

# Analysis of the NICHD Vitamin D Pregnancy Cohort on a Per-Protocol vs. Intent-to-Treat Basis: The Effect of Adherence on Trial Results

Michael J Abercrombie, Judith R Shary, Myla D Ebeling, Bruce W Hollis and Carol L Wagner\*

Medical University of South Carolina, Charleston, USA

## Abstract

**Objective:** To perform per-protocol analysis of data obtained from the NICHD vitamin D pregnancy study published by Hollis et al., which found via intent-to-treat analysis that 4000IU/day vitamin D supplementation is safe and effective in achieving sufficiency in women and neonates. This study hypothesizes that differential adherence as examined by per-protocol analysis will affect the magnitude of differences in maternal and neonatal vitamin D status between treatment groups.

**Study design:** A double-blind, RCT of vitamin D supplementation (400, 2000 or 4000IU/day) in 350 Caucasian, African American and Hispanic women with singleton pregnancies was conducted. This study defines adherence as 75% and 85% of pills taken between visits and examines the effect of adherence on vitamin D status across treatment groups. The primary outcome, measured by radioimmunoassay, is maternal serum 25(OH)D throughout pregnancy, one month prior to delivery (PTD) and neonatal serum 25(OH)D at delivery.

**Results:** No statistically significant difference in maternal 25(OH)D throughout pregnancy, 1-month PTD, or neonatal 25(OH)D were found between 75% adherent participants and nonadherent participants regardless of supplementation. At 85% adherence, maternal 25(OH)D throughout pregnancy, one month PTD, and neonatal 25(OH)D were significantly higher in the 4000IU group compared to nonadherent participants ( $p=0.0002$ ,  $p=0.0074$ ,  $p=0.0068$ , respectively). No significant differences were found with 400 or 2000IU supplementation regardless of adherence.

**Conclusions:** Participants 85% adherent to protocol and receiving 4000IU vitamin D were the only group that demonstrated significantly higher vitamin D status for each outcome: maternal 25(OH)D throughout pregnancy, 1-month PTD and neonatal 25(OH)D. Compared to intent-to-treat, this powerful per-protocol analysis demonstrates the impact that nonadherence can have on study results and has implications for how clinical trial data are analyzed and presented.

**Keywords:** Pregnancy; Vitamin D; Radioimmunoassay; Menstrual period

## Introduction

Since its Nobel prize worthy discovery in 1928, vitamin D research has lagged behind other similar essential compounds, in large part due to concerns about its toxicity to the human body [1]. More recent research, however, has shown that vitamin D and its active metabolites have various effects in the body that, in addition to its well-characterized skeletal effects, include immune modulation [2-4]. Studies involving diverse groups of individuals have shown that vitamin D plays a prominent role in health, including the health of pregnant women and their infants [5-14].

Since vitamin D supplementation has been shown to be beneficial during pregnancy, the question arose as to how much vitamin D should be administered to mothers to ensure benefits without the development of hypervitaminosis D. In a randomized controlled trial, 350 women of African American, Caucasian, or Hispanic descent were randomized to receive 400, 2000, or 4000 IU of vitamin D per day starting at 12-16 weeks of gestation [7]. The research team found that 4000 IU supplementation was the most effective dose regardless of race in bringing total circulating 25(OH)D concentrations to sufficiency, or above 80 nmol/L, as suggested by The Endocrine Society [15]. The analysis of the data was, however, performed as an intention-to-treat study rather than per protocol. In other words, the study did not look at only those women in the study who were adherent to the protocol set forth in the study, but rather all women enrolled regardless of whether or not they were compliant with the prescribed daily vitamin

D supplement prescribed by the study. Therefore, the aim of this *post hoc* analysis was to analyze the existing data on a “per protocol” basis using pill count as a measure of adherence. The hypothesis of this study was that, upon analysis on a “per protocol” basis, the circulating 25(OH)D concentrations would be significantly higher than those found using an intention-to-treat approach in both the 2000 and 4000 IU supplementation groups.

## Methods

### Trial design

The original vitamin D supplementation trial was performed at a single center, the Medical University of South Carolina (MUSC), and was double blind, controlled, and randomized. Women who had not yet reached sixteen weeks gestation with a single pregnancy were allowed to participate in this study.

