
*Numbers may not add up to total number of cases or cohort members due to missing

Table 2.5: Odds ratios (OR) and 95% confidence intervals (CI) for gestational diabetes mellitus (GDM) according to maternal serum 25[OH]D_3 concentrations (ng/ml) in early pregnancy

	GDM Cases (N=135)	**Sub-Cohort (N=517)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**	**Adjusted++ OR (95%CI)**	**Adjusted+++ OR (95%CI)**
25[OH]D_3 (categorical)						
Sufficient (≥30)	33 (24.41)	178 (34.4)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Insufficient (20-29)	53 (39.3))	221 (42.8)	1.29 (0.80-2.08)	1.33 (0.81-2.18)	1.19 (0.72-1.96)	1.13 (0.68-1.88)
Deficient (<20)	49 (36.3)	118 (22.8)	**2.24 (1.36-3.69)**	**1.95 (1.16-3.29)**	**1.78 (1.04-3.02)**	1.64 (0.96-2.81)
P for trend			**0.002**	**0.012**	**0.034**	0.073
25[OH]D_3 (continuous)						
Per 5 ng/ml increase			0.84 (0.75-0.94)	0.86 (0.76-0.96)	0.88 (0.78-0.99)	0.90 (0.79-1.01)

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes
++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, Pre-pregnancy BMI
+++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, BMI at 18-22 weeks pregnancy

Table 2.6: Odds ratios (OR) and 95% confidence intervals (CI) for Gestational Diabetes Mellitus (GDM) according to maternal serum 25[OH]D_3 quartiles measured in early pregnancy

	GDM Cases (N=135)	**Sub-Cohort (N=517)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**	**Adjusted++ OR (95%CI)**	**Adjusted+++ OR (95%CI)**
25[OH]D_3 (ng/ml)						
1st Quartile(≥32.5)	23 (17.0)	129 (25.0)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd Quartile (26.4-32.4)	29 (21.5)	130 (25.0)	1.25 (0.69-2.28)	1.14 (0.62-2.12)	1.03 (0.55-1.93)	1.0 3(0.55-1.93)
3rd Quartile (20.6-26.3)	28 (20.7)	129 (25.0)	1.22 (0.67-2.22)	1.21 (0.65-2.26)	1.02 (0.54-1.92)	0.95 (0.50-1.81)
4th Quartile (<20.6)	55 (40.7)	129 (25.0)	**2.39 (1.39-4.12)**	**2.03 (1.15-3.58)**	**1.79 (1.01-3.19)**	1.68 (0.94-3.00)
P for trend			**0.001**	**0.010**	**0.034**	**0.072**

§ Quartiles based on distribution of the comparison sub-cohort
+ Adjusted for season of blood draw maternal age, race/ethnicity, family history of diabetes
++ Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes, Pre-pregnancy BMI
+++ Adjusted for season of blood draw maternal age, race/ethnicity, family history of diabetes, BMI at 18-22 weeks pregnancy

Table 2.7: Interaction of overweight/obesity status and current/former smoking status with maternal serum 25[OH]D_3 on gestational diabetes mellitus (GDM)

	GDM		**Stratified model**		**Joint model**	
Characteristics	**Yes (N=135)**	**No (N=517)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	23 (17.0)	144 (27.9)	Referent	Referent	Referent	Referent
No, BMI <25	53 (39.3)	253 (48.9)	1.29	(0.75-2.23)	1.29	(0.75-2.23)
Yes, BMI ≥25	10 (7.4)	34 (6.6)	Referent	Referent	1.76	(0.75-4.17)
No, BMI ≥25	49 (36.3)	82 (16.6)	2.02	(0.85-4.76)	**3.24**	**(1.80-5.83)**
P-value for interaction						0.487

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, family history of diabetes
*Numbers may not add up to total number of cases or cohort members due to missing

CHAPTER 3

Associations of Early Pregnancy Maternal Vitamin D with Mood or Anxiety Disorders

ABSTRACT

Background: Accumulating evidence suggests inverse associations of vitamin D with mood or anxiety disorders (MAD) among men and non-pregnant women. However, relatively little is known about these associations among pregnant women. In addition, whether pre-pregnancy overweight/obese status (ppOS) or maternal smoking modify these associations is unknown.

Methods: Using a cross-sectional study design, we examined associations of early pregnancy (16 weeks of gestation, on average) vitamin D with MAD among participants of a pregnancy cohort study. Cases (N=148) with MAD, diagnosed before or during pregnancy, were identified based on physician-diagnosis and/or self-report using medical records. Sub-cohort (N=554) members were selected randomly from the entire pregnancy cohort. Liquid chromatography-tandem mass spectroscopy techniques were used to measure maternal serum vitamin D (total 25[OH]D and 25[OH]D_3). Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Stratified models and interaction terms were used to evaluate interactions of vitamin D with ppOS or maternal smoking on MAD.

Results: Mean serum total 25[OH]D and 25[OH]D_3 concentrations were higher among women with MAD compared with women without MAD (31.4 vs.29.2 ng/ml for total 25[OH]D, and, 29.2 vs.26.4 ng/ml for 25[OH]D_3, respectively) (both P-values<0.05). Increases of 5 ng/ml in 25[OH]D or 25[OH]D_3 were associated with 1.14 and 1.16-fold higher in risk of MAD, respectively (both P-values<0.05). Similarly, linear decreases in risk of MAD were observed across decreasing quartiles of total 25[OH]D (trend P-value=0.044) and 25[OH]D_3 (trend P-value=0.067). We observed significant interactions of vitamin D with maternal ppOS or maternal

smoking history on risk of MAD (interaction P-values<0.05). Decreased risk in MAD related to vitamin D insufficiency/deficiency was observed only among participants who were over-weight/obese or were current/former smokers. Results of analyses on data that excluded cases that were diagnosed at or before blood draw were similar to results reported above, although associations did not reach statistical significance.

Conclusions: Contrary to previous reports and expectations, serum total 25[OH]D and $25[OH]D_3$ concentrations were directly associated with diagnosed MAD. We also observed potential interactions between maternal pre-pregnancy obesity/overweight status or smoking status and maternal vitamin D on MAD. Future prospective studies that employ standardized and validated measures of mood or anxiety symptoms are needed.

INTRODUCTION

According to previous reports, 15-30% of women experience mood or anxiety disorders (MAD) during pregnancy [1-3]. Self-reported maternal symptoms of MAD during pregnancy have been associated with preeclampsia [4, 5], low birth weight, inconsolability and excessive crying in infants [6]. While a number of risk factors have been identified for MAD (such as lack of social support, poverty, family violence, and substance abuse), risk factors for MAD during pregnancy are not fully described [7]. Recent research on novel risk factors of MAD in the general population indicates potentially significant roles of vitamin D [8].

Among men and non-pregnant women, decreased vitamin D concentrations have been associated with diagnosed depression or symptoms of depression [9-13]. These findings are supported by evidence from experimental studies [14, 15] that provide potential biological mechanisms for hypothesized relationships between vitamin D and MAD. First, vitamin D deficiency has been associated with decreased concentrations of insulin, a hormone that facilitates serotonin production (by regulating the transport of tryptophan- the serotonin precursor) in the brain [16]. Serotonin has well demonstrated roles in mood and anxiety disorders. Second, vitamin D crosses the blood brain barrier and vitamin D receptors (VDR) in the brain are most concentrated in the hypothalamus [17], part of the hypothalamic-pituitary-adrenal axis involved in neuroendocrine functions related to mood and emotions [11]. Third, investigations have pointed to anxiety related behavioral impairment (including decreased grooming and aggression) in VDR-knockout mice [14, 15]. Since vitamin D deficiency is potentially preventable, investigations of vitamin D and

MAD in pregnancy can provide potential opportunities to improve MAD related pregnancy outcomes.

To our knowledge, only one previous study investigated early pregnancy vitamin D and maternal depressive symptoms [18]. This study, conducted by Brandenbarg et al.[18] , reported that early pregnancy maternal vitamin D deficiency was associated with higher rate of self-reported symptom of depression. We examined cross-sectional associations of maternal early pregnancy vitamin D with MAD, diagnosed before or during pregnancy, among participants of a pregnancy cohort study. We also evaluated whether maternal pre-pregnancy obesity/overweight status or smoking status modify these associations.

METHODS

Study setting and study population

This study [19-21] was conducted among participants of the Omega Study, a prospective cohort study (1996-2008) of pregnant women designed to examine risk factors for pregnancy complications. Detailed study procedures were described before [22]. Briefly, study participants were women attending prenatal care clinics at Swedish Medical Center in Seattle, Washington and Tacoma General Hospital in Tacoma, Washington. Women who initiated prenatal care before completion of 20 weeks of gestation were included in the study. Women who were <18 years of age, could not speak and read English, did not plan to carry the pregnancy to term, and did not plan to deliver at one of the study institutions were not eligible for participation in the study.

