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Original Research Article

The Impact of Baseline 25-Hydroxyvitamin D Level and Gestational Age on Prenatal Vitamin D Supplementation to Prevent Offspring Asthma or Recurrent Wheezing

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A B S T R A C T

Background: Prenatal vitamin D deficiency is associated with asthma or recurrent wheezing in offspring. However, evidence from randomized trials on the efficacy of vitamin D supplementation is inconclusive.

Objectives: We aimed to examine the differential efficacy of prenatal vitamin D supplementation based on the maternal baseline vitamin D status and the starting time of supplementation to prevent early life asthma or recurrent wheezing.

Methods: We conducted a secondary analysis of the Vitamin D Antenatal Asthma Reduction Trial (VDAART), a randomized double-blind trial of prenatal vitamin D supplementation initiated at 10–18 weeks (wks) of gestation (4400 IU of intervention/day compared with 400 IU of placebo/day) to prevent offspring asthma or recurrent wheezing by the age of 6 years. We assessed the effect of modification of supplementation by maternal baseline vitamin D status at enrollment and the timing of initiation of supplementation.

Results: An inverse relationship was observed between maternal 25-hydroxyvitamin D (25(OH)D) levels at trial entry and 25(OH)D levels during late pregnancy (32–38 wks of gestation) in both supplementation arms ($P < 0.001$). Overall, supplementation efficacy was not dependent on the maternal baseline 25(OH)D status. However, a trend toward the reduction of asthma or recurrent wheezing was observed across the baseline groups in the intervention arm ($P = 0.01$), with the greatest reduction observed in the most severely vitamin D–deficient women (25(OH)D < 12 ng/mL; adjusted odds ratio [aOR] = 0.48; confidence interval [CI]: 0.17, 1.34). Gestational age at trial enrollment modified supplementation efficacy, showing a greater reduction of offspring asthma or recurrent wheezing with earlier intervention during pregnancy (aOR = 0.85; CI = 0.76, 0.95), particularly in women who were 9–12 wk pregnant (aOR = 0.45; CI = 0.24, 0.82).

Conclusions: Pregnant women with severe vitamin D deficiency show the greatest 25(OH)D improvement because of supplementation. In these women, a vitamin D dose of 4400 IU might have a preventive role in the development of early life offspring asthma or recurrent wheezing. Gestational age is suggested to modify the efficacy of prenatal vitamin D supplementation, showing the highest beneficial effect if supplementation is started during the first trimester of pregnancy.

This study is an ancillary analysis from the VDAART, which is registered in ClinicalTrials.gov as NCT00902621.

Keywords: pregnancy, 25(OH)D, vitamin D supplementation, childhood asthma, recurrent wheeze

Abbreviations: aOR, adjusted odds ratio; $COPSAC_{2010}$, Copenhagen Prospective Studies on Asthma in Childhood 2010; ITT, intention-to-treat; RCT, randomized controlled trial; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

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Introduction

Evidence from several observational studies suggests that low serum vitamin D concentrations in pregnant women contributes to early life offspring recurrent wheeze and asthma, a strong predictor of later onset of persistent wheeze and childhood asthma [1]. Vitamin D could act as a modulator of fetal lung and immune system development, which are critical components in wheezing and asthma onset and disease progression [2]. Vitamin D deficiency is common during pregnancy [3]. Observational studies have shown that higher vitamin D concentrations during pregnancy and in neonates, as measured by the circulating metabolite 25(OH)D, reduce the risk of wheezing and allergic phenotypes in neonates and subsequent asthma development [4–6]. Meta-analyses of these studies supported a protective effect of maternal vitamin D intake and sufficiency status on asthma development in offspring [7–9]. However, it was noted that larger studies with longer-term follow-ups were necessary to better establish the true effect size of prenatal vitamin D supplementation [9]. Consistently, a meta-analysis over an extended period that pooled estimates from 33 long-term cohort studies suggested that the beneficial effect of prenatal vitamin D on asthma or recurrent wheezing is most pronounced after the age of 5 years (y) [10].

The results from randomized controlled trials (RCTs) of prenatal vitamin D supplementation are not in complete agreement with the findings from observational studies. Grant et al. [11] conducted an RCT on prenatal vitamin D supplementation initiated at 27 wks of gestation, and postnatally continued the supplementation in neonates by 6 months (mo). Pregnant women and their offspring pairs (women/infants) were randomized to treatment with 1000 IU/400 IU, 2000 IU/800 IU of daily vitamin D, or placebo/placebo doses. At 18 mo after birth, the investigators found that vitamin D supplementation during pregnancy and infancy reduced the proportion of children with allergy as well as the primary care visits where childhood asthma was diagnosed [11]. In contrast, Goldring et al. [12] found no effect on wheezing phenotypes for a single high dose of prenatal vitamin D supplementation (22,000 IU) at 27 wks of gestation [12]. Similarly, the Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) showed no significant effect of daily 2800 IU vitamin D dosage during the third trimester on persistent wheezing or asthma in offspring aged 3 and 6 y, although higher maternal serum vitamin D concentrations were associated with a lower risk of wheezing at the age of 3 y [13,14]. Of note, these trials started low daily or single high prenatal dose supplementation during late pregnancy (third trimester) and had small sample sizes or did not reach their target sample size. Although observational studies suggest that higher vitamin D concentrations may lead to improved health outcomes, it is unclear whether supplementation benefits those with lower initial concentrations more than those with higher concentrations or whether starting treatment earlier during pregnancy might increase its efficacy.

Achieved concentrations of vitamin D after supplementation will depend on the dosage, the initial vitamin D concentration prior to starting supplementation, and the timing of supplementation initiation [15]. It remains unclear whether the dose–response relationship of supplementation in pregnant women changes between those who are low initially or those who already have a level deemed to be sufficient. To investigate whether the vitamin D sufficiency status and the starting time of intervention during early pregnancy affect the efficacy of prenatal vitamin D in reducing the risk of early life recurrent wheezing and asthma in offspring, we conducted a secondary analysis of one of the largest RCTs to date, i.e., the Vitamin D Antenatal Asthma Reduction Trial (VDAART) [16]. The trial's 3- and 6-y follow-up results were published, and the primary intention-to-treat (ITT) analysis showed no significant effect of vitamin D supplementation at 3 and 6 y. However, in an adjusted sensitivity analysis, VDAART noted a significant effect of supplementation on the prevention of asthma or recurrent wheezing by the age of 3 y among children who provided complete 3-y information [17]. The stratified analysis by mean prenatal 25(OH)D concentrations during pregnancy (mean of 25(OH)D at 10–18 wks and 32–38 wks) of the 6-y outcome did not show an effect of prenatal vitamin D supplementation. However, the effect of supplementation was not specifically explored based on maternal baseline 25(OH)D concentrations and the starting time of supplementation [18]. The trial results for both the 3- and 6-y primary outcomes suggest further investigation on the impact of early pregnancy maternal baseline 25(OH)D status and the timely correction of these concentrations on the overall effect of prenatal vitamin D supplementation in the prevention of asthma or recurrent wheezing over an extended time frame.