\*Corresponding author: Carol L Wagner, MD, Shawn Jenkins Children's Hospital, Medical University of South Carolina, 165 Ashley Avenue, MSC 917, Charleston, SC 29425, USA, Tel: (843) 792-2112; Fax: (843) 792-8801; E-mail: [wagnercl@musc.edu](mailto:wagnercl@musc.edu)

Received May 18, 2018; Accepted May 21, 2018; Published May 31, 2018

**Citation:** Abercrombie MJ, Shary JR, Ebeling MD, Hollis BW, Wagner CL (2018) Analysis of the NICHD Vitamin D Pregnancy Cohort on a Per-Protocol vs. Intent-to-Treat Basis: The Effect of Adherence on Trial Results. J Nutr Food Sci 8: 696. doi: [10.4172/2155-9600.1000696](https://doi.org/10.4172/2155-9600.1000696)

**Copyright:** © 2018 Abercrombie MJ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Participants

The study was conducted at MUSC in Charleston, South Carolina between January 2004 and July 2009 after approval from the IRB (HR # 10727; clinical-trials.gov # NCT00292591). Each mother in the study had to be at least sixteen years of age, as well as fewer than 16 completed weeks of gestation at the first visit. She also had to be able to give written consent to participate in the study and to receive her prenatal care in the Charleston area. The baseline 25(OH)D concentrations was taken at the first visit, and supplementation was started between weeks 12 and 16 of pregnancy, as defined by maternal last known menstrual period.

## Exclusion criteria

Women who were beyond 16 weeks in their pregnancy were not allowed to participate, as well as those women who had preexisting parathyroid conditions, calcium conditions, or chronic hypertension. Women who had thyroid conditions but were on medication with hormone levels within the normal parameters were allowed to participate.

## Study protocol

**Gestational age at enrollment:** Women were enrolled between 12 and 16 weeks gestation, beginning at 12 0/7 weeks and ending at 16 0/7 weeks. Age was determined by using the last known menstrual period and, if this was not known, the obstetrical estimate was used and verified at the twenty-week ultrasound. If the estimate was deemed incorrect, it was adjusted and all changes were documented.

**Initial and subsequent study visits:** The initial visit consisted of consent followed by blood and urine samples to collect baseline total circulating 25(OH)D concentrations. Supplementation was never started earlier than 12 0/7 weeks, nor later than 16 0/7 weeks. The subsequent visits occurred monthly and coincided with obstetrical visits if care was provided at MUSC. For those patients seeking care outside of MUSC, the visits for the study were performed in addition to the obstetrical visits.

**Completion of questionnaires:** Upon consent for the study, questionnaires were completed with sociodemographic information, medical history, general health status, and diet. Interim questionnaires were completed at each visit regarding maternal health including updating current medications and analysis of recent doctor visits. The interim questionnaires assessed any adverse events experienced during the pregnancy as well as any illnesses experienced by the mother. Mode of delivery, birth weight, level of care provided to each newborn, and any adverse events were recorded.

**Blood and urine samples:** Maternal blood and urine samples were collected at each visit, and, when possible, umbilical cord blood was collected. If the umbilical cord blood was not available for collection, a sample was taken from the newborn within two days of birth prior to discharge and served as the surrogate value.

## Intervention

**Multivitamin and vitamin D supplementation:** All patients that were accepted into the study and supplemented between 12 and 16 weeks gestation were randomized into either the 400, 2000, or 4000 IU group. The doses were given in two pills a day, one 400 IU multivitamin tablet, which was the current recommended daily dose of vitamin D for pregnant women. The other pill taken contained 3600 IU, 1600 IU, or 0 IU vitamin D<sub>3</sub>. 25(OH)D concentrations were monitored, and

women with 25(OH)D below 100 nmol/L were eligible to participate in either the 400, 2000, or 4000 IU group. Those between 100 and 150 nmol/L were limited to the 2000 or 400 IU groups, and those over 150 nmol/L were specifically placed in the 400 IU arm of the study. 400 IU was chosen to represent the then current recommendation; 2000 IU to study the then current suggested upper limit of vitamin D supplementation, and 4000 IU was chosen based on prior pilot data that showed optimized vitamin D status in all mothers in this group by 20 weeks' gestation.