For the current study, we initially selected a random sub-cohort of 675 participants and all available cases of MAD (N=214), diagnosed before and during pregnancy, among Omega Study participants (**Figure 3.1**). Participants who miscarried before 20 weeks (n=9) and reported major chronic diseases (to avoid confounding) such as prior diabetes mellitus (n=10), chronic hypertension (n=36), renal disease (n=10), thyroid conditions (n=105), chronic liver disease (n= 8), rheumatoid arthritis (n=6), and lupus (n=3) were excluded. The final analytic group included 148 MAD cases diagnosed prior to blood collection and 554 women in the comparison sub-cohort (**Figure 3.1**). To clarify temporal relationships between vitamin D and MAD, we excluded all MAD cases (N=102) that were diagnosed before or at blood draw in early pregnancy. We included 46 MAD cases that were diagnosed after blood collection and 554

women in the sub-cohort for these analyses. All study participants provided informed consent. Study procedures were approved by the Institutional Review Boards of Swedish Medical Center and Tacoma General Hospital.

Data Collection

At the time of enrollment, study personnel conducted in-person interviews using structured questionnaires to collect information on maternal characteristics, including socio-demographic characteristics, past medical history, and family history. Study personnel also collected maternal non-fasting peripheral blood specimens. All blood specimens were processed within 30 minutes and aliquots of serum stored at -80°C until vitamin D measurements. After delivery, maternal and infant medical records were abstracted for information on the course and outcome of the pregnancy.

Vitamin D Measurement

Maternal early pregnancy vitamin D concentrations were assessed by measuring total 25[OH]D and 25[OH]D_3 in maternal serum collected shortly after study enrollment (16 weeks gestation on average). Serum samples were analyzed using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods at ZRT Laboratories (ZRT, Portland, OR). According to previously published criteria [23], pregnant women were categorized into vitamin D sufficient (≥30 ng/ml), insufficient (20-29 ng/ ml) and deficient (<20 ng/ml) groups using early pregnancy vitamin D measurements.

Mood or Anxiety Disorder (MAD) Diagnosis

MAD diagnosis was based on reports in pregnant women's antenatal clinic or hospital medical records. Reports of positive diagnoses included: (1) faxed diagnostic notes from psychiatrists or other physicians which were included as part of the antenatal or hospital medical record and (2) participants' self-reported medical history with details such as date of diagnosis and treatment (including medication use) indicated in the antenatal medical records [24].

Statistical Analyses

We examined distribution of categorical (number and percentage) and continuous (mean and standard deviation) variables among MAD cases and sub-cohort members using descriptive statistics. Multivariable logistic regression was used to determine the association between early pregnancy maternal vitamin D concentrations (either total 25[OH]D or 25[OH]D3) with MAD. Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated. In addition to *a priori* selected potential confounders, covariates that altered unadjusted odds ratios (ORs) by 10% or more were included in final adjusted models [25]. We fit two models, an unadjusted model and a model adjusted for season of blood draw, maternal age, race/ethnicity, and pre-pregnancy body mass index (pp-BMI). In these analyses, we modeled exposure variables (both total 25[OH]D and $25[OH]D_3$) in three ways: (1) continuous variables, (2) as categorical variables according to previously published criteria [23], either vitamin D sufficient (≥30 ng/ml) or insufficient/deficient (<30 ng/ml), and (3) quartiles based on distributions among the sub-cohort. Since the vitamin D deficient category had relatively small numbers and similar magnitude/direction of associations with MAD were observed comparing either the vitamin D deficient or insufficient categories with the vitamin D sufficient category, vitamin D insufficient and deficient categories were combined.

To evaluate potential effect modification of vitamin D and MAD associations by pre-pregnancy overweight/obese status (>25kg/m^2) or current maternal smoking status (current or former smoker vs. never smoked), we conducted analyses stratified by vitamin D deficiency status and pre-pregnancy overweight/obese or maternal smoking status [26]. These analyses were used to examine independent and joint effects of vitamin D and the two potential effect modifiers. We also fit models that include interaction terms of vitamin D with each effect modifier. Interaction term P-values were used to infer statistical significance (if P-value < 0.05) of interactions.

We repeated the statistical analyses steps described above by excluding MAD cases that were diagnosed before or at blood collection. We also conducted sensitivity analyses to assess possibility of different relationships among subgroups (e.g. only among non-Hispanic Whites or only among current/former smokers vs. non-smoked). Our findings from these analyses were similar to the primary analyses reported in this manuscript. All reported CIs were calculated at the 95% level and all reported P-values are two-tailed. Analyses were performed using STATA 11.0 (STATA, College Station, TX).

RESULTS

Among the 148 MAD cases, 117 had depression only, 22 had anxiety disorders only, 7 women had both mood and anxiety disorders, and 2 women had bipolar disorder (**Table 3.1**). Eighty-four percent (84%) of women with MAD reported taking psychotropic medications (n=124). Women with diagnosed MAD were more likely to be non-Hispanic White, current or former cigarette smokers, and have a higher pre-pregnancy BMI (**Table 3.2**). In early pregnancy, women with MAD had higher concentrations of total 25[OH]D (31.2 vs. 29.1 ng/ml, P –value = 0.010) and 25[OH]D_3 (29.0 vs. 26.4 ng/ml, P –value = 0.003) compared with women without MAD (**Table 3.2**).

Women with total 25[OH]D insufficiency/deficiency had a reduced, albeit statistically insignificant, risk of MAD compared to women with sufficient total 25[OH]D (adjusted OR=0.77; 95% CI: 0.53, 1.12) (**Table 3.3**). Each 5 ng/ml increase in total 25[OH]D was associated with a statistically significant 16% increase in MAD risk (adjusted OR=1.14; 95% CI: 1.02-1.27) (**Table3. 3**). Women who were insufficient or deficient in 25[OH]D_3 had a lower risk of MAD (OR= 0.71 95% CI: 0.48, 1.04) that was not statistically significant (**Table 3.3**). We observed that each 5 ng/ml increase in 25[OH]D_3 was associated with a statistically significant 1.2-fold increase in MAD risk (adjusted OR= 1.16; 95%CI: 1.05, 1.29) (**Table 3.3**).

Decreasing quartiles of total 25[OH]D concentrations were associated with lower risk of MAD (trend P-value=0.044) (**Table 3.4**). Women in the highest, referent, quartile for total 25[OH]D concentrations had a 60% lower risk of MAD compared with women in the lowest quartile (95% CI: 0.35-1.00) (**Table 3.4**). We also observed a marginally significant linear decrease in MAD

risk in relation to decreasing quartiles of 25[OH]D_3 concentrations (trend P-value=0.067) (**Table 3.4**).

We observed evidence of statistically significant interactions between total 25[OH]D sufficient status and maternal overweight/obese status (interaction P-value=0.029) or maternal smoking (interaction P-value=0.042) on risk of MAD (**Table 3.5**). Lower risk of MAD associated with vitamin D insufficiency/deficiency was observed only among participants who were overweight/obese or were current/former smokers. Similar interactions between 25[OH]D_3 sufficient status and maternal overweight/obese status (interaction P-value=0.014) or maternal smoking (interaction P-value=0.094) on risk of MAD were observed (**Table 3.6**).

We repeated these analyses by excluding MAD cases who were diagnosed before or at blood collection for vitamin D measurements (**Tables 3.7-3.10**). In general, findings (estimates of associations and directions of relationships) from these analyses were similar to those reported for all MAD cases. However, observed associations did not reach statistical significance.

DISCUSSION

In this study we found that higher vitamin D, measured using either total 25[OH]D or 25[OH]D_3 concentrations was associated with higher risk of diagnosed MAD. We also found significant interactions between vitamin D concentrations and pre-pregnancy overweight/obese status or current/former smoking status on MAD diagnosis. Higher risk of MAD associated with vitamin D sufficient status was observed only among participants who were overweight/obese or current/former smokers.

In 2012, Brandenbarg et al.[18] reported that women who were vitamin D deficient (25[OH]D ≤ 29.9 nM) or insufficient (30-49.9 nM) at 13 weeks gestation were more likely to report high levels of depressive symptoms (score ≥ 16 on the Center for Epidemiologic Studies Depression Scale, CES-D) at 16 weeks gestation compared to women who were vitamin D sufficient (<50 nM) (adjusted OR=1.48; 95% CI: 1.13-1.95 and adjusted OR= 1.44; 95% CI: 1.12-1.85 for vitamin D deficient and insufficient, respectively). Findings from several studies of non-pregnant women or women and men provide evidence of associations of lower vitamin D concentrations with MAD [11, 12, 27]. In a case-control study of premenopausal women in Washington DC, those with major depressive disorders (MDD) had lower total 25[OH]D concentrations compared to women without MDD (27 ± 10.1 ng/ml vs. 34 ± 14.4 ng/ml; P-value= 0.002) [27]. Among a population-based cohort of men and women ages 65 to 95 years in the Netherlands, concentrations of 25[OH]D were 14% lower among cases with minor depression and 14% lower in cases with major depressive disorder compared with control participants (all P-value<0.001) [11]. In that cohort, depression severity, measured using the CES-D, was significantly associated with decreased serum 25[OH] D concentrations (P-value =0.03). In their study of overweight and

obese men and women, Jorde et.al [12] reported that participants with serum 25[OH]D concentrations < 40 nmol/L self-reported higher depressive traits on the Beck Depression Inventory ($P < 0.05$).