Therefore, we conducted a secondary analysis of VDAART for the primary outcome of asthma or recurrent wheezing by the age of 6 y to investigate the differential effect of vitamin D supplementation by maternal baseline 25(OH)D sufficiency status and the fetal gestational age at the start of supplementation. We hypothesized that vitamin D intervention efficacy in the prevention of offspring asthma or recurrent wheezing by the age of 6 y might be moderated by maternal baseline 25(OH)D at trial entry and/or gestational age at early pregnancy. We additionally assessed whether the change in maternal vitamin D status at late pregnancy because of supplementation was dependent on the maternal baseline 25(OH)D concentrations and whether the pattern of early life 25(OH)D concentrations in offspring followed initial maternal 25(OH)D concentrations.

Methods

VDAART design and participants

Pregnant women were recruited from 3 clinical sites across the United States: Boston Medical Center (Boston, MA, United States), Kaiser Permanente Southern California Region (San Diego, CA, United States), and Washington University at St. Louis (St. Louis, MO, United States). Inclusion criteria were pregnant nonsmoking women from the age of 18–40 y with a pregnancy gestational age of 10–18 wk. Mothers and/or the biological fathers had a medical history of asthma, eczema, or allergic rhinitis. Pregnant women with other chronic disorders, such as chronic hypertension, were excluded from the trial. The details of VDAART and eligibility criteria for participants are previously published [16]. Participants were screened for eligibility between October 2009 and July 2011. Follow-up of the last child for the 3-y primary outcome—asthma, recurrent wheezing, or both—was completed in January 2015. The primary outcome was extended in a follow-up study by the age of 6 y and completed in January 2018.

After the child's birth, families were questioned through telephone interviews every 3 mo to monitor the child's symptoms, diagnoses, and medicine use. Additionally, the mother and child annually visited the clinic for follow-up during the child's first 3 y of life. Child's blood was drawn in years 1, 3, and 6 for 25(OH)D measurements. The children continued to be followed for assessment of their asthma or recurrent wheezing status by the age of 6 y. Maternal-offspring pairs with

offspring follow-up data available for the primary trial outcome of asthma or recurrent wheezing were used as the source population for this analysis ($N = 806$ ITT population]).

VDAART vitamin D supplementation and 25(OH)D measurement

Pregnant women were randomized 1:1 at the gestational age of 10–18 wk to a daily dose of 4000 IU vitamin D3 along with 1 multivitamin containing 400 IU vitamin D3 or matching placebo in addition to 1 multivitamin containing 400 IU vitamin D3; thus 4400 IU (hereafter referred to as the "intervention") compared with 400 IU (hereafter referred to as the "placebo") vitamin D3 supplementation per day until delivery. Prescription adherence was monitored using Medication Events Monitoring Systems caps, smart caps that recorded each instance the container was opened and with serum vitamin D 25(OH)D concentrations.

Prenatal vitamin D concentrations were measured as per the VDAART protocol [16]. Maternal blood for the determination of the prenatal 25(OH)D concentrations was drawn at trial enrollment (10–18 wk of gestational age) and at the visit in the third trimester (32–38 wk of gestational age). The absolute increase in 25(OH)D concentrations at the third trimester was calculated. DiaSorin Liaison chemiluminescence immunoassay was used for the quantitative analysis, which is a validated chemiluminescence assay performed at the Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, United States [19]. The assay is cospecific for 25(OH)D_3 and 25(OH)D_2. The interassay CV for 25(OH)D_2 and 25(OH)D_3 were all 6.8% or lower at various concentrations of samples processed; the total 25(OH)D values were used for the analysis reported here.

VDAART primary end point

The primary end point was asthma or recurrent wheezing in the first 6 y of the offspring's life. In VDAART, offspring's asthma was defined as a maternal or caregiver report of physician-diagnosed asthma, and the time of onset was defined as the first report of wheezing or the first report of the use of any asthma medication. Recurrent wheezing was defined as maternal or caregiver report of wheezing or the use of any asthma medication in 2 separate years over the first 6 y, and the time of onset was the first report of wheezing or the first report of the use of any asthma medication [18]. More extensive information about the doctor's diagnoses can be found in the original VDAART rationale [16].

Statistics

The analysis was conducted at the Channing Division of Network Medicine/Brigham and Women's Hospital, the VDAART coordinating center. T tests or chi-squared tests were used to assess the differences between the characteristics of participants of both supplementation groups after randomization.

To investigate the modification effect of maternal baseline 25(OH)D concentrations on supplementation efficacy, clinically interpretable groups were constructed based on the early-pregnancy 25(OH)D status and treated as an ordinal variable in the analysis with the highest rank being sufficiency (≥30 ng/mL). According to the evidence-based vitamin D guidelines recommended by the Institute of Medicine and National Academy of Medicine [20,21], we defined vitamin D sufficiency as having 25(OH)D concentrations of >30 ng/mL, insufficiency as 25(OH)D concentrations between 20 and 30 ng/mL, deficiency as 25(OH)D concentrations between 12 and 20 ng/mL, and severe deficiency as 25(OH)D concentrations of <12 ng/mL, respectively. Effect modification of the time of supplementation initiation on asthma or recurrent wheezing was examined using gestational age at entry as a continuous variable, and we further explored the effect across its quartiles for the effect stratification by cross-classification.

To test our main hypothesis, we applied a global test using a logistic regression (full) model with the inclusion of both main effects of supplementation arm, maternal baseline 25(OH)D groups, and gestational age at initiation of supplementation as well as the interaction terms for "supplementation arm*maternal baseline 25(OH)D group" and "supplementation arm*gestational age at initiation of supplementation." Furthermore, we cross-classified the pregnant women by using the quartiles of gestational age at enrollment stratified by the supplementation arm, and examined the association of the quartile parts with offspring asthma or recurrent wheezing. To assess whether there is a trend in nutritional response to vitamin D supplementation in pregnant women, based on changes in 25(OH)D concentrations from baseline, we conducted linear regression analyses across maternal baseline groups. Additionally, we examined the relationship between maternal baseline 25(OH)D concentrations and offspring 25(OH)D concentrations in the early years of life (1, 3, and 6 y). Furthermore, we conducted a sensitivity analysis using the outcome of asthma or recurrent wheezing by the age of 3 y to ensure robustness of our findings in the primary analysis of the 6-y outcome.