**Adherence to medication regimen:** Women were considered compliant with the study if they had brought back their pill bottles at 3 of the 5 analyzed visits and had taken 75% of their pills between visits on average. Women who did not take at least 75% of their pills on average and had brought their pills back at less than 3 of the 5 analyzed visits were not included. If a woman missed one visit, the additional month supply of pills was mailed to her or delivered to her residence. Adherence in these cases was defined by measuring the total number of pills taken between the two visits. If a subject missed two visits, or at any point took fewer than fifty percent of her allotted pills for the month, she was exited from the study.

**Randomization:** Stratified blocked randomization was used to balance by ethnicity and enrollment, the latter to prevent bias from the time of year the subject was enrolled.

A randomization scheme was developed separately for each of the three ethnic groups (i.e., the strata). Within each stratum, the treatments were assigned within blocks. Because there were three treatment groups, the block size had to be divisible by 3; the data team selected a block size of six, which was unknown to the investigators or the pharmacists. In this way, at the end of each block (i.e., enrollment of six subjects), each ethnic group was balanced in the number randomly assigned to the 400-, 2000-, and 4000-IU treatment groups.

## Sample size and power considerations

As described in the earlier publication of this study [7], the sample size was designed to detect a statistically significant increase in 25(OH)D by 10 ng/mL between any two groups and was calculated to require a minimum of 32 patients per group at 90% power,  $\alpha=0.05$ , two tailed tests for the primary analysis. This calculation assumed that the standard deviation of 25(OH)D measurements at a single time point was approximately 10, that there would be a low correlation ( $r=0.25$ ) between the baseline and final measurements, and that a substantial proportion (up to 50%) of participants could be lost to follow-up due to moving, termination of care, or discontinuation of participation. Because the primary outcome-maternal and neonatal vitamin D status at or around the time of delivery, a prerequisite for inclusion in the final analysis was that the mother had to have had a livebirth and had to have been a study participant until the day of delivery.

## Statistical analysis

In this per protocol analysis, the main variables of interest were:

(1) differences in mean maternal and infant total circulating 25(OH)D concentration at the time of delivery between supplement groups (ANOVA); and

(2) differences, between supplement groups, in the proportion of women achieving 25(OH)D of  $\geq 80$  nmol/L within one month and at the time of delivery (Chi-square).

Area under the curve for 25(OH)D concentration was calculated

from the values of 25(OH)D from visit 3 (when 25(OH)D was at steady-state) until delivery. Secondary analyses employed Chi-square for categorical variables, ANOVA or Student's t-test, as appropriate, for normally distributed variables (with the Bonferonni option for pair-wise analysis in ANOVA), and paired Student's t-test for within group changes from baseline to delivery. Analyses were conducted using SAS statistical software (version 9.4; SAS Institute, Cary, NC). Statistical significance was determined by comparing two-sided p-values to  $\alpha=0.05$ .

