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Maternal vitamin D sufficiency and reduced placental gene expression in angiogenic biomarkers related to comorbidities of pregnancy

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ABSTRACT

Introduction: Maternal circulating 25-hydroxyvitamin D [25(OH)D] has been shown to optimize production of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] during pregnancy at approximately 100 nmoles/L, which has pronounced effects on fetal health outcomes. Additionally, associations are noted between low maternal 25(OH)D concentrations and vascular pregnancy complications, such as preeclampsia. To further elucidate the effects of vitamin D activity in pregnancy, we investigated the role of maternal 25 (OH)D, the nutritional indicator of vitamin D status, in relation to placental maintenance and, specifically, expression of placental gene targets related to angiogenesis and vitamin D metabolism.

Methods: A focused analysis of placental mRNA expression related to angiogenesis, pregnancy maintenance, and vitamin D metabolism was conducted in placentas from 43 subjects enrolled in a randomized controlled trial supplementing 400 IU or 4400 IU of vitamin D_3 per day during pregnancy. Placental mRNA was isolated from biopsies within one hour of delivery, followed by quantitative PCR. We classified pregnant women with circulating concentrations of $<$100 nmoles/L as deficient and those with $\geq$100 nmoles/L as sufficient. The value of each gene's change in the PCR cycle threshold (ΔCT), which is a relative measure of target concentration, was compared with maternal 25(OH)D concentrations $<$100 nmoles/L and $\geq$100 nmoles/L based on a two-sample Wilcoxon test.

Results: Soluble FMS-like tyrosine kinase 1 (sFlt-1) and vascular endothelial growth factor (VEGF) gene expression was significantly downregulated in the maternal subgroup with circulating 25(OH)D $\geq$100 ng/mL compared to the subgroup $<$100 ng/mL.

Discussion: Here, we report a significant association between maternal vitamin D status and the expression of sFlt-1 and VEGF at the mRNA level. Achieving maternal circulating 25(OH)D $\geq$100 nmoles/L suggests the impact of maternal vitamin D_3 supplementation on gene transcription in the placenta, thereby potentially decreasing antiangiogenic factors that may contribute to vascular pregnancy complications.

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1. Introduction

Pregnancy has been shown to be a critical life stage in which dietary supplementation with vitamin D appears to have a pronounced effect on fetal health outcomes, including a reduction in the risks of premature labor/birth, and additional maternal comorbidities such as gestational diabetes, hypertensive disorders, and infection [1–6]. Little is known, however, about the role of maternal vitamin D sufficiency *on the fetus* and its role in pregnancy protection/maintenance throughout gestation. In a recent NICHD-sponsored, 6-year randomized, double-blind, placebo-controlled trial investigating serum concentrations of active, hormonal vitamin D during pregnancy, circulating maternal 25 (OH)D concentrations were found to be optimized at 100 nmol/L (40 ng/mL), which is twice the level normally observed in non-pregnant women [7]. In the present study, we aimed to examine the effect of maternal vitamin D status on hormones with vital roles in placental development and maintenance.

The association of lower vitamin D concentrations with non-cardiovascular disease demonstrates a diverse range of pathologies in observational studies, including infectious diseases, obesity, bone health, cancer and multiple sclerosis [8–10]. Likewise, there is

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extensive evidence from laboratory studies to suggest that vitamin D influences the vascular system, supported by observational studies in humans revealing the association of vitamin D insufficiency with increased arterial stiffness and endothelial dysfunction in the conductance and resistance of blood vessels [11]. As endothelial dysfunction is a hallmark of pregnancy complications that potentially lead to premature labor and delivery, such as preeclampsia, we hypothesized that maternal circulating vitamin D concentrations may affect the expression of an array of genes linked with angiogenesis and the potential for placental insufficiency secondary to its abnormal vasculature.

Consistent with this hypothesis, multiple studies have shown associations between low maternal 25(OH)D concentrations and the risk of preeclampsia [12–14]. Mirzakhani H, et al. [15] demonstrated in their study of over 800 participants that higher maternal circulating vitamin D concentrations both at the start of the study (first trimester) and in late pregnancy were associated with a lower risk of preeclampsia [15]. There is still little known, however, about the effects of maternal circulating vitamin D concentrations on the fetus/fetal tissue; although new and developing studies are attempting to combat the controversy of vitamin D's relation to pregnancy outcomes. Al-Garawi A, et al. [16] recently described the gene expression profiles of healthy pregnancy women in the *V*itamin *D A*ntenatal *A*sthma *R*eduction *T*rial (VDAART). The conclusions of this study suggest maternal vitamin D levels influence transcriptional profiles and these alterations of the maternal transcriptome may contribute to fetal immune imprinting [16]. It is proposed that these transcriptional changes may be related to pregnancy comorbidities, including vascular complications. Women with preeclampsia (a known significant vascular complication of pregnancy) are recognized to be at great risk for adverse pregnancy outcomes with a 20-fold increased risk for maternal mortality and several-fold higher risk for neonatal morbidity and mortality, depending on the gestational age at delivery and the presence of growth restriction in the fetus [17]. Therefore, the placenta was chosen, due to its innately high vascularization and as the interface between maternal and fetal tissue, to determine how maternal vitamin D status affects both maternal and fetal tissue on a molecular level.