Analyses were presented as unadjusted analyses and adjusted for additional study variables that were selected as a priori determinants or potential confounders in relation to the outcome, "asthma or recurrent wheezing." These variables included maternal age, maternal education, maternal asthma, paternal asthma, study site, preterm birth (<37 wks of gestational age), maternal race, sex of the child, gestational age at enrollment, and season of blood drawn [4].

Adjusted odds ratios (aORs) and their corresponding 95% CIs were calculated. The trend tests were examined weighed by the inverse of the estimated variance of the log odds derived from the adjusted analyses. We used the statistical software R version 4.0.3 with the R packages of "effects" and "sjPlot" to visualize interactions [22]. All tests were 2-sided, and the significance level was prespecified at P value of < 0.05.

Results

Trial participants

The flow chart of participants for VDAART is shown in Figure 1, and the main trial characteristics are described in Table 1. Specifications of the cohort's participants are reported in Table 2. A total of 806 children, the ITT cohort of VDAART, was included in this analysis. Prenatal baseline 25(OH)D was available in 801 (99%) of the mothers of these children. Data on gestational age at enrollment were collected for 803 (99%) children. There were no statistical differences by comparisons of baseline characteristics between the intervention and placebo groups (Table 2).

In the study cohort, 176 women (22%) had an initial 25(OH)D concentration of ≥30 ng/mL (95 in the intervention group and 81 in the placebo group), 295 women (37%) had concentrations between 20 and 30 ng/mL (147 in the intervention group and 148 in the placebo group), 223 women (28%) had concentrations between 12 and 20 ng/mL (105 in the intervention group and 118 in the placebo group), and 107 (13%) had concentrations of <12 ng/mL at the trial entry (55 in the intervention group and 52 in the placebo group). The first quartile of gestational age values at entry to the trial included 205 women (26%) with the second, third, and fourth quartiles, including 202 women (25%), 196 women (24%), and 200 women (25%), respectively.

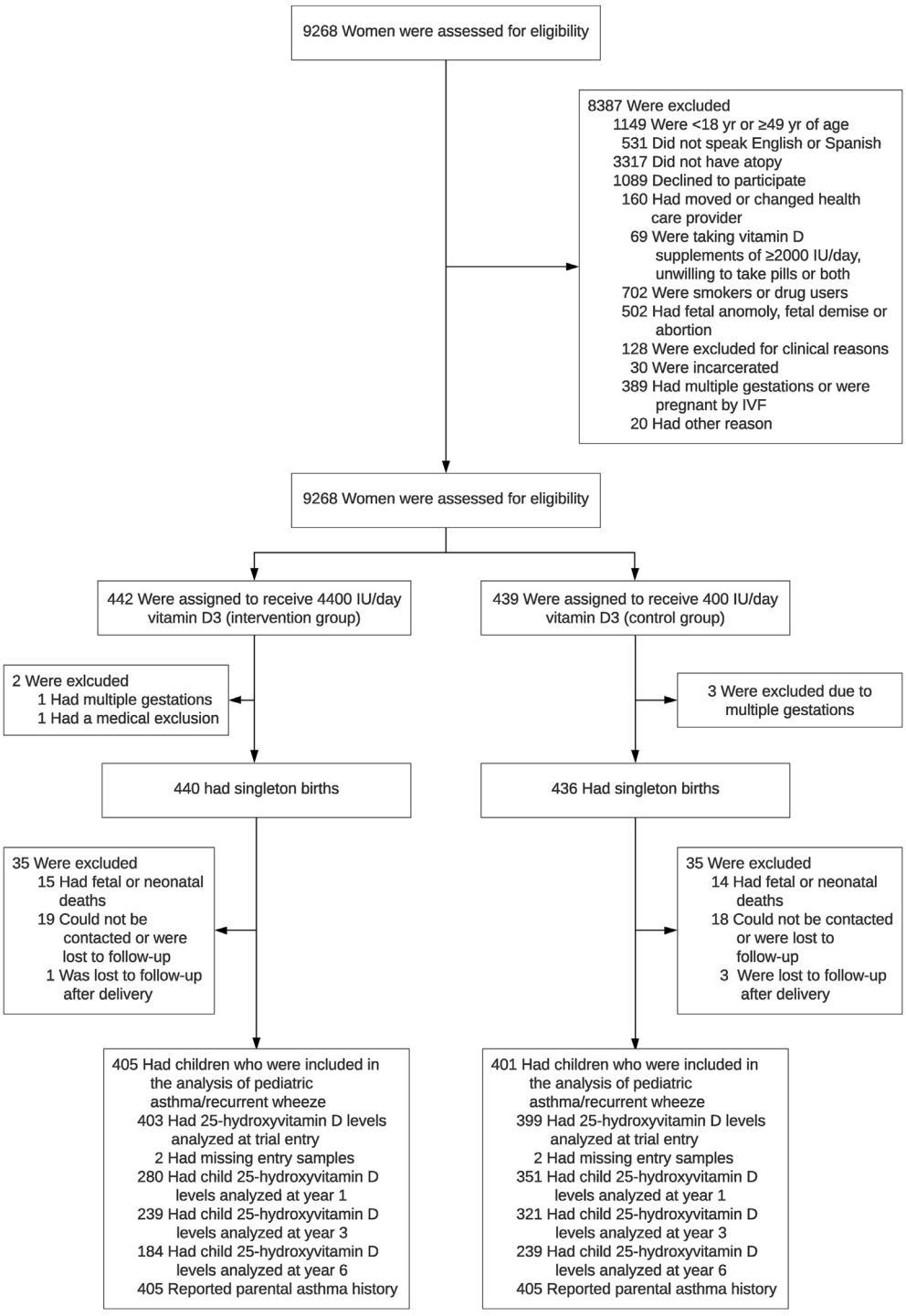

FIGURE 1. Flow chart of participants' enrollment, randomization, and follow-ups of VDAART. VDAART, Vitamin D Antenatal Asthma Reduction Trial.