## Results

### Study population

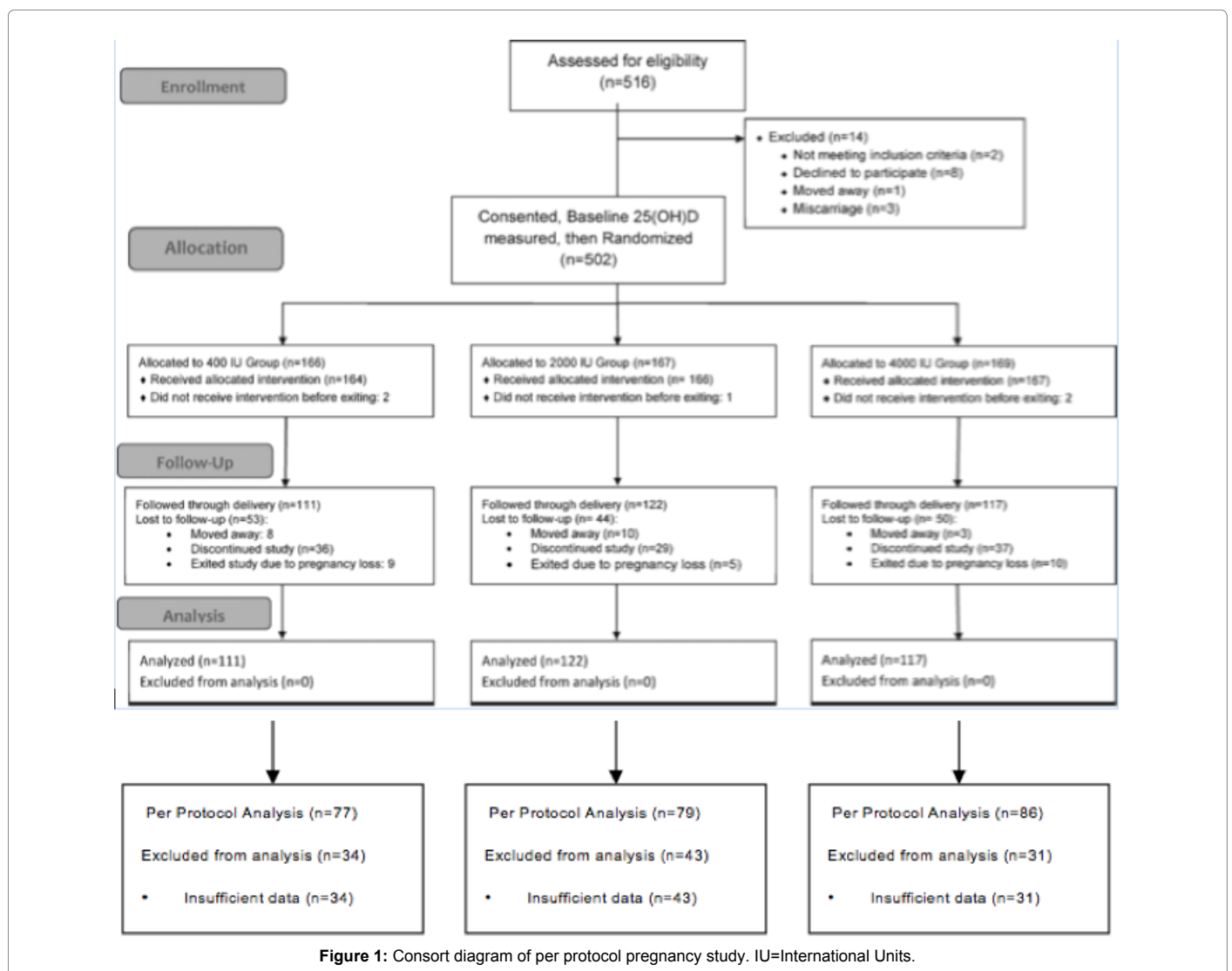
Figure 1 shows enrollment of women who participated in the trial. The selection process began by interviewing 516 women, of whom 502 consented to the study. These 502 were then further allocated to one of three treatment groups: 166 to the 400-IU group, 167 to the 2000-IU group, and 169 to 4000-IU group. There were 23 women in the initial 502 consenting women who had 25(OH)D concentrations of greater than 100 nmol/L and were thus ineligible for enrollment in the 4000-IU group. This group of 23 women consisted of 2 African American, 6

Hispanic, and 15 Caucasian. Of those, 12 were enrolled into the 400-IU group, 10 were enrolled into the 2000-IU group, and 1 was enrolled in the 4000-IU group (the latter being a protocol deviation early in the study where one woman with a baseline 25(OH)D concentration of 41 ng/mL was randomized to the 4000- IU group).

After allocation into treatment groups, there were no statistically significant differences among the groups who continued in the study through delivery, those who dropped out, or those who experienced pregnancy losses. In all, 350 women who continued in the study through delivery were analyzed in the original study published in 2011. For the per protocol analyses, the total number of subjects was as follows: 77 in the 400 IU group, 79 in the 2000 IU group, and 86 in the 4000 IU group. There were no statistically significant difference found between the three groups upon initial analysis, and those that were excluded from analysis were excluded due to insufficient pill count data. Figure 1 below represents this information.

### Study outcomes

Figure 2 demonstrates that when mothers are compared at 75% adherence to the study protocol, there are no statistically significant