Our study primarily investigated the role of maternal vitamin D status on placental expression of target genes related to vascular complications of pregnancy; however, a total of three groups of target genes were chosen based on previously reported functions within the placenta. The first group chosen has critical function in the angiogenesis pathway related to pregnancy, and included vascular endothelial growth factor (VEGF), placental growth factor (PGF), and soluble fms-like tyrosine kinase 1 (sFlt-1). Additionally, regulatory genes known for their role in placental (and thus pregnancy) maintenance, which included progesterone receptor B (PRB), estrogen receptor 1 (ESR1), human chorionic gonadotropin β (hCGβ), and human placental lactogen (hPL) were investigated. Finally, genes related to vitamin D metabolism, including vitamin D receptor (VDR), glucocorticoid receptor (GRα, aka NR3C1), 24-hydroxylase (CYP24A1), and CYP27B1 were evaluated. Of note, CYP27B1 is responsible for the production of $1,25(OH)_2D$ from 25 (OH)D, while CYP24A1 catalyzes the conversion of $1,25(OH)_2D$ into 24-hydroxlated products, constituting the degradation of the vitamin D molecule [18]. We hypothesized that increased mRNA expression of proangiogenic genes and decreased mRNA expression of antiangiogenic factors would be observed in vitamin D sufficient women compared to vitamin D deficient women.

2. Methods

2.1. Study design

This study was part of a randomized, placebo-controlled clinical trial (NCT 01932788) in which women provided informed consent and were followed from time of enrollment through delivery. The Institutional Review Board at the Medical University of South Carolina approved this study protocol (Pro 00020570). Enrolled mothers were 18–45 years of age who presented at 8-14 weeks'

Table 1
Comparison of maternal and infant characteristics of women classified as 25(OH)D sufficient (≥100 nmoles/L) or deficient (<100 nmoles/L) prior to delivery.

	Maternal 25(OH)D <100 nmoles/L	Maternal 25(OH)D ≥100 nmoles/L	Total	*p*-value*
	N (%)	N (%)	N (%)	
Ethnicity				**0.04**
African American	5 (11.6)	6 (13.9)	11 (25.6)	
Hispanic	6 (13.9)	11 (25.6)	17 (39.5)	
Caucasian	1 (2.3)	13 (20.2)	14 (32.5)	
American Indian	1 (2.3)	0 (0.0)	1 (2.3)	
Insurance				0.10
Private	2 (4.7)	13 (30.2)	15 (34.9)	
Medicaid	7 (16.3)	7 (16.3)	14 (32.6)	
Self-Pay	4 (9.3)	10 (23.3)	14 (32.6)	
Marital Status				0.76
Single	4 (9.3)	5 (11.6)	9 (20.9)	
Married	6 (13.9)	18 (41.9)	24 (55.8)	
Cohabitating	3 (7.0)	7 (16.3)	10 (23.3)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Maternal age (years)	36.9 ± 4.2	28.9 ± 4.2	28.3 ± 4.3	0.31
Maternal BMI	30.3 ± 8.2	28.7 ± 8.1	29.2 ± 8.0	0.56
Maternal 25(OH)D baseline (ng/mL)	58.4 ± 22.7	71.9 ± 23.7	67.9 ± 23.9	0.10
Maternal 25(OH)D V6/7 (ng/mL)	60.9 ± 26.2	140.5 ± 31.2	116.6 ± 47.2	
Infant gestational age (weeks)	39.2 ± 1.2	38.5 ± 1.8	38.7 ± 1.7	0.24
Infant birth weight (grams)	3381 ± 562	3363 ± 517	3369 ± 524	0.64

*p-values are included to identify the association between maternal vitamin D status and other demographic variables of interest. Of note, African Americans and Hispanics are more likely to be vitamin D deficient ($p = 0.043$). The type of insurance ($p = 0.104$), maternal marriage status ($p = 0.759$), maternal age ($p = 0.306$), gestational age ($p = 0.239$), baseline 25(OH)D ($p = 0.096$), maternal BMI ($p = 0.563$), birthweight ($p = 0.644$) were not significantly different between the vitamin D sufficient and vitamin D deficient patients.