However, several other studies of non-pregnant women or women and men concluded that there was no association between vitamin D and MAD [28-30]. In a study of bone mineral density and depression among menopausal women, investigators reported total 25[OH]D concentrations were similar among women with current major depression, diagnosed by the Diagnostic and Statistical Manual of Mental Disorders (third edition, revised), compared with women with no history of major depression [28]. A study by Herrán et. al. [29] on bone remodeling and major depression among a population of Spanish men and women reported no difference in total 25[OH]D between patients receiving care for suspected first depressive episode and comparable controls (27.3 ± 16.1 vs. 23.0 ± 7.8 ng/ml; $P = 0.2$). Among a group of middle-aged and elderly study participants in Beijing and Shanghai, China, Pan et al. [30] reported no association between the prevalence of depressive symptoms using the CES-D and 25[OH]D concentrations.

Our findings are contrary to what we expected, based on reports from majority of previous studies and potential biological mechanisms. Some considerations that may explain our observation that MAD are associated with higher concentrations of serum total 25[OH]D and $25[OH]D_3$ are as follows. First, participants with MAD in our study are under the care of a physician, as evidenced by their medical records and self-report of taking prescription medication for the disorder. Given previous reports of associations between vitamin D and MAD, it is possible that physicians (or patients themselves) had recommended vitamin D

supplementation for their patients' ailments. In this case, confounding-by-indication, as is common in cross-sectional studies such as ours, may lead to the observed findings. However, our findings of analyses among MAD cases that were newly diagnosed (after blood collection), which was in general similar to our primary findings, though not statistically significant, does not support this hypothesis. Larger prospective studies, like the Brandenbarg et al. study [18], are needed to assess these relationships. The contributions of other risk factors are also important in evaluating these relationships. Of note, we did not observe associations between higher vitamin D and MAD risk among women who were at low risk (non-overweight/obese or no history of smoking). Therefore, associations between vitamin D and MAD risk may vary by distributions of other risk factors.

Our study is only the second study to examine the relationship between vitamin D status and MAD among pregnant women. We used state-of-the-art liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods to measure both maternal early pregnancy serum total 25[OH]D and 25[OH]D_3 concentrations. Our analyses adjusted for relevant covariates and assessed effect modification by overweight/obese status and smoking history. Besides the potential confounding by indication bias that may have accounted for observed unexpected findings, other limitations of our study include the following. First, we did not use standardized criteria to determine MAD status among all women in the study. Women in the comparison group may have undiagnosed MAD. In Kessler et al.'s [30] study of the prevalence and treatment of mental disorders from 1990 to 2003, the prevalence of mental disorders among people 18-54 did not change, but the rate of treatment increased to 32.9 % and among those who received treatment, only about 50% met diagnostic criteria for a mental disorder. Second, we did not

distinguish different subtypes of MAD in our analyses, due to small number issues. Therefore, different relationship of vitamin D with MAD subtypes is a possibility. Third, assessing symptoms of MAD may be a more sensitive way to explore potential relationships between maternal vitamin D and MAD. Data on symptom and frequency/severity were not available in the current study. Finally, our study findings are generalizable to other populations with similar risk factor (e.g. socio-economic status, race, etc.) distributions.

In this cross-sectional study, higher maternal serum total 25[OH]D and 25[OH]D_3 in early pregnancy were associated with higher prior diagnosis of MAD. This association was modified by pre-pregnancy overweight/obese status or maternal smoking history. Future prospective studies that use validated assessment tools to track the occurrence of episodes of MAD observed in pregnant women both before and after the time of their blood serum vitamin D assessments are warranted. A greater understanding of maternal vitamin D and MAD relationship has the potential to facilitate preventative efforts to improve the health and wellbeing of mothers and infants.

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Figure 3.1: Sampling and analytic groups, MAD study

	Sampled Sub-cohort N=675	**Sampled Mood or Anxiety Cases N=214**
Renal Disease	4	6
Thyroid Disease	70	35
Liver Disease	7	1
GA at delivery <20 weeks	5	4
Chronic Hypertension	19	17
Prior Diabetes	9	1
Rheumatoid Arthritis	5	1
Lupus	2	1
CROSS-SECTIONAL ANALYSES	**Analysis Sub-cohort N=554**	**Analysis All Mood or Anxiety Cases N=148**
CASE-COHORT ANALYSES	**Analysis Sub-cohort N=554**	**Analysis Newly Diagnosed Mood or Anxiety Cases N=46**

Table 3.1: Distribution of study participant's by mood or anxiety disorder diagnosis

Participants psychiatric diagnoses	**ALL MAD Cases (N= 148)**	**Newly Diagnosed MAD Cases (N= 46)**
Depression Only	117	10
Anxiety Disorder Only	22	33
Both Depression & Anxiety Disorder	7	3
Bipolar Disorder	2	0

Table 3.2: Characteristics of study participants according to mood or anxiety disorder: cross-sectional study

Characteristics	**All MAD Cases (N=148)** n (%)	**Newly Diagnosed MAD Cases (N= 46)** n (%)	**Sub-Cohort (N= 554)** n (%)	**P-value[§] (All vs.cohort)**	**P-value[§] (Newly vs. cohort)**
Maternal Age (years)	33.1 ± 5.1	33.3 ± 5.4	32.6 ± 4.5	0.27	0.35
≥35	60 (40.5)	19 (41.3)	188 (33.9)	0.13	0.295
Married	117 (79.1)	35 (76.1)	472 (85.2)	0.07	0.093
Household Income ≥$70,000	89 (69.0)	30 (79.0)	385 (75.6)	0.12	0.62
Post High School Education	124 (93.9)	35 (89.7)	501 (96.4)	0.22	0.04
White/Non-Hispanic	139 (95.2)	43 (93.3)	468 (84.5)	**0.01**	**0.25**
Nulliparous	83 (56.1)	25 (54.4)	342 (61.7)	0.21	0.32
Leisure Time Physical Activity	101 (76.5)	31 (79.5)	419 (80.7)	0.28	0.86
Prenatal Vitamin Use	127 (96.2)	39 (100.0)	507 (97.7)	0.34	0.34
Calcium (mg/day)	1344.1 ± 670.3	1174.01 ±716.5	1235.0 ± 618.4	0.11	0.60
Current Cigarette Smoker	21 (15.9)	3 (7.7)	29 (5.6)	**<0.001**	**<0.33**
Pre-pregnancy BMI (kg/m^2)	24.7 ± 6.0	24.4 ± 6.2	23.3 ± 5.1	**0.01**	**0.27**
GA at Blood Draw (weeks)	15.4 ± 2.6	14.2 ±2.5	15.2 ± 2.8	0.52	0.0092
Season of Blood Draw					
Spring (March-May)	38 (25.7)	10 (21.7)	157 (28.3)	0.21	0.57
Summer (Jun-Aug)	29 (19.6)	13 (28.3)	141 (25.5)		
Autumn (Sept- Nov)	40 (27.0)	15 (32.6)	140 (25.3)		
Winter (Dec-Feb)	41 (27.7)	8 (17.4)	116 (20.9)		
Maternal Serum Total 25[OH]D (ng/ml)	31.2 ± 9.4	30.4 ± 10.0	29.1 ± 8.2	**0.01**	**0.41**
Maternal Serum 25[OH]D$_3$ (ng/ml)	29.0 ± 9.9	28.1 ± 10.8	26.4 ± 8.7	**0.003**	**0.35**

§ P-values (chi-square or Students T-test) comparing MAD cases to sub-cohort
Mean ± SD (standard deviation)
Abbreviations: **BMI**, body mass index; **GA**, gestational age

Table 3.3: Odds ratios (OR) and 95% confidence intervals (CI) for mood or anxiety disorder (MAD) according to total maternal serum 25[OH]D and 25[OH]D_3 concentrations (ng/ml) in pregnancy: all MAD cases

	MAD Cases (N=148)	**Sub-Cohort (N=554)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**
TOTAL 25[OH]D (categorical)				
Sufficient (≥30)	78 (52.7)	246 (44.4)	Referent	Referent
Insufficient/Deficient (<30)	70 (0.47)	308 (55.6)	0.72 (0.50-1.03)	0.77 (0.53-1.12)
TOTAL 25[OH]D (continuous)				
Per 5 ng/ml increase			**1.16 (1.04-1.29)**	**1.14 (1.02-1.27)**
25[OH]D_3 (categorical)				
Sufficient (≥30)	63 (42.6)	183(33.0)	Referent	Referent
Insufficient/Deficient (<30)	85 (57.4)	371(67.0)	0.67 (0.46-0.96)	0.71 (0.48-1.04)
25[OH]D_3 (continuous)				
Per 5 ng/ml increase			**1.17 (1.06-1.30)**	**1.16 (1.05-1.29)**

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+Adjusted for season of blood draw, maternal age, race/ethnicity, Pre-pregnancy BMI

Table 3.4: Odds ratios (OR) and 95% confidence intervals (CI) for mood or anxiety disorder (MAD) according to total maternal serum 25[OH]D and 25[OH]D_3 quartiles measured in early pregnancy: all MAD cases