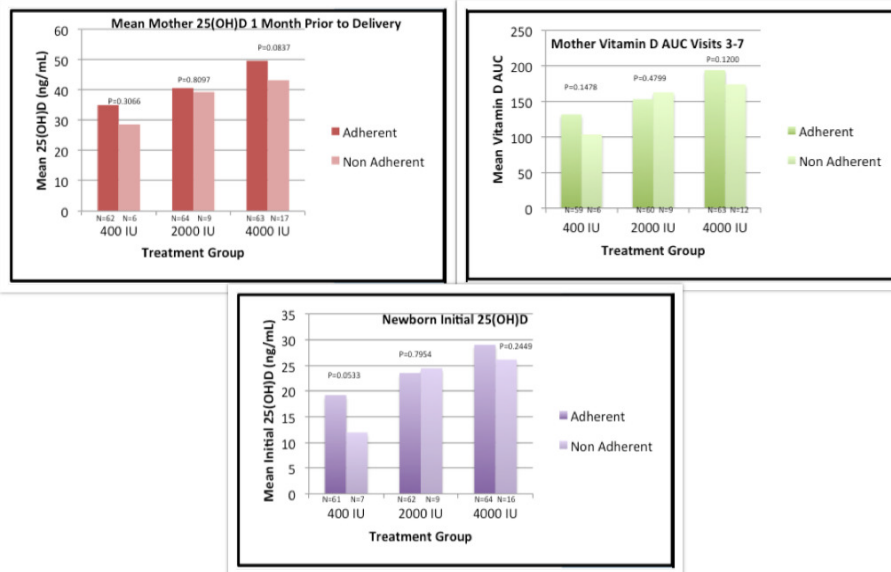


Figure 2: Outcome data for each treatment group, 75% adherent vs. non-adherent.

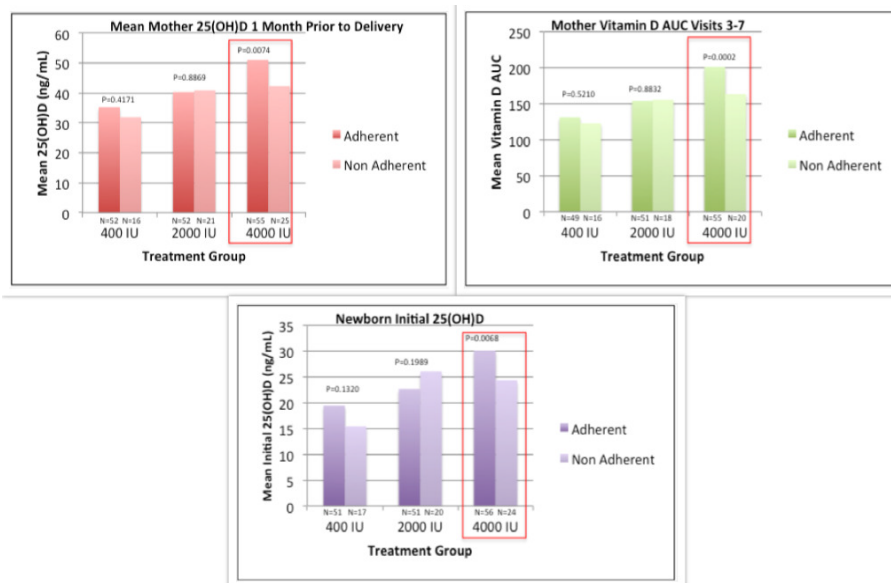


Figure 3: Outcome data for each treatment group, 85% adherent vs. non-adherent.

differences in total circulating 25(OH)D concentrations throughout pregnancy between those who were and were not 75% adherent in the 400 IU, 2000 IU, or 4000 IU groups. The same results were found when comparing the 75% adherent subjects to the non-adherent subjects within the 400 IU, 2000 IU, and 4000 IU groups in regard to the mother's circulating 25(OH)D concentration at one month before delivery and the neonatal cord blood 25(OH)D concentration. Finally, the figure shows no significant data was found when comparing neonatal vitamin D levels among the three treatment groups. Using the definition of adherence used in this study at 75% of supplement taken over the course of the study, Figure 2 shows no significant differences between those who were and those who were not adherent.

The data were then further analyzed, using 85% pill count to define

adherence. When comparing subjects who were and were not 85% adherent to the study protocol within treatment groups, there were statistically significant differences in maternal circulating 25(OH)D one month before delivery and, in 25(OH)D concentrations across pregnancy in the 4000 IU group. In addition, in the 4000 IU group, neonatal circulating 25(OH)D concentrations were higher for infant of the women who were 85% adherent. Images for the findings are presented in Figure 3.