TABLE 1
Characteristics of the Vitamin D Antenatal Asthma Reduction Trial

Trial	*N*	Inclusion/exclusion criteria	Duration of supplementation	Follow-up	Supplementation
VDAART, double-blinded, multicenter RCT, United States (2009–2015)	806 paired pregnant women and their offspring included in the intent-to-treat analysis for the primary outcome of asthma or recurrent wheeze	Inclusion: parental asthma, allergy, or eczema Exclusion: maternal chronic disease	From 10–18 wks of gestation until birth	6 y of follow-up by quarterly telephone calls and annual visits to the clinic	Intervention ($n = 405$): 4000 + 400 IU/d Placebo ($n = 401$): 400 IU/d

Abbreviations: RCT, randomized controlled trial; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

TABLE 2
Baseline characteristics of VDAART's intent-to-treat population for the primary outcome of offspring asthma or recurrent wheezing by the age of 6 y (*N* paired mother and child = 806)[1]

	4400 IU/d of vitamin D *n* = 405	400 IU/d of vitamin D *n* = 401	*P* value
Maternal age in y (mean ± SD)	27.5 ± 5.5	27.2 ± 5.6	0.452
Gestational age at enrollment in wks (mean ± SD)	14.1 ± 2.8	14.2 ± 2.7	0.787
Maternal asthma: yes	171 (42.2)	151 (37.7)	0.211
Paternal asthma: yes	100 (24.7)	87 (21.7)	0.370
Maternal 25(OH)D at enrollment in ng/mL (mean ± SD)	23.3 ± 10.3	22.6 ± 10.2	0.308
			0.583
<12 ng/mL	55 (13.7)	52 (13.0)	
12–20 ng/mL	105 (26.1)	118 (29.6)	
20–30 ng/mL	147 (36.6)	148 (37.1)	
≥30 ng/mL	95 (23.6)	81 (20.3)	
Mother's race			0.886
African American	175 (43.2)	172 (42.9)	
White	161 (39.8)	165 (41.1)	
Other	69 (17.0)	64 (16.0)	
Maternal education college or higher	134 (33.1)	139 (34.7)	0.69
Marital status			0.593
Married	177 (43.9)	190 (47.4)	
Divorced or separated	12 (3.00)	10 (2.50)	
Not married	214 (53.1)	201 (50.1)	
Household income			0.791
<$50,000	171 (42.2)	170 (42.4)	
≥$50,000	139 (34.3)	130 (32.4)	
Refused to say or unknown	95 (23.5)	101 (25.2)	
Study site			0.651
Boston, MA	117 (28.9)	123 (30.7)	
San Diego, CA	135 (33.3)	139 (34.7)	
St Louis, MO	153 (37.8)	139 (34.7)	
Male sex of child	201 (49.6)	220 (54.9)	0.157
Preterm birth (<37 wk): yes	40 (9.9)	31 (7.7)	0.331
Season of blood drawn at enrollment			0.783
Winter	93 (23.0)	101 (25.2)	
Spring	134 (33.1)	130 (32.4)	
Summer	99 (24.4)	88 (21.9)	
Fall	79 (19.5)	82 (20.4)	

Abbreviation: VDAART, Vitamin D Antenatal Asthma Reduction Trial.
[1] Data are given as a number (percentage) of individuals, unless otherwise specified.

Effect of prenatal vitamin D supplementation on maternal 25(OH)D concentrations

Postsupplementation 25(OH)D concentrations were higher in the intervention group than that in the placebo group (mean difference = 12.5 ng/mL; 95% CI: 10.6, 14.4 ng/mL; $P < 0.001$). Figure 2A shows the achieved postsupplementation levels per maternal baseline 25(OH)D group according to the 25(OH)D cutoffs. In both supplementation arms, postsupplementation levels increased in all 25(OH)D groups, and the highest achieved levels at the third trimester were observed in mothers with 25(OH)D concentrations of ≥30 ng/mL (*P*-trends < 0.001). The deficiency cutoff at 20 ng/mL showed that only the women randomized to placebo in the lowest maternal baseline group (<12 ng/mL), on average, remained deficient (25(OH)D <20 ng/mL) in the third trimester. The absolute increase in the 25(OH)D concentration for each maternal baseline group of interest is shown in Figure 2B. The absolute increase in maternal 25(OH)D concentration was negatively associated with higher baseline 25(OH)D values in both supplementation arms (*P*-trends < 0.001, Figure 2B).

Effect of maternal baseline 25(OH)D concentrations on prenatal vitamin D supplementation efficacy and risk of asthma or recurrent wheezing by age of 6 y

By 6 y of age, 357 (357/801 = 46%) children were diagnosed with asthma or recurrent wheezing. Of these children, a total of 72 (72/176 = 41%) were born to women with sufficient vitamin D status at 10–18 wks of gestation (baseline 25[OH]D ≥ 30 ng/mL), of whom 40 were in the intervention arm (56%) and 32 were in the placebo arm (44%). Pregnant women with insufficiency (baseline 25[OH]D > 20–30 ng/mL) gave birth to 119 (119/295 = 40%) children with a diagnosis of asthma or recurrent wheezing, of whom 56 received the intervention (47%) and 63 placebo (53%). Vitamin D–deficient women (baseline 25[OH]D > 12–20 ng/mL) gave birth to a total of 109 (109/223 = 49%) children with asthma or recurrent wheezing, 54 of whom received the intervention (50%) and 55 placebo (50%). Of the offspring born to women who were severely deficient in vitamin D at trial entry, 57 (57/107 = 53%) were diagnosed with asthma or recurrent wheezing, 25 in the intervention arm (44%), and 32 in the placebo arm (56%).

The odds of asthma or recurrent wheezing at the age of 6 y, as well as the effect estimates of the supplementation, varied across maternal 25(OH)D baseline groups (Table 3), although the interaction between maternal baseline groups and supplementation arms was not statistically significant (Table 3). Similar results were obtained when testing maternal baseline 25(OH)D concentrations as a continuous variable in the model (Supplemental Table 1). Notably, a decreasing trend in the odds of offspring asthma or recurrent wheezing was observed across baseline 25(OH)D groups among women in the intervention arm (adjusted *P*-trend = 0.01). The trend implied an effect modification of the baseline 25(OH)D on the vitamin D intervention, with the greatest risk reduction among pregnant women with baseline severe deficiency treated with 4400 IU of vitamin D daily (Supplemental Figure 1). In a stratified analysis by the maternal baseline group, the vitamin D intervention, independent of maternal age at the enrollment and other potential confounders, reduced the odds of asthma or recurrent wheezing in offspring born to pregnant women who were severely deficient, i.e., 25(OH)D concentrations of <12 ng/mL (aOR = 0.26; 95% CI: 0.10, 0.73; $P = 0.01$; Supplemental Figure 2).