	MAD Cases (N=148)	**Sub-Cohort (N=554)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**
TOTAL 25[OH]D				
1st Quartile (>34.7)	53 (36.5)	139 (25.1)	Referent	Referent
2nd Quartile (28.9-34.7)	35 (23.0)	137 (24.7)	0.64 (0.39-1.04)	0.68 (0.41-1.13)
3rd Quartile (23.3-28.8)	32 (21.6)	139 (25.1)	**0.59 (0.36-0.97)**	0.66 (0.40-1.12)
4th Quartile (<23.3)	28 (18.9)	139 (25.1)	**0.52 (0.31-0.87)**	**0.59 (0.35-1.00)**
P for trend			**0.009**	**0.044**
25[OH]D_3				
1st Quartile (>32.2)	49 (33.1)	136 (24.5)	Referent	Referent
2nd Quartile (26.1-32.2)	37 (25.0)	140 (25.3)	0.73 (0.45-1.19)	0.82 (0.50-1.34)
3rd Quartile (20.4-26.0)	38 (25.7)	140 (25.3)	0.70 (0.43-1.15)	0.74 (0.45-1.23)
4th Quartile (< 20.4)	24 (16.2)	138 (24.9)	**0.56 (0.33-0.94)**	0.62 (0.36-1.05)
P for trend			**0.029**	0.067

§ Quartiles based on distribution of the comparison sub-cohort
+Adjusted for season of blood draw, maternal age, race/ethnicity, Pre-pregnancy BMI

Table 3.5: Interaction of overweight/obesity status and current/former smoking status with maternal serum total 25[OH]D on mood or anxiety disorders : all MAD cases, cross-sectional study

	Mood or Anxiety Disorder		**Stratified model**		**Joint model**	
Characteristics	**Yes (N=148)**	**No (N=554)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	47 (31.8)	202 (36.5)	Referent	Referent	Referent	Referent
No, BMI <25	45 (30.4)	215 (38.8)	0.94	(0.60 – 1.49)	0.94	(0.60 – 1.49)
Yes, BMI ≥25	30 (20.3)	44 (7.9)	Referent	Referent	**3.04**	**(1.70 - 5.41)**
No, BMI ≥25	25 (16.9)	92 (16.6)	**0.47**	**(0.24 – 0.92)**	1.42	(0.81 – 2.47)
P-value for interaction						**0.029**
Vitamin D Sufficient and Cigarette Smoking						
Yes, Never Smoked	38 (25.7)	178 (32.1)	Referent	Referent	Referent	Referent
No, Never Smoked	28 (18.9)	55 (9.9)	1.03	(0.63 – 1.69)	1.03	(0.63 – 1.68)
Yes, Current /Former Smoker	40 (27.0)	200 (36.1)	Referent	Referent	**2.59**	**(1.44 - 4.65)**
No, Current /Former Smoker	26 (17.6)	86 (15.5)	0.56	(0.30 – 1.07)	1.48	(0.84 – 2.61)
P-value for interaction						**0.042**

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, pre-pregnancy BMI
*Numbers may not add up to total number of cases or cohort members due to missing

Table 3.6: Interaction of overweight/obesity status and current/former smoking status with maternal serum 25[OH]D_3 on mood or anxiety disorders: all MAD cases, cross-sectional study

	Mood or Anxiety Disorder		Stratified model		Joint model	
Characteristics	**Yes (N=148)**	**No (N=554)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	37 (25.0)	150 (27.1)	Referent	Referent	Referent	Referent
No, BMI <25	55 (37.2)	267 (48.2)	0.84	(0.53 – 1.35)	0.84	(0.53 – 1.35)
Yes, BMI ≥25	25 (16.9)	33 (6.0)	Referent	Referent	**3.00**	**(1.56 – 5.75)**
No, BMI ≥25	30 (20.3)	103 (18.6)	**0.48**	**(0.24 – 0.96)**	1.40	(0.81 – 2.45)
P-value for interaction						**0.014**
Vitamin D Sufficient and Cigarette Smoking						
Yes, Never Smoked	29 (19.6)	134 (24.2)	Referent	Referent	Referent	Referent
No, Never Smoked	49 (33.1)	244 (44.0)	0.99	(0.59-1.64)	0.99	(0.59-1.64)
Yes, Current /Former Smoker	22 (14.9)	27 (4.9)	Referent	Referent	**3.06**	**(1.55-6.03)**
No, Current /Former Smoker	32 (21.6)	104 (18.8)	**0.49**	**(0.25 – 0.96)**	1.47	(0.83-2.59)
P-value for interaction						**0.094**

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, pre-pregnancy BMI
*Numbers may not add up to total number of cases or cohort members due to missing

Table 3.7: Odds ratios (OR) and 95% confidence intervals (CI) for mood or anxiety disorder (MAD) according to total maternal serum 25[OH]D concentrations (ng/ml) in pregnancy: newly diagnosed MAD cases

	MAD Cases (N=46)	**Sub-Cohort (N=554)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**
TOTAL 25[OH]D (categorical)				
Sufficient (≥30)	24 (52.2)	250 (45.1)	Referent	Referent
Insufficient/Deficient (<30)	22 (47.8)	304 (54.9)	0.74 (0.41-1.36)	0.73 (0.40-1.35)
TOTAL 25[OH]D (continuous)				
Per 5 ng/ml increase			1.09 (0.91-1.31)	1.12 (0.93-1.36)
25[OH]D_3 (categorical)				
Sufficient (≥30)	20 (43.5)	188 (33.9)	Referent	Referent
Insufficient/Deficient (<30)	26 (56.5)	366 (66.1)	0.66 (0.36-1.20)	0.60 (0.32-1.13)
25[OH]D_3 (continuous)				
Per 5 ng/ml increase			1.11 (0.94-1.31)	1.14 (0.96-1.36)

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+Adjusted for season of blood draw, maternal age, race/ethnicity, Pre-pregnancy BMI

Table 3.8: Odds ratios (OR) and 95% confidence intervals (CI) for mood or anxiety disorder (mad) according to maternal serum total 25[OH]D and 25[OH]D_3 quartiles measured in early pregnancy: newly diagnosed MAD cases

	MAD Cases (N=46)	**Sub-Cohort (N=554)**	**Unadjusted OR (95%CI)**	**Adjusted+ OR (95%CI)**
Total 25[OH]D				
1st Quartile (>34.7)	15 (32.6)	139 (25.1)	Referent	Referent
2nd Quartile (28.9-34.7)	12 (26.1)	140(25.3)	0.82 (0.37-1.82)	0.82 (0.37-1.83)
3rd Quartile (23.3-28.8)	7 (15.2)	139 (25.1)	0.47 (0.18-1.18)	0.49 (0.19-1.25)
4th Quartile (< 23.3)	12(26.1)	136 (24.5)	0.83 (0.37-1.83)	0.91 (0.41-2.03)
P for trend			0.409	0.557
25[OH]D_3				
1st Quartile (>32.2)	17 (37.0)	143 (25.8)	Referent	Referent
2nd Quartile (26.1-32.2)	9 (19.6)	137 (24.7)	0.56 (0.24-1.29)	0.59 (0.25-1.37)
3rd Quartile (20.4-26.0)	8 (17.4)	137 (24.7)	0.49 (0.20-1.17)	0.49 (0.21-1.18)
4th Quartile (< 20.4)	12(26.1)	137(24.7)	0.71 (0.33-1.54)	0.75 (0.35-1.64)
P for trend			0.321	0.372

§ Quartiles based on distribution of the comparison sub-cohort
+Adjusted for season of blood draw, maternal age, race/ethnicity, Pre-pregnancy BMI

Table 3.9: Interaction of overweight/obesity status and current/former smoking status with maternal serum total 25[OH]D on mood or anxiety disorders: newly diagnosed MAD cases

	Mood or Anxiety Disorder		Stratified model		Joint model	
Characteristics	**Yes (N=46)**	**No (N=554)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	15 (33.3)	204 (36.8)	Referent	Referent	Referent	Referent
No, BMI <25	16 (35.6)	211 (38.1)	1.05	(0.50 -2.18)	1.05	(0.50 -2.18)
Yes, BMI ≥25	9 (20.00)	46 (8.3)	Referent	Referent	2.69	(1.10 – 6.55)
No, BMI ≥25	5 (11.1)	90 (16.2)	0.36	(0.11 – 1.18)	0.88	(0.31-2.51)
P-value for interaction						0.922
Vitamin D Sufficient and Cigarette Smoking						
Yes, Never Smoked	12 (30.8)	179 (32.3)	Referent	Referent	Referent	Referent
No, Never Smoked	12 (30.8)	200 (36.1)	1.01	(0.44-2.31)	1.01	(0.44-2.31)
Yes, Current /Former Smoker	8 (20.5)	58 (10.5)	Referent	Referent	2.20	(0.85-5.69)
No, Current /Former Smoker	7 (18.0)	85 (15.3)	0.59	(0.20 – 1.74	1.30	(0.49-3.45)
P-value for interaction						0.735

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, pre-pregnancy BMI
*Numbers may not add up to total number of cases or cohort members due to missing

Table 3.10: Interaction of overweight/obesity status and current/former smoking status with maternal serum 25[OH]D_3 on mood or anxiety disorders: newly diagnosed MAD cases