## Discussion

The 2011 publication by Hollis and Wagner et al. reported a significant increase in total circulating 25(OH)D and vitamin D levels throughout pregnancy as well as maternal 25(OH)D one month before

delivery and initial newborn 25(OH)D levels in those mothers given the 4000 IU supplement when compared to the 2000 IU or 400 IU groups [7]. The aim of this *post hoc* analysis was to reproduce those results using a *per protocol* rather than intention-to-treat analysis. The first step in this process was to define adherence to the study protocol, which was defined by cumulative pill count. The data analysis using 75% adherence did not yield statistically significant differences when comparing those who were and were not adherent in the 400, 2000, or 4000 IU groups across all vitamin D variables analyzed. When analyzed using a higher adherence rate of 85%, statistically significant differences in maternal and neonatal vitamin D status within the 4000IU treatment groups were noted.

In each case, the adherent 4000 IU women had significantly higher vitamin D concentrations than the non-adherent women. Initial newborn 25(OH)D concentration was also higher in the adherent group compared to the non-adherent group when an adherence rate of 85% was used.

This study was limited by a few factors. First, all women selected to participate were in Charleston, South Carolina or at least had medical care at the Medical University of South Carolina. Furthermore, in selecting only those who followed the treatment protocol, the study may have introduced selection bias, although randomization and double blinding attempted to limit this bias as much as possible. Another limitation was, due to the long duration of the trial, some subjects were inevitably lost to follow up or transferred care to another facility. While the populations in each group were found to be large enough to compare, one could argue that a larger population sample size would be desirable and thus limited the study's generalizability.

## Conclusion

This randomized controlled trial of vitamin D supplementation during pregnancy, intent-to-treat analyses may underestimate 25(OH)D concentrations achieved by those who were adherent to the study protocol. Such differences in adherence could ultimately affect dosing recommendations.

## Funding

Funded by the National Institute of Children's Health and Human Development (NICHD) #R01 HD47511, NIH RR01070, MUSC Department of Pediatrics, and by the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, NIH/NCAT Grant number UL1 TR000062.

## References

1. Wolf G (2004) The discovery of vitamin D: the contribution of Adolf Windaus. *J Nutr* 134: 1299-1302.
2. Holick MF (2011) Vitamin D: evolutionary, physiological and health perspectives. *Curr Drug Targets* 12: 4-18.
3. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, et al. (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311: 1770-1773.
4. Hewison M (2012) An update on vitamin D and human immunity. *Clin Endocrinol* 76: 315-325.
5. Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD (2010) Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol* 203: 366 e1-366 e6.
6. Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF (2009) Association between vitamin D deficiency and primary cesarean section. *J Clin Endocrinol Metab* 94: 940-945.
7. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL (2011) Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* 26: 2341-2357.
8. Wagner CL, McNeil RB, Johnson DD, Hulsey TC, Ebeling M, et al. (2013) Health characteristics and outcomes of two randomized vitamin D supplementation trials during pregnancy: a combined analysis. *J Steroid Biochem Mol Biol* 136: 313-320.
9. Wagner CL, Baggerly C, McDonnell S, Baggerly KA, French CB, et al. (2016) Post-hoc analysis of vitamin D status and reduced risk of preterm birth in two vitamin D pregnancy cohorts compared with South Carolina March of Dimes 2009-2011 rates. *J Steroid Biochem Mol Biol* 155: 245-251.
10. Wolsk HM, Harshfield BJ, Laranjo N, Carey VJ, O'Connor G, et al. (2017) Vitamin D supplementation in pregnancy, prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring: Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. *J Allergy Clin Immunol* 140: 1423-29.
11. Hollis BW, Wagner CL (2017) Vitamin D supplementation during pregnancy: Improvements in birth outcomes and complications through direct genomic alteration. *Mol Cell Endocrinol* 453: 113-130.
12. McDonnell SL, Baggerly KA, Baggerly CA, Aliano JL, French CB, et al. (2017) Maternal 25(OH)D concentrations  $\geq 40$  ng/mL associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. *PLoS One* 12: e0180483.
13. Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, et al. (2017) Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: a combined analysis of two randomized controlled trials. *PLoS One* 12: e0186657.
14. Blighe K, Chawes BL, Kelly RS, Mirzakhani H, McGeachie M, et al. (2017) Vitamin D prenatal programming of childhood metabolomics profiles at age 3 y. *Am J Clin Nutr* 106: 1092-1099.
15. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, et al. (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 96: 1911-1930.