Effect of gestational age on prenatal vitamin D supplementation efficacy and risk of asthma or recurrent wheezing

We observed an interaction between gestational age at the initiation of supplementation and the intervention arm in the full model ($P = 0.004$, Table 3). No effect of the gestational age at the time of supplementation was observed on the efficacy of 400 IU vitamin D supplementation ($P = 0.43$; Figure 3). Pregnant women showed a reduction in the odds of having offspring with asthma or recurrent wheezing by the age of 6 y if they received daily vitamin D supplementation of 4400 IU at an earlier time point in their pregnancy (Table 3 and Figure 3). The adjusted odds of asthma or recurrent wheezing among offspring whose mothers received 4400 IU vitamin D was 15% lower for each week of the earlier start of supplementation between 10 and 18 wks of gestation (aOR, 0.85; 95% CI: 0.76, 0.95). Examining the quartiles of gestational age at 10 and 18 wk in both supplementation arms, we observed a 55% reduction in the odds of offspring asthma or recurrent wheezing among pregnant women who received 4400 IU of vitamin D before the second trimester (1st quartile: 8.6–11.9 wk), compared with women who received 400 IU vitamin D before the second trimester (aOR, 0.45; 95% CI: 0.24, 0.82; $P = 0.01$).

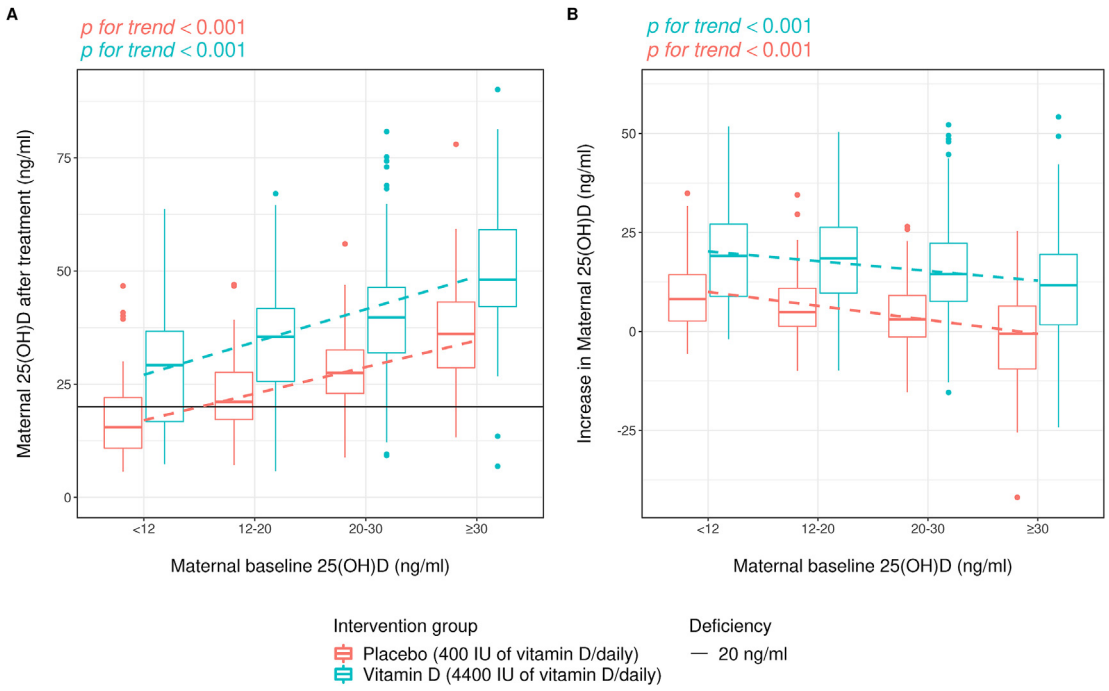

FIGURE 2. Treatment efficacy of vitamin D supplementation (4400 compared with 400 IU/daily) in improving 25(OH)D according to maternal baseline 25(OH)D status. (A) Maternal baseline 25(OH)D concentrations at 10–18 wks of gestation compared with paired third trimester concentrations (32–38 wks of gestation) after supplementation, the black line represents a deficiency cutoff at 20 ng/mL, (B) Absolute increase in maternal 25(OH)D concentrations from the baseline per supplementation arm and across maternal baseline 25(OH)D groups. *P* values demonstrate the significance of trends across maternal baseline 25(OH)D cutoffs obtained from linear regression models adjusted for the potential confounders; maternal age, maternal education, maternal asthma, paternal asthma, study site, preterm birth, maternal race, sex of the child, gestational age at the enrollment, and season of blood drawn.

Furthermore, a trend toward a reduction in offspring asthma or recurrent wheezing by the age of 6 y across quartiles of the gestational age of 10 and 18 wk was observed among pregnant women who received 4400 IU vitamin D (adjusted *P*-trend = 0.02; Table 4). We did not observe a similar trend among women who received 400 IU of vitamins (adjusted *P*-trend = 0.72, Table 4).

Compared with the primary analysis of 6-y outcome (*n* cases = 357), the sensitivity analysis also showed a similar effect direction in the association of the interaction between gestational age at enrollment and the vitamin D intervention with the outcome of asthma or recurrent wheezing by the age of 3 y (*n* cases = 215). Although the measure of interaction did not reach statistical significance (Supplemental Table 2), the lowest odds of the 3-y outcome, in line with the main analysis, were observed among children whose mothers started to receive 4400 IU/d in the 1st trimester (aOR = 0.47; 95% CI: 0.23, 0.94; *P* = 0.039; Supplemental Table 3).