	Mood or Anxiety Disorder		**Stratified model**		**Joint model**	
Characteristics	**Yes (N=46)**	**No (N=554)**	**Adjusted OR**	**95% (CI)**	**Adjusted OR**	**95% (CI)**
Vitamin D Sufficient and BMI						
Yes, BMI <25	11 (24.4)	152 (27.4)	Referent	Referent	Referent	Referent
No, BMI <25	20 (44.4)	263 (47.5)	1.03	(0.48 – 2.20)	1.03	(0.48 – 2.20)
Yes, BMI ≥25	9 (20.00	36 (6.5)	Referent	Referent	3.41	(1.31 – 8.93)
No, BMI ≥25	5 (11.10	100 (18.1)	**0.23**	**(0.07-0.79)**	0.77	(0.22 -1.92)
P-value for interaction						0.581
Vitamin D Sufficient and Cigarette Smoking						
Yes, Never Smoked	9 (23.1)	136 (24.9)	Referent	Referent	Referent	Referent
No, Never Smoked	15 (38.5)	243 (43.9)	1.00	(0.42-2.36)	0.99	(0.42-2.34)
Yes, Current /Former Smoker	7 (18.0)	40 (7.2)	Referent	Referent	2.90	(1.00-8.41)
No, Current /Former Smoker	8 (20.5)	103 (18.6)	0.43	(0.14-1.27)	1.20	(0.45-3.24)
P-value for interaction						0.218

Vitamin D sufficient, ≥ 30 ng/ml
Adjusted for season of blood draw, maternal age, race/ethnicity, pre-pregnancy BMI
*Numbers may not add up to total number of cases or cohort members due to missing

CHAPTER 4

Early Pregnancy Maternal Vitamin D Concentrations and Risk of Preeclampsia

ABSTRACT

Background: Vitamin D has been associated with cardiovascular outcomes, including hypertension, in the general population. Findings from several studies that examined associations of maternal vitamin D with risk of preeclampsia (PE) were inconsistent. Whether pre-pregnancy overweight/obesity status modifies associations of vitamin D with risk of PE is unknown.

Methods: In a case-cohort study, participants (73 PE cases and 600 randomly chosen sub-cohort members) were selected among a prospective cohort of pregnant women. Liquid chromatography-tandem mass spectroscopy was used to measure maternal vitamin D concentrations (both total 25[OH]D and 25[OH]D_3) in early pregnancy (16 weeks of gestation, on average). PE diagnosis was made based on the American College of Obstetricians and Gynecologists guidelines. Logistic regression models were used to calculate unadjusted and adjusted odds ratios and 95% confidence intervals (CI). Stratified models and interaction terms were used to assess effect modification of vitamin D and PE risk associations by pre-pregnancy overweight/obesity status.

Results: Early pregnancy mean serum total 25[OH]D and 25[OH]D_3 concentrations were lower among women who subsequently developed PE (28.6 and 25.7 ng/ml, respectively) compared with women who did not (29.2 and 26.6 ng/ml, respectively), although the differences were not statistically significant (both p-values>0.05). Risk estimates indicated consistently higher risk of PE (10-40% higher) among women with vitamin D deficiency or women in the lowest quartile for vitamin D concentrations compared with women who were vitamin D sufficient or were in the upper three quartiles for vitamin D concentrations, respectively. However, none of the risk

estimates were statistically significant. Associations between vitamin D and PE risk did not vary depending upon women's pre-pregnancy overweight/obesity status (interaction p-value>0.05).

Conclusions: In the current study, we did not observe significant associations between early pregnancy vitamin D and risk of PE. Given the inconsistency of findings to date and strong mechanistic evidence for this association, future larger prospective studies are warranted.

INTRODUCTION

In the general population, vitamin D has been associated with cardiovascular diseases (CVD), including hypertension[1-4], all-cause and CVD mortality [5], and CVD risk factors (e.g. type 2 diabetes mellitus)[6]. Mechanistic investigations have provided evidence supporting cardiovascular roles of vitamin D that involve inflammation[7-11] and the renin-angiotensin-aldosterone (RAS) system[1, 12, 13]. However, the role of vitamin D in development of cardiovascular pregnancy complications, such as preeclampsia, has not been fully investigated.

Preeclampsia complicates 5-8% of pregnancies [14-16]and has serious consequences in the mother and her offspring. These complications include increased risk for pulmonary edema, hepatic and/or renal failure, and/or coagulation abnormalities in the mother and intrauterine growth retardation in the fetus [14, 17-20]. Further, it has been shown that both the mother and her child have an increased risk of developing hypertensive disorders later in life as a result of PE[21, 22]. Despite tremendous progress by researchers to identify risk factors of PE and understand underlying mechanisms, prevention has been elusive. A number of novel risk factors, such as vitamin D deficiency, have been proposed to contribute to this multi-factorial complex disorder.

Previous studies of maternal vitamin D and PE reported inconsistent findings. While findings from some studies indicate inverse associations of vitamin D concentrations with risk of PE [23-28], findings from other studies did not [29, 30]. Differences in findings could, at least in part, result from inherent differences in study population characteristics (e.g. dietary habit or genetic susceptibility factors), differences in study design (e.g. cross-sectional vs. case-control study designs), time of vitamin D measurements (early or late pregnancy), method of vitamin D

assessment (mass spectroscopy or radioimmuno assay), differences in guidelines used for PE diagnosis, and inadequate assessment of potential confounders or effect modifiers. Using a case-cohort study design, we investigated associations of early pregnancy maternal vitamin D concentrations with subsequent risk of PE among participants of a pregnancy cohort study. We also examined whether pre-pregnancy obesity is an effect modifier of these associations.

METHODS

Study Setting and Study Population

The current case-cohort study [31-33] was conducted among participants of the Omega Study, a well-characterized prospective cohort study of pregnant women designed to examine the risk factors of preeclampsia and other pregnancy complications. In the Omega Study, participants were recruited from 1996 to 2008 (N=4,000) among women attending prenatal care clinics affiliated with Swedish Medical Center in Seattle, Washington and Tacoma General Hospital in Tacoma, Washington. Women were eligible for participation if they met the following criteria: initiated prenatal care before completion of 20 weeks of gestation, were 18 years of age or older, could speak and read English, planned to carry the pregnancy to term, and planned to deliver at one of the study institutions. Study protocols were approved by Institutional Review Boards of participating institutions. Study participants provided informed consent.

We randomly selected a sub-cohort of 750 participants and all cases of PE (n=122) among Omega study participants (**Figure 4.1**). Thirty-three PE cases were among the 750 selected sub-cohort members. We excluded participants with renal disease (n=10), thyroid condition (n= 98), chronic liver disease (n=7), women who had pre-existing chronic hypertension (n= 45), and women who delivered prior to 20 weeks gestation (n= 6) (**Figure 4.1**). After exclusions, we had a total of 73 PE cases and a sub-cohort of 600 women comparison controls for analyses.

Data Collection

Upon enrollment, participants completed an interviewer-administered structured questionnaire. The questionnaires were used to gather information on socio-demographic, reproductive, medical

and family history. Information on pre-pregnancy BMI was obtained by dividing pre-pregnancy weight (in kgs) by height squared (in meters). Near the time of enrollment, study personnel collected maternal non-fasting blood specimens for biomarker measurements. All blood specimens were processed within 30 minutes, and aliquots of serum stored at -80°C until vitamin D measurements. After delivery, maternal and infant medical records were abstracted for information on the course and outcomes of the pregnancy.

Vitamin D Measurement

We assessed early pregnancy maternal vitamin D levels by measuring serum vitamin D concentrations using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods at ZRT Laboratory in Portland, Oregon. LC-MS/MS is capable of measuring $25[OH]D_2$, $25[OH]D_3$ and total 25[OH]D ($25[OH]D_2$ + $25[OH]D_3$). Few samples had detectable concentrations of $25[OH]D_2$. Therefore, our analyses evaluated only total 25[OH]D and $25[OH]D_3$. Inter-assay coefficients of variation (CV%) for $25[OH]D_2$ and $25[OH]D_3$ measurements were <10% [34].

Preeclampsia Diagnosis

PE was diagnosed using recommendations of the American College of Obstetricians and Gynecologists (ACOG) [35]. According to the recommendations, pregnant women were diagnosed with PE if they had (1) sustained (on ≥2 occasions) blood pressure of ≥ 140/90 mmHg with readings performed ≥ 6 hours apart and 2) urine protein concentrations of ≥ 300 mg/dl or 1+ on a urine dipstick on ≥ 2 urine specimens collected ≥ 4 hours apart, after 20 weeks gestation.

Statistical Analyses

We examined the distribution of categorical (number and percentage) and continuous (mean and standard deviation) variables among PE cases and sub-cohort members. We used unadjusted and adjusted multivariable logistic regression models to examine associations between early pregnancy maternal serum 25[OH]D concentrations and subsequent risk of PE.

Exposure variables (total 25[OH]D and 25[OH]D_3) were modeled as (1) continuous variables (2) categorical variables defined according to previously published criteria for vitamin D deficiency [36] as vitamin D sufficient (≥30 ng/ml), insufficient (20–29 ng/ ml), and deficient (20 ng/ml), and (4) quartiles based on vitamin D distribution among the sub-cohort. Linear associations were evaluated in (2) and (3) by examining linear trend p-values across increasing categories of vitamin D status and quartiles, respectively.

In addition to *a priori* selected potential confounders, covariates which altered the unadjusted odd ratios (OR) by 10% or more were included in the final adjusted models [37]. We fit three separate logistic regression models to calculate ORs and 95% confidence intervals (CI) [38]. These models were one unadjusted model (Model 1) and two adjusted models. We adjusted for race/ethnicity (white non-Hispanic or other), maternal age (years), season (spring: March-May, summer: June-August, autumn: September-November, winter: December- February), and gestational age at blood draw in the first adjusted model (Model 2). In Model 3, we included Model 2 adjustment variables along with pre-pregnancy body mass index (BMI).