Discussion

In this study, we present a secondary analysis of one of the largest RCTs of prenatal vitamin D supplementation to prevent childhood

TABLE 3
The association of maternal baseline 25(OH)D groups, gestational age at the initiation of treatment and their interactions (*) with vitamin D treatment arm, and "asthma or recurrent wheezing" by the age of 6 y using logistic regression models (*N* paired mother and child = 801)

	Unadjusted (interaction) model		Adjusted (interaction) model	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Vitamin D intervention**	0.08 (0.02, 0.42)	0.028	0.06 (0.01, 0.31)	0.0012
GA at the start of treatment	0.98 (0.91, 1.06)	0.65	0.98 (0.90, 1.06)	0.55
Baseline 25(OH)D <12 ng/mL	2.46 (1.21, 5.09)	0.014	1.63 (0.73, 3.08)	0.24
Baseline 25(OH)D 12–20 ng/mL	1.34 (0.76, 2.39)	0.32	1.10 (0.58, 2.08)	0.77
Baseline 25(OH)D 20–30 ng/mL	1.14 (0.66, 1.98)	0.65	1.12 (0.62, 1.78)	0.72
VD intervention*GA at the start of treatment**	1.15 (1.03, 1.27)	0.011	1.18 (1.05, 1.31)	0.0037
VD intervention*baseline 25(OH)D <12 ng/mL	0.48 (0.18, 1.28)	0.14	0.48 (0.17, 1.34)	0.16
VD intervention*baseline 25(OH)D 12–20 ng/mL	0.99 (0.44, 2.21)	0.97	0.83 (0.35, 1.95)	0.67
VD intervention*baseline 25(OH)D 20–30 ng/mL	0.72 (0.33, 1.55)	0.40	0.71 (0.31, 1.61)	0.42

Reference of maternal baseline 25(OH)D categories is a sufficient woman with 25(OH)D of >30 ng/mL. The references for the interaction terms are vitamin D–sufficient women randomly assigned to the vitamin D intervention group. Unadjusted analysis included only the covariates shown in the table. The adjusted analysis was adjusted for the potential confounders: maternal age, maternal education, maternal asthma, paternal asthma, study site, preterm birth, maternal race, sex of the child, gestational age at the enrollment, and season of blood drawn. ORs are for a 1-wk difference in GA at the start of treatment and for 1 ng/mL difference in 25(OH)D at baseline. **P-value less than 0.05.

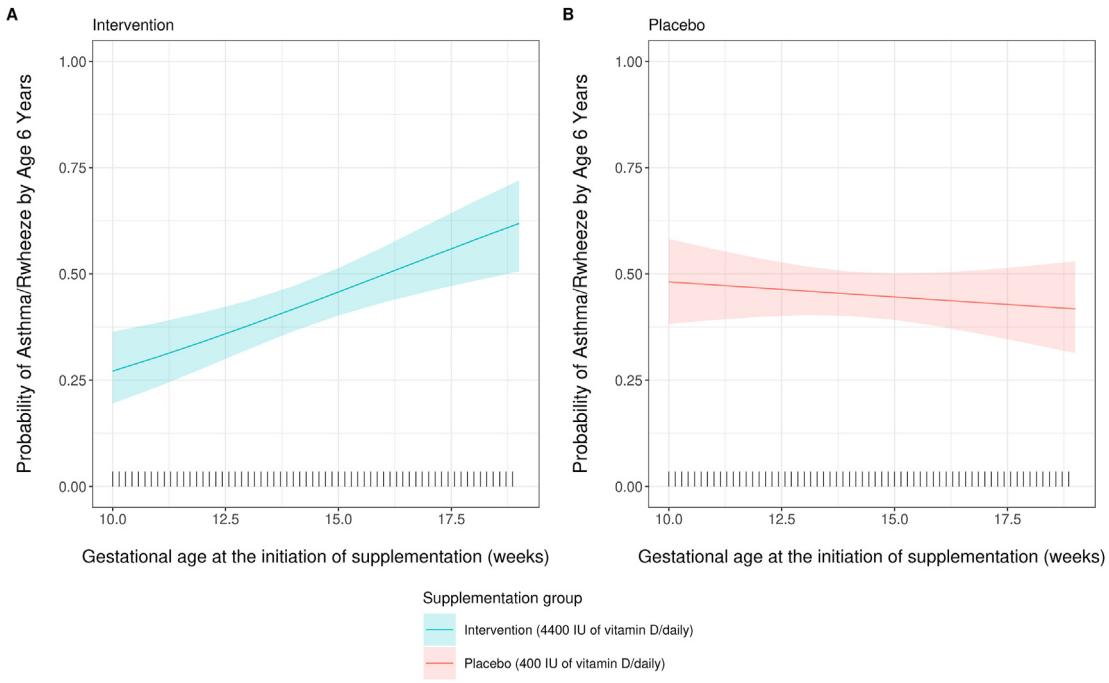

FIGURE 3. The effect of timing of supplementation initiation on the probability of offspring asthma or recurrent wheeze per supplementation group. The association between offspring's gestational age at the initiation of supplementation at trial enrollment and asthma or recurrent wheeze by age 6 y is demonstrated for the intervention arm (A) and placebo arm (B). Population means and 95% CIs are shown as respectively colored lines and shading. Inward ticks on the x-axis mark the data range and distribution. Vitamin D supplementation group*gestational age at the enrollment, adjusted for: maternal baseline 25(OH)D, maternal age, maternal education, maternal asthma, paternal asthma, study site, preterm birth, maternal race, sex of the child, and season of blood drawn.

TABLE 4
The association of gestational age at the initiation of supplementation with asthma or recurrent wheezing by the age of 6 y stratified by the supplementation arm and ordered by quartiles of GA

Supplementation group	GA at start treatment in wks (quartiles)	Adjusted OR (95% CI)
Placebo[1]	8.6–11.9 (1st)	1
	11.9–13.7 (2nd)	0.95 (0.53, 1.72)
	13.7–16.4 (3rd)	0.80 (0.44, 1.45)
	16.4–19 (4th)	0.90 (0.49, 1.63)
Intervention[2]	8.6–11.9 (1st)**	0.45 (0.24, 0.82)
	11.9–13.7 (2nd)	0.70 (0.38, 1.26)
	13.7–16.4 (3rd)	1.07 (0.59, 1.94)
	16.4–19 (4th)	1.21 (0.66, 2.22)

ORs for each category are shown compared with the reference (first quartile of gestational age at the initiation of supplementation in the placebo arm). The logistic regression model is adjusted for the potential confounders, including maternal age, maternal education, maternal asthma, paternal asthma, study site, preterm birth status, maternal race, child sex, and the season of blood drawn. **P-value less than 0.05.

[1] Trend test for odds of asthma or recurrent wheeze across quartiles of gestational age at enrollment in the placebo arm (first quartile as reference): Adjusted $P = 0.72$.