Potential effect modification of vitamin D and PE risk associations by pre-pregnancy overweight/obesity status was evaluated using stratified models [39], stratified by vitamin D

sufficient (≥30ng/ml) and pre-pregnancy overweight/obesity (≥25kg/m^2) status. Models were also fit that included interaction terms for vitamin D sufficient and pre-pregnancy overweight/obesity status. P-values of interaction terms in these models were used to assess statistically significant interactions.

In addition to these analyses, we completed sensitivity analyses by conducting analyses exclusively among non-Hispanic Whites. We also repeated analyses by including cases and sub-cohort members with prior history of chronic hypertension (29 cases and 16 sub-cohort members, respectively). Results from these analyses were similar to those that are reported in this manuscript. All reported CIs were calculated at the 95% level, and all reported p-values are two-tailed. Analyses were performed using STATA 11.0 (STATA, College Station, TX).

RESULTS

Women who developed PE were more likely to be nulliparous, to have higher pre-pregnancy BMI, and were less likely to have post high school education compared with women in the comparison sub-cohort (all p-values < 0. 05) (**Table 4.1**). Early pregnancy maternal serum total 25[OH]D and 25[OH]D_3 concentrations were lower among women who later developed PE compared with concentrations among women who did not, although differences were not statistically significant (28.6 vs. 29.2 ng/ml, ; P-value= 0.59 for total 25[OH]D and 25.7 vs. 26.6 ng/ml; P-value=0.38 for 25[OH]D3, respectively). A higher proportion of women were deficient in total 25[OH]D (16% vs. 12% (**Table 4.2**) in early pregnancy among PE cases compared with women who did not develop PE. Similarly, a higher proportion of women were deficient in 25[OH]D_3 (29% vs. 23%, **Table 4.5**) in early pregnancy among PE cases compared with women who did not.

Consistently higher risk of PE (10-40% higher) was observed among women with vitamin D deficiency or women in the lowest quartile for vitamin D concentrations, compared with women who were vitamin D sufficient or were in the upper three quartiles for vitamin D concentrations, respectively. However, we did not observe statistically significant associations between vitamin D (both total 25[OH]D and 25[OH]D3) and risk of PE (**Tables 4.2-4.3 and 4.5-4.6**). Adjustment for pre-pregnancy BMI further attenuated the estimates towards the null. Linear trend tests for associations between vitamin D and PE risk were not significant (all trend p-values > 0.05) (**Tables 4.3 and 4.6**). We also did not observe significant interactions between vitamin D sufficient status and pre-pregnancy overweight/obesity status on PE risk (p-values of interaction 0.68 and 0.21 for 25[OH]D and 25[OH]D3, respectively) (**Tables 4.4 and 4.7)**.

DISCUSSION

In the current case-cohort study nested within a prospective cohort, we did not find significant associations of vitamin D (both total 25[OH]D and 25[OH]D_3) with risk of PE. The confidence intervals for the odds ratios relating decreasing concentrations of vitamin D to PE risk crossed one, although the estimates themselves were mostly greater than one, indicating potential inverse associations of vitamin D with PE risk. We did not find significant interactions between vitamin D and pre-pregnancy BMI on PE risk.

Findings from previous studies (**summarized in Table 8**) investigating association of maternal vitamin D with PE risk were inconsistent. There were eight available published studies in this topic area. Of these, six reported significant inverse associations between maternal vitamin D and PE risk [23-28], while the remaining two did not [29, 30]. Cruikshank et al.[23] reported lower 25[OH]D levels among PE cases compared to controls (21.1 vs. 31.4 ng/ml, $p< 0.001$). In 2007, Bodnar et al. [24], in a case-control study, reported that maternal vitamin D deficiency at less than 22 weeks gestation was a strong and independent risk factor for PE. Geometric mean of serum 25[OH]D concentrations in early pregnancy (adjusted for maternal race/ethnicity, pre-pregnancy body mass index, education, season, and sample gestational age) were lower in women who later developed PE (n=49) compared with controls (n=216) (45.4 nmol/L; 95% CI: 38.6, 53.4 nmol/L, vs. 53.1; 95% CI: 47.1, 59.9 nmol/L; P <0.01) (Conversion: 1 nmol/L = 2.496). After adjustment for race/ethnicity, season, sample gestational age, pre-pregnancy BMI, and education, maternal 25[OH]D concentration less than 37.5 nmol/L was associated with a 5-fold increase in the odds of PE (adjusted OR= 5.0; 95% CI: 1.7, 14.1). Sensitivity analysis to evaluate the impact of unmeasured confounding due to calcium intake resulted in slightly attenuated estimate (adjusted

OR = 4.6; 95% CI: 1.6, 13.1). There was also a strong inverse monotonic dose-response relationship between serum 25[OH]D concentrations at less than 22 weeks and risk of PE (P = 0.02). After confounder adjustment, a 50 nmol/l decline in 25[OH]D concentration was associated with a 2.4-fold increased risk of PE (adjusted OR = 2.4; 95% CI: 1.1, 5.4).

However, a more recently published article of a nested case-control study (among 39 PE cases and 131 controls) by Powe et al.[30] reported no association between first trimester serum 25[OH]D or vitamin D binding protein levels (VDBP) with first trimester blood pressure or subsequent risk of PE. Wei et al.'s [26] 2012 prospective cohort study is the only study that measured vitamin D at two time points during pregnancy. The study included 32 cases of PE and 697 cohort members. The investigators reported that Women with 25[OH]D< 50 nmol/l at 24–26 weeks gestation experienced an increased risk of PE (adjusted OR:3.24, 95% CI: 1.37, 7.69). However, the association was not significant for maternal 25[OH]D level at 12–18 weeks of gestation (51.1 ± 14.8 nmol/l for women without preeclampsia vs. 56.0 ± 19.1 nmol/l for women with preeclampsia, P = 0.16).

Several factors may contribute to observed inconsistencies of findings in previous studies. First, PE case definition was not similar across the studies. For example the study by Seely et al.[29] did not define PE, but merely stated that "none had severe PE". Some studies focused only on the most severe cases of PE compared to normotensive comparison groups [27, 28]. Others, such as the study by Powe et al.[30] defined PE similar to the way we defined PE in the current study. Second, timing of vitamin D measurement is different across the studies. For example, serum 25[OH]D was measured just before labor induction or during spontaneous labor in the study by Cruikshank

et al.[23]. Third, the number of PE cases included in these studies varied across the studies contributing to difference in study power to demonstrate significant associations. For instance, some of the earliest studies included as few as 12 PE cases [29]. Although investigators used different methods to assess vitamin D concentrations (e.g. LC/MS or RIA), reports of significant associations were not related to any specific vitamin D measurement method. Finally, differences across study populations, which may be related to other potential modifying characteristics (e.g. dietary and lifestyle habit) may play roles in inconsistency of findings.

Potential association of vitamin D levels with PE risk is biologically plausible for a number of reasons. First, vitamin D plays a role in inflammatory processes [40, 41, 44] and insulin resistance [42, 43] which are implicated in the development of PE. Second, vitamin D has been associated with decreased T-regulatory cell activity which supports immune tolerance and allows placental implantation [45]. Third, investigators have shown that alterations of vitamin D metabolism and vitamin D receptor expression play a role in abnormal trophoblastic invasion found in pregnancies with PE [46-48].

Our study has several strengths. First, we used LC-MS/MS to determine 25[OH]D_3 concentrations. This method is less susceptible to some of the issues with immunoassays including sample matrix effects, decreased specificity, and cross-reactivity with nonspecific compounds [36]. Second, we were able to assess temporal relationships between 25[OH]D and PE, since blood was collected in early pregnancy before PE diagnosis. Third, available detailed information on covariates ensured our ability to adjust for a number of potential confounders in

our analyses. Finally, we examined potential effect modification by pre-pregnancy BMI, an important risk factor that is related to vitamin D levels and PE.

Several limitations of our study deserve mention. First, our study may have been underpowered to detect statistically significant differences in this population. Second, vitamin D was assessed at single time point during pregnancy (before 20 weeks gestation) and therefore we were unable to examine potential associations between vitamin D and PE across time. Misclassification due to single measurements, contributing to random error that may reduce study power, is also a possibility. Third, our study may have been limited by persistent confounding (unmeasured and residual). Fourth, generalizability of findings may be limited due to our population characteristics (i.e. higher socioeconomic status and higher percentage of non-Hispanic Whites).

In conclusion, our findings do not support prior published reports from the majority of studies that maternal vitamin D in early pregnancy is inversely associated with risk of PE. However, the estimates, while not statistically significant, point to an inverse relationship between the two. Future studies, with larger number of PE cases are needed to better understand the association between maternal vitamin D and risk of PE.