[2] Trend test for odds of asthma or recurrent wheeze across quartiles of gestational age at enrollment in the intervention arm (first quartile as reference): adjusted $P = 0.02$.

asthma or recurrent wheezing by the age of 6 y. We found that initiation of the 4400 IU/d vitamin D intervention at early- to midpregnancy lowered the odds of offspring asthma and recurrent wheezing by 15% for every week that the intervention was started earlier. More specifically, the initiation of 4400 IU of vitamin D between 9 and 12 wk compared with 400 IU of the vitamin was associated with the largest reduction of 55% in the odds of asthma or recurrent wheezing. Pregnant women with early to midpregnancy (baseline) vitamin D deficiency had the highest absolute and relative increase in 25(OH)D concentrations compared with those with insufficiency or sufficiency status. Our results imply an effect of 4400-IU vitamin D supplementation in the prevention of offspring asthma or recurrent wheeze among pregnant women who had severe vitamin D deficiency at trial entry. These findings indicate that early-pregnancy supplementation of vitamin D, as early as the 1st trimester, could potentially prevent asthma or recurrent wheezing in offspring at age 6 y and that the effect might be the most beneficial for severely deficient women during early pregnancy. Furthermore, the results from the sensitivity analysis of the 3-y outcome were in line with the findings of the main analysis of the 6-y outcome showing the effect of treatment on reducing the odds of the 3-y outcome among children whose mothers received treatment in the first trimester. The lack of statistical significance for the interaction between gestational age and the intervention arm at year 3 may be due to reduced statistical power (40% fewer cases) at this earlier age.

To date, 4 major RCTs have investigated prenatal supplementation on asthma or recurrent wheezing, including VDAART [11,12,14,18]. No significant effect of supplementation was found in these trials using an ITT analysis, except for a reduction in primary care visits for childhood asthma by Grant et al. [11] These trials applied different dosing regimens and gestational age starting times of the intervention during pregnancy that could have affected the efficacy of

supplementation. Except for VDAART and COPSAC sample sizes were small, and all trials enrolled pregnant women with vitamin D sufficiency in addition to those with insufficiency or deficiency. Notably, nutrient supplementation trials differ from drug trials because patients have a baseline level of the nutrient at trial entry which creates misclassification and reduces power unless accounted for in the design and/or analysis of the trial [23]. Furthermore, the high variability in biosynthesized nutrients like vitamin D because of many mediating factors (e.g., diet, season, latitude, outdoor activity, skin pigmentation, and gut microbiome) necessitate a large sample size to achieve adequate power across all groups with differing vitamin D status [24]. In particular, vitamin D requirements during pregnancy surge by 50%–100% in the second trimester and again 100% in the third trimester to maintain fetal calcium homeostasis and support bone development, which further increases the deficit that vitamin D–deficient women have to overcome and thus increases the need for very early-pregnancy correction in this group [25,26]. Our findings show that (severely) deficient women quantitively gained the most from vitamin D supplementation, with preliminary indications of qualitative gain. On the other hand, pregnant women with insufficiency or sufficiency at baseline were shown to have a lesser increase in their 25(OH)D concentrations by supplementation than those with deficiency, implying that they might need a relatively larger dose than what was administered in these trials to achieve high enough concentrations of 25(OH)D to prevent asthma in their offspring. Therefore, as suggested by this analysis, it is likely essential to account for vitamin D status in pregnant women at trial entry to maximize the treatment efficacy of supplementation throughout pregnancy; the fact that this was not prioritized in any of the prior RCTs might explain the null effect of vitamin D supplementation in these studies.

Of note, meta-analyses of vitamin D supplementation studies on different health outcomes in adults and nonpregnant women are also suggestive of the effectiveness of intervention specifically in those with deficiency or severe deficiency status, although in this instance, it should be emphasized that pregnancy presents a unique physiologic state [27]. On the contrary, the VITAL trial in older adults in United States showed no benefit of vitamin D supplementation on cancer or cardiovascular outcomes [28]. However, only 13% of VITAL subjects (with 89% compliance) had baseline 25(OH)D concentrations of <20 ng/mL and the intervention and placebo dosages were closer, i.e., 2000 IU/d and 800 IU/d, respectively. Another study that investigated the dose–response relationship of vitamin D with all-cause mortality in a European population demonstrated that 25(OH)D concentrations of <10 ng/mL are causal for mortality, implicating that, more probably, the severely deficient population could benefit the most from supplementation [29].

In a meta-analysis of VDAART and $COPSAC_{2010}$ by the age of 3 y, Wolsk et al. [30] showed that supplementation reduced the risk of asthma or recurrent wheezing in the offspring in the combined ITT populations (OR: 0.74; 95% CI: 0.57, 0.96) and that the effect was more prominent in women with higher baseline 25(OH)D (>30 ng/mL) at trial entry (aOR: 0.54; 95% CI: 0.33, 0.88). By the age of 6 y, we did not observe such an effect of supplementation among vitamin D–sufficient women at enrollment. This observation by 3 y and among offspring with maternal baseline sufficient status suggests that the observed protective effect in the 3-y analysis might have been driven by prenatal baseline vitamin D status rather than supplementation during the trial [4]. By the age of 6 y, the protective effect of maternal baseline vitamin D might wear off and the protective effect of supplementation during the trial becomes more prominent, especially among those pregnant women with baseline 25(OH)D deficiency. In a comparison of the offspring who developed asthma or recurrent wheeze with those who did not between the ages of 3 and 6 y, we also observed that a history of maternal asthma was more prevalent in the group who did develop asthma (χ^2, $P = 0.016$). The development of these diseases around years 5 or 6 is commonly observed in allergic diseases with a genetic predisposition [31]. Therefore, it is likely that not enough time had elapsed in the follow-up of Wolsk et al. [30] by 3 y of age to capture enough cases to demonstrate the effect of supplementation on offspring asthma or recurrent wheezing among vitamin D–deficient pregnant women at this time point. Finally, Wolsk et al. [30] did not consider the important effect of the timing of the intervention initiation on the asthma wheezing outcome at the age of 3 y.

The optimal timing of the vitamin D supplementation for pregnancy and continuation of supplementation after a child's birth are still matters of discussion. Our study showed a modification effect of prenatal gestational age on the vitamin D supplementation efficacy. Genetic research has shown that vitamin D pathways are associated with fetal lung programing during development (branching morphogenesis) in the pseudoglandular and canalicular stage occurring in the first trimester and continuing into the second trimester and that the genes associated with vitamin D and lung development are differentially expressed in children with asthma [32]. The early and lasting effects of vitamin D on lung development have been observed in longitudinal studies [33–35]. Vitamin D deficiency at 18 wks of gestation in pregnant women from the Western Australian Pregnancy Cohort (Raine) study was associated with impaired lung development in their offspring at the age of 6 y [33]. Considering the onset of vitamin D's influence on fetal lung development, initiation of the intervention at 10–18 wks of gestation in VDAART was likely not early enough for an optimal effect on lung development. Our results support this notion by demonstrating that the odds of asthma or recurrent wheezing were lower among children whose mothers received 4400 IU of vitamin D earlier during the pregnancy (Figure 3). This notion also concurs with our finding of a relatively strong association between maternal and offspring 25(OH)D concentrations in early childhood up until the age of 6 y, which showed that despite the correction of maternal 25(OH)D concentrations in maternal baseline groups with deficient status, paired offspring 25(OH)D concentrations remained low (Supplemental Figure 3).

Human lung development begins in the fifth fetal week and the alveolar phase continues throughout the first few years of life [36]. Therefore, sufficient vitamin D status as early as the embryonic stage (4–7 wks of gestation) might be necessary to maximize the effect of vitamin D on placenta development, fetal airway, and lung development and thus prevent asthma/recurrent wheezing [37,38]. In a variety of species, vitamin D is also critical to successful pregnancy and implantation of the fertilized egg, and vitamin D modulates the maternal immune system's response to the fetus throughout pregnancy [39], suggesting that the ideal time to initiate vitamin D supplementation is prior to pregnancy.

As the alveolar stage of lung development continues into the second year of life, it raises the question of whether supplementation should be continued after delivery. Prenatal compared with postnatal vitamin D deficiency in in vivo mouse models has shown that prenatal deficiency causes structural impairment of the lungs and that although postnatal supplementation improved lung function, it could not correct the

abnormal fetal development, suggesting that postnatal supplementation might be beneficial in neonates but should foremost be accompanied by prenatal correction to limit vitamin D deficiency–induced developmental lung abnormalities [40]. This is emphasized by our finding that deficiency in early- to midpregnancy impacted offspring 25(OH)D concentrations up until the age of 6 y. Throughout the offspring's early life in VDAART, we observed a positive correlation between maternal baseline and offspring's 25(OH)D concentrations (Supplemental Figure 4). A similar trend over time was observed in $COPSAC_{2010}$, as demonstrated by Brustad et al. [41]. This observation indicates that prenatal supplementation in deficient pregnant women might be necessary to be continued postnatally to prevent the risk of childhood asthma more efficiently. Grant et al. [11] continued prenatal vitamin D supplementation until 6 mo of life and observed a reduction in asthma visits to the physician, which hints at the synergistic potential of extending prenatal supplementation into early childhood.

The current recommended dose of vitamin D used as a supplement during pregnancy is 600 IU per day by the Institute of Medicine, with an upper limit of 4000 IU daily [20]. The Endocrine Society revised these recommendations specifically for pregnant women at the risk of vitamin D deficiency and advises a dosage of up to 2000 IU daily with an upper limit of 10,000 IU [21]. However, because most pregnant women have vitamin D insufficiency or deficiency despite ingesting prenatal vitamins that typically contain ~400 IU of vitamin D, one could argue that significantly higher doses are necessary [42]. Lee et al. [43] reported that of 40 pregnant women ingesting 600 IU vitamin D daily according to Institute of Medicine, 76% were deficient at birth along with 81% of their offspring. VDAART has shown that dosages up to 4400 IU daily can be safely administered to correct a deficient status, and that even at this dose, 25% of pregnant women failed to achieve a serum concentration of 30 ng/mL at the end of the trial [17]. We observed an inverse relationship between the maternal baseline 25(OH)D concentration and 25(OH)D increase in concentration in late pregnancy. Nevertheless, having higher baseline 25(OH)D concentrations at the trial entry had a stronger positive association with post-supplemental concentrations, implying that the initial baseline maternal 25(OH)D concentration had a greater impact on inducing sufficiency status in the offspring after vitamin D supplementation than the applied doses at the time of intervention. This emphasizes the need for supplementation at the earliest opportunity to correct vitamin D deficiency during pregnancy. Nevertheless, additional trials could help evidently determine the optimal dosage regimen and timing of supplementation.

VDAART has many strengths, including a relatively large population sample with high rates of asthma or recurrent wheezing, high daily vitamin D dosing at a relatively early time during the pregnancy (as early as first trimester), baseline and follow-up blood samples of pregnant women and offspring with high rates of follow-up and adherence over a long-time frame, and achieved mean 25(OH)D concentrations in the target range for most women. Secondary analysis of longitudinal cohorts has the potential to provide answers to the most pressing questions for future well-designed trials. Nevertheless, there are some study limitations to be considered. Subgroup analyses of these data may experience decreased statistical power to detect modification effects, particularly among subgroups with a smaller effect size. The subgroup analysis in this study was justified based on biologic and physiologic rationales and appropriate statistical modeling for main variables of interest that are in relationship with the primary trial outcome to minimize the multiplicity. Nevertheless, future confirmatory investigations are warranted. Finally, VDAART was a high-risk cohort that prioritized parents with a history of allergic diseases and recruited a large group of African Americans. African Americans are at a higher risk of developing asthma and had lower baseline 25(OH)D concentrations at trial entry [30,44]. Therefore, some caution is mandated in extrapolating the results presented here to the general population.

In conclusion, pregnant women with the lowest baseline 25(OH)D concentrations (severe deficiency) had the most significant improvement in their vitamin D levels due to the intervention of 4400 IU vitamin D. Our results implicate that these women might also benefit from daily vitamin D intervention of 4400 IU to reduce the odds of asthma or recurrent wheezing by the age of 6 y among their children. Additionally, earlier supplementation of vitamin D before the second trimester might improve the overall efficacy of the vitamin D intervention in the prevention of offspring asthma or recurrent wheezing. Hence, timely correction of vitamin D deficiency in early pregnancy should be considered. Further trials of vitamin D supplementation are warranted in pregnant women at a high risk of vitamin D deficiency to identify the optimal timing and dose of vitamin D supplementation for deficiency correction during pregnancy and its potential effect on offspring health outcomes.

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Author contributions

The authors' responsibilities were as follows – BC, HB, RSZ, GTO, LBB, AAL, STW, HM: designed the research; ILS, NB, ML, BC, HB, RSZ, GTO, LBB, AAL, STW, HM: conducted the research; ILS, NB, ML, HM: analyzed the data; ILS, HJG, STW, HM: wrote the article; HM: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Conflict of interest

RSZ reports grants from the NHLBI for the present work; other grants and personal fees from Merck & Co. MedImmune/AstraZeneca, Genentech/Novartis, and GSK; personal fees from Regeneron Pharmaceuticals, UpToDate, and DBV Technologies; and grants from ALK Pharma, Teva, and Quest. ML currently works at Vertex Pharmaceuticals. The remaining authors report no conflicts of interest.

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Data availability

The data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.04.019.

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