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Figure 4.1: Sampling and Analytic Groups, PE study

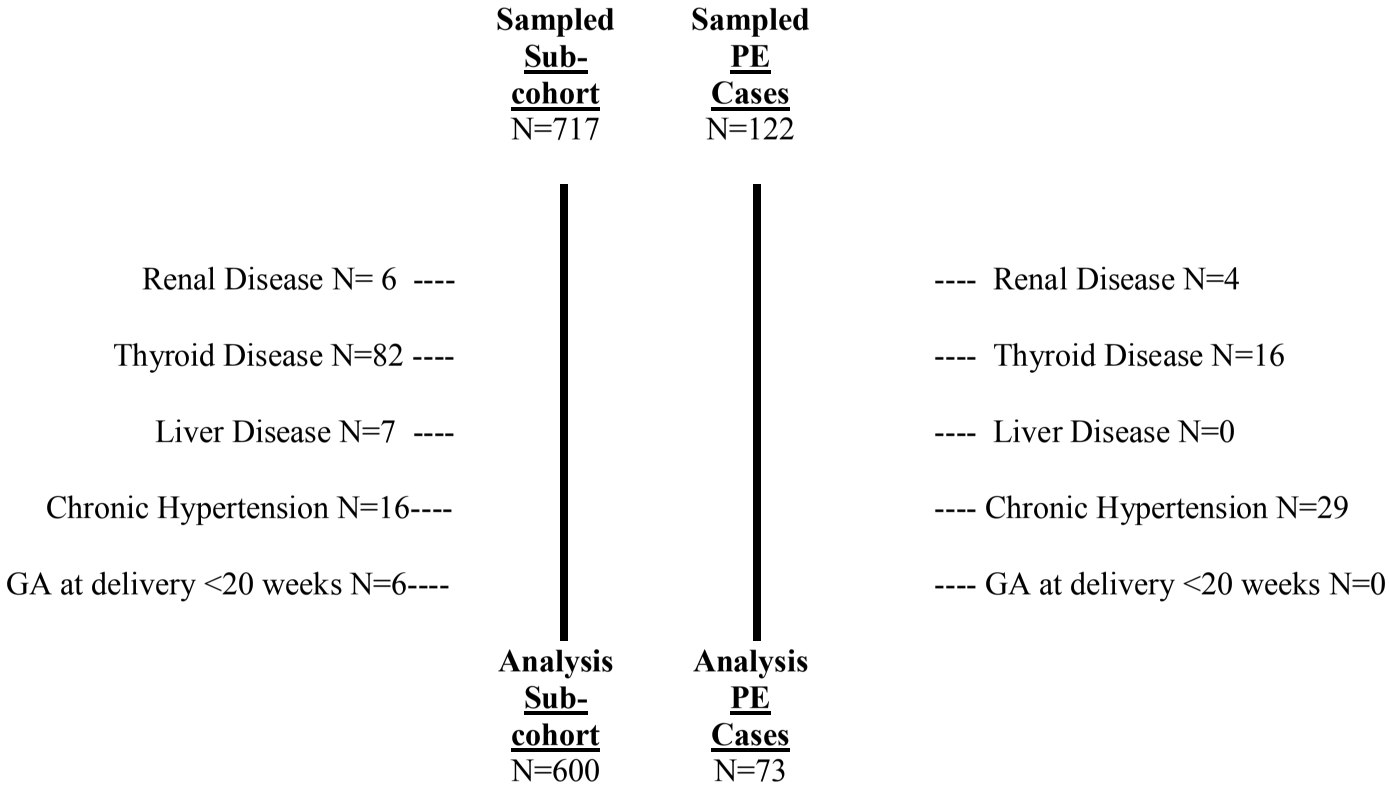

Table 4.1: Selected characteristics of study participants by preeclampsia case sub-cohort status

Characteristics	**PE Cases (N=73)** n (%)	**Sub-Cohort (N= 600)** n (%)	**P-value[§]**
Maternal Age (years)*	32.0 ± 5.6	32.7 ± 4.5	0.18
<35	49 (67.1)	392 (65.3)	0.76
≥35	24 (32.9)	208 (34.7)	
Married	60 (82.2)	507 (84.5)	0.61
High School Education or less	8 (11.4)	18 (3.2)	**0.001**
White/Non-Hispanic	63 (86.3)	510 (85.0)	0.20
Nulliparous	57 (78.1)	367 (61.2)	**0.005**
Leisure Time Physical Activity	60 (85.7)	449 (79.8)	0.24
Took Prenatal Vitamin	70 (95.9)	547 (97.2)	0.15
Smoking Status During Pregnancy			
Never	56 (80.0)	403 (71.6)	0.06
Former	7 (10.0)	121 (21.49)	
Current	7 (10.0)	39 (6.9)	
Family History of Diabetes Mellitus	12 (16.4)	90 (15.0)	0.75
Family History of Hypertension	41 (56.2)	268 (44.7)	0.06
Pre-pregnancy BMI (kg/m^2)*	27.5 ± 7.9	23.4 ± 5.1	**<0.001**
≥25 kg/m^2	38 (52.1)	150 (25.0)	**<0.001**
GA at Blood Draw (weeks)*	14.8 ± 2.7	15.2 ± 2.9	0.19
Season of Blood Draw			
Spring (March-May)	11 (15.3)	173 (28.8)	0.26
Summer (Jun-Aug)	22 (30.6)	154 (25.7)	
Autumn (Sept- Nov)	19 (26.4)	145 (24.2)	
Winter (Dec-Feb)	20 (27.7)	128 (21.3)	
Total Maternal Serum 25[OH]D	28.6 ± 9.4	29.2 ± 8.4	0.56
Maternal Serum 25[OH]D_3	25.7 ± 8.3	26.6 ± 8.9	0.41

§ P-values comparing PE cases to sub-cohort (t-test p-values for continuous variables and chi-square p-values for categorical variables0
*Mean ± SD (standard deviation), otherwise number (percent)
Abbreviations: **BMI**, body mass index; **GA**, gestational age

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Table 4.2: Associations of maternal serum total 25[OH]D concentrations with preeclampsia (PE) risk

`	PE Cases (N= 73)	Sub-Cohort (N=600)	Unadjusted OR (95%CI)	Adjusted+ OR (95%CI)	Adjusted++ OR (95%CI)
TOTAL 25[OH]D (categorical, ng/ml))					
Sufficient (≥30)	35 (47.9)	267 (44.5)	Referent	Referent	Referent
Insufficient (20-29)	26 (35.6)	262 (43.7)	0.76 (0.44-1.29)	0.76 (0.44-1.31)	0.67 (0.38-1.18)
Deficient (<20)	12 (16.4)	71 (11.8)	1.29 (0.64-2.61)	1.39 (0.67-2.90)	0.92 (0.41-2.06)
P for trend			0.89	0.80	0.47
TOTAL 25[OH]D (continuous)					
Per 5 ng/ml increase			0.96 (0.83-1.11)	0.95 (0.82-1.11)	1.03 (0.88-1.20)

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+ Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw
++Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw, Pre-pregnancy BMI

Table 4.3: Odds ratios (OR) and 95% confidence intervals (CI) for preeclampsia (PE) according to total maternal serum 25[OH]D quartiles

	PE Cases (N=73)	Sub-Cohort (N=600)	Unadjusted OR (95%CI)	Adjusted+ OR (95%CI)	Adjusted++ OR (95%CI)
TOTAL 25[OH]D					
1st Quartile (≥34.8)	18 (24.7)	151(25.2)	Referent	Referent	Referent
2nd Quartile (28.9-34.7)	18 (24.7)	151 (25.2)	.99 (0.50-1.99)	0.95 (0.47-1.93)	0.85 (0.41-1.76)
3rd Quartile (23.3-28.8)	13 (17.8)	151 (25.2)	0.71 (0.34-1.51)	0.71 (0.33-1.51)	0.63 (0.29-1.35)
4th Quartile (<23.3)	24 (32.9)	147 (24.5)	1.32 (0.69-2.54)	1.33 (0.68-2.58)	1.02 (0.51-2.04)
P for trend			0.539	0.535	0.904

§ Quartiles based on distribution of the comparison sub-cohort
+ Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw
++ Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw, Pre-pregnancy BMI

Table 4.4: Interaction of overweight/obesity status with maternal serum total 25[OH]D on preeclampsia (PE)

Characteristics	PE Yes (N=73)	PE No (N=600)	Stratified model Adjusted OR	Stratified model 95% (CI)	Joint model Adjusted OR	Joint model 95% (CI)
Vitamin D Sufficient and BMI						
Yes, BMI <25 kg/m^2	21 (28.8)	217 (36.2)	Referent	Referent	Referent	Referent
No, BMI <25 kg/m^2	14 (19.2)	233 (38.8)	0.66	(0.33-1.35)	0.66	(0.33-1.35)
Yes, BMI ≥25 kg/m^2	14 (19.2)	50 (8.3)	Referent	Referent	**3.25**	**(1.52-6.95)**
No, BMI ≥25 kg/m^2	24 (33.9)	100 (16.7)	0.87	(0.40-1.91)	**2.68**	**(1.38-5.19)**
P-value forinteraction						0.682

Vitamin D sufficient, ≥ 30 ng/ml
BMI: Body mass index, ≥ 25 overweight or obese
Adjusted for season of blood draw, maternal age, race/ethnicity, gestational age at blood draw
*Numbers may not add up to total number of cases or cohort members due to missing

Table 4.5: Associations of maternal serum 25[OH]D_3 concentrations with preeclampsia (PE) risk

	PE Cases (N= 73)	Sub-Cohort (N=600)	Unadjusted OR (95%CI)	Adjusted+ OR (95%CI)	Adjusted++ OR (95%CI)
25[OH]D_3 (categorical, ng/ml)					
Sufficient (≥30)	26 (35.6)	204 (34.0)	Referent	Referent	Referent
Insufficient (20-29)	26 (35.6)	259 (43.2)	0.79 (0.44-1.40)	0.75 (0.42-1.34)	0.67 (0.37-1.22)
Deficient (<20)	21 (28.8)	137 (22.8)	1.20 (0.65-2.22)	1.09 (0.58-2.07)	0.87 (0.44-1.69)
P for trend			0.64	0.90	0.59
25[OH]D_3 (continuous)					
Per 5 ng/ml increase			0.94 (0.82-1.08)	0.95 (0.83-1.10)	1.00 (0.86-1.16)

Vitamin D deficiency was defined according to the cut-points given by Holick, 2007
+ Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw
++ Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw, Pre-pregnancy BMI

Table 4.6: Odds ratios (OR) and 95% confidence intervals (CI) for preeclampsia (PE) according to maternal serum 25[OH]D_3 quartiles measured in early pregnancy

	PE Cases (N=73)	Sub-Cohort (N=600)	Unadjusted OR (95%CI)	Adjusted* OR (95%CI)	Adjusted** OR (95%CI)
25[OH]D_3 (ng/ml)					
1st Quartile (≥32.4)	17 (23.3)	150 (25.0)	Referent	Referent	Referent
2nd Quartile (26.3-32.3)	14 (19.2)	148 (24.8)	0.83 (0.40-1.75)	0.84 (0.40-1.78)	0.73 (0.33-1.58)
3rd Quartile (20.4-26.2)	20 (27.4)	152 (25.4)	1.16 (0.59-2.30)	1.11 (0.56-2.23)	0.93 (0.46-1.89)
4th Quartile (<20.4)	22 (30.1)	150 (25.0)	1.29 (0.66-2.53)	1.20 (0.60-2.40)	0.92 (0.45-1.89)
P for trend			0.313	0.460	0.992

§ Quartiles based on distribution of the comparison sub-cohort
* Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw
** Adjusted for season, maternal age, race/ethnicity, gestational age at blood draw, Pre-pregnancy BMI

Table 4.7: Interaction of overweight/obesity status with maternal serum 25[OH]D_3 on preeclampsia (PE)

Characteristics	PE Yes (N=73)	PE No (N=600)	Stratified model Adjusted OR	Stratified model 95% (CI)	Joint model Adjusted OR	Joint model 95% (CI)
Vitamin D Sufficient and BMI						
Yes, BMI <25 kg/m^2	17 (23.3)	164 (27.3)	Referent	Referent	Referent	Referent
No, BMI <25 kg/m^2	18 (24.7)	286 (47.7)	0.55	(0.27-1.12)	0.55	(0.27-1.12)
Yes, BMI ≥25 kg/m^2	9 (12.3)	40 (6.7)	Referent	Referent	2.26	(0.93-5.52)
No, BMI ≥25 kg/m^2	29 (39.7)	110 (18.3)	1.24	(0.51-3.01)	**2.57**	**(1.32-5.00)**
P-value for interaction						0.205

Vitamin D sufficient, ≥ 30 ng/ml
BMI: Body mass index, ≥ 25 overweight or obese
Adjusted for season of blood draw, maternal age, race/ethnicity, gestational age at blood draw
*Numbers may not add up to total number of cases or cohort members due to missing

Table 4.8: Major findings of epidemiologic studies of vitamin D and preeclampsia

Author (Year) Country	Study Design, Population	Maternal Vitamin D Measurement & Assay	PE Assessment/ Criteria	Major Findings
Seely[29] (1992) USA	Case-control N=12 PE N=24 NT	Serum 25[OH]D, 3rd trimester Assay: RIA (Nichols Institute)	Hypertension (SBP>140 or DBP> 85mm Hg) after 24 weeks w/ documented NT 1st trimester & 24-h urinary protein >300mg	Women with PE and NT had equivalent levels of 25[OH]D (PE 73.9±7.5, NT 89.8±11.7 nmol/L).
Cruikshank[23] (1993) USA	Case-control N=15 PE N=7 NT	Serum 25[OH]D, just before labor induction or during spontaneous labor Assay: competitive binding protein RIA	PE was not clearly defined, however authors noted that none had severe PE	Lower 25[OH]D at baseline in preeclamptics compared to those who were not (21.11±5.00 vs. controls 31.40±3.57 ng/ml, p< 0.001).
Bodnar[24] (2007) USA	Nested Case-control N=55 PE N=220	Serum 25[OH]D, <22 weeks Assay: ELISA (Immunodiagnostic Systems Limited)	Preeclampsia was defined as gestational hypertension and proteinuria and return of all abnormalities to normal by 12 weeks postpartum. Ajury of clinical experts reviewed medical records of all women in the cohort to assign preeclampsia diagnoses.	25[OH]D< 37.5 nmol/L was associated with a 5-fold increase in the odds of PE (aOR(1), 5.0; 95% CI, 1.7-14.1). There was also a strong inverse monotonic dose-response relation between serum 25[OH]D concentrations <22 weeks and risk of PE (P=0.02). After adjustment(1), a

				50-nmol/L decline in 25[OH]D doubled the risk of PE (aOR, 2.4; 95% CI, 1.1-5.4).
Haugen[25] (2009) Norway	Cross-sectional N=23,423	Daily intake from FFQ and reported vitamin intake (µg/day) at 22 weeks	Birth registry indication of PE according to guidelines issued by the Society for Gynecology (defined as>140/90 after 20 weeks' gestation, combined with proteinuria greater than &1 dipstick on at least 2 occasions	Vitamin D supplementation was associated with a 27% reduction in risk of PE (OR=0.73 [0.58-0.92]) for women taking 10-15 µg/day as compared to women who did not take supplements.
Powe[30] (2010) USA	Nested case-control N=39 PE N=131	Serum 25[OH]D, 1st trimester Assay: liquid chromatography-tandem mass spectrometry	Cases of PE were normotensive at the first prenatal visit (BP ≥140/90 mm Hg), w/ HPTN (SBP≥140 or DBP≥90 mm Hg) & proteinuria (≥2+ protein on urine dipstick or 300 mg of proteinuria per 24-h) after 20 weeks	No association between first trimester serum 25[OH]D, vitamin D binding protein levels (VDBP) and PE 25[OH]D was not associated with first trimester BP nor subsequent risk of PE
Robinson[27] (2010) USA	Case-control N=50 EOSPE N=100 control	Plasma 25[OH]D at the time of EOSPE diagnosis Assay: RIA (DiaSorin)	EOSPE cases met American College of Obstetrics and Gynecology criteria for severe PE <34 weeks	Women with EOSPE had decreased 25[OH]D levels compared to controls (P< 0 .001), remained significant after adjustment [(2)]
Baker[28] (2010)	Nested case-control	Serum 25[OH]D, at 15-20 weeks gestation	Severe PE (defined as PE and 1+ of the following: pulmonary	25[OH]D concentrations were lower in women who developed severe PE

USA	N=51 SPE N=204 control	Assay: liquid chromatography-tandem mass spectrometry	edema, seizures, oliguria, elevated liver enzymes with right upper quadrant pain, thrombocytopenia or persistent cerebral symptoms	compared to controls (median IQR range, 75 (47-107) nmol/L vs. 98 (68-113) nmol/L; P=0.01]. Maternal 25[OH]D <50 nmol/L was associated with an almost 4-fold odds of severe PE (unadjusted odds ratio, 3.63; 95% Cl, 1.52-8.65) compared with 25[OH]D levels of at least 75 nmol/L. Adjustment(3) strengthened the association (aOR, 5.41 95% CI 2.02-14.52).
Wei[26] (2012) CANADA	Prospective Cohort N= 697	Plasma total 25[OH]D at 12–18 and 24–26 weeks of gestation using Assay: Chemiluminescence immunoassay	PE defined as gestational Hypertension (two or more readings of diastolic blood pressure ≥90 mmHg taken 4 hours apart but within 72 hours occurring at ≥20 weeks of gestation) with proteinuria (urine protein dipstick test ≥2+, or the urinary excretion of ≥0.3 g in a 24-hour urine collection)	Women with 25[OH]D < 50 nmol/l at 24–26 weeks gestation experienced an increased risk of preeclampsia (adjusted odds ratio 3.24, 95% confidence interval 1.37–7.69), whereas the association was not statistically significant for maternal 25[OH]D level at 12–18 weeks of gestation

***Abbreviations**: **25[OH]D**, 25-hydroxyvitamin D; **aOR**, adjusted odds ratio; **BMI**, body mass index; **BP**, blood pressure; **CI**, confidence interval; **DBP**, diastolic blood pressure; **EOSPE**, early onset preeclampsia; **FFQ**, food frequency questionnaire; **IQR**, interquartile range;
MDD, Major Depressive Disorder; **NT**, Normotensive; **OR**, odds ratio; **PE**, preeclampsia; **RIA**, radioimmunoassay; **SBP**, systolic blood pressure; **SPE**, severe preeclampsia;
****SI Conversions**: 1 ng/mL = 2.496 nmol/L;

[1] Adjusted for race/ethnicity, season, sample gestational age, prepregnancy BMI, & education.
[2] Adjusted for BMI, maternal age, African American race, & gestational age at sample collection
[3] Adjusted for season of blood draw, maternal age, multiparity, BMI, & gestational age at collection