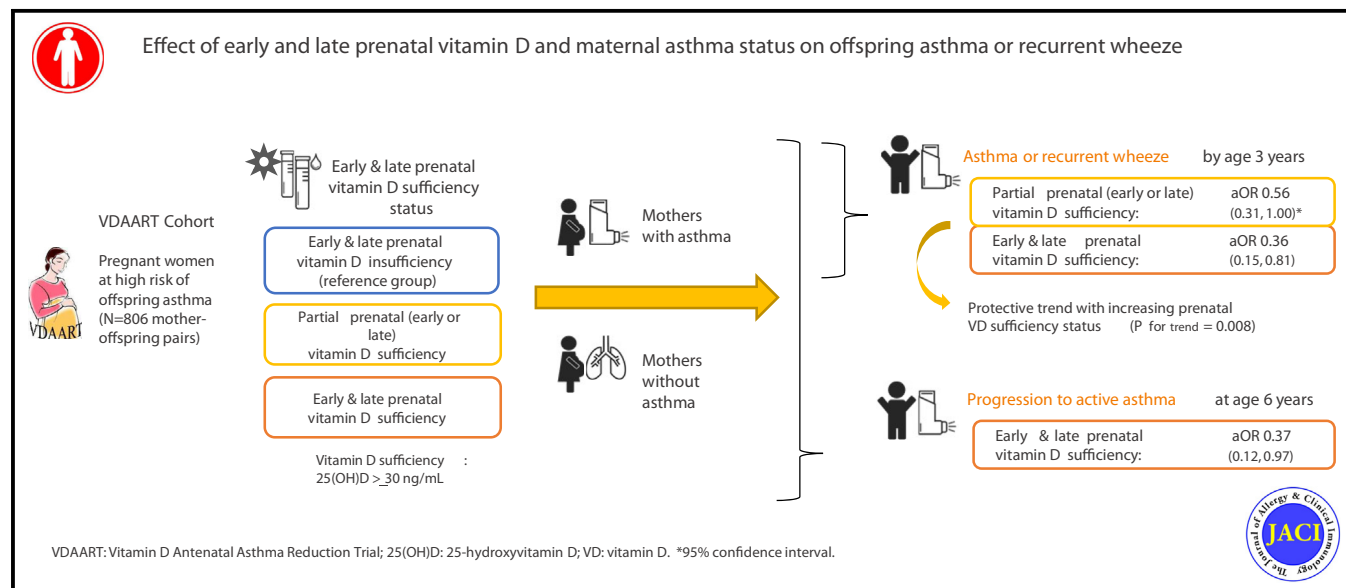


Effect of early and late prenatal vitamin D and maternal asthma status on offspring asthma or recurrent wheeze

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GRAPHICAL ABSTRACT



Background: Childhood asthma developmental programming is complex. Maternal asthma is a strong risk factor for childhood asthma, whereas vitamin D (VD) has emerged as a modifiable prenatal exposure.

Objective: Our aim was to examine the combined effect of early and late prenatal VD status in during pregnancies in women with and without asthma on childhood asthma or recurrent wheeze development.

Methods: We conducted a cohort study using prospectively collected data from the Vitamin D Antenatal Asthma Reduction Trial, a randomized, double-blinded, placebo-controlled VD supplementation trial in pregnant women at high risk of offspring asthma (N = 806 mother-offspring pairs). 25-Hydroxyvitamin-D (25(OH)D) level was measured in early and late pregnancy. Our main exposure was an ordered variable representing early and late prenatal VD sufficiency (25(OH)D level ≥ 30 ng/mL) status during pregnancy in women with and without asthma. The primary outcome was offspring with asthma or recurrent wheeze by age 3 years. We also examined the effect of prenatal VD level on early life asthma or recurrent wheeze progression to active asthma at age 6 years.

Results: Among mothers with asthma versus among mothers with early and late prenatal VD insufficiency, those with early or late VD sufficiency (adjusted odds ratio = 0.56; 95% CI = 0.31-1.00) or early and late VD sufficiency (adjusted odds ratio = 0.36; 95% CI = 0.15-0.81) had a lower risk of offspring with

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asthma or recurrent wheeze by age 3 years ($P_{\text{for trend}} = .008$). This protective trend was reiterated in asthma or recurrent wheeze progression to active asthma from age 3 to 6 years ($P_{\text{for trend}} = .04$).

Conclusion: This study implies a protective role for VD sufficiency throughout pregnancy, particularly in attenuating the risk conferred by maternal asthma on childhood asthma or recurrent wheeze development. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Pregnancy, maternal asthma, prenatal, vitamin D, childhood, recurrent wheeze, asthma

Childhood asthma is a complex, chronic disease of the airways with multiple associated risk factors that potentially interact with each other. It is the most common chronic disease of childhood, affecting approximately 6.2 million children (age <18 years) in the United States, with a prevalence of 8.3% of children in 2017; similar rates are reported globally.¹⁻⁴ The high economic cost of asthma was estimated at more than \$81 billion in 2013.⁵ The medical burden of asthma and wheezing illness in childhood may extend into adolescence and adulthood, with sequelae including lung function abnormalities.^{6,7} Therefore, preventive measures targeting modifiable risk factors in at-risk populations could reduce the global burden of asthma.

Maternal asthma is a well-established risk factor for childhood wheezing and asthma.⁸ Nevertheless, the developmental programming of childhood asthma is far more complex, encompassing prenatal and postnatal environmental factors. Among these factors, the role of vitamin D (VD) in childhood asthma is increasingly being highlighted as more is known about its pleiotropic effects on immunomodulation, lung development, and the microbiome.⁹⁻¹² VD is now known to exert effects throughout pregnancy and perhaps as early as the pseudoglandular stage (5-17 weeks of gestational age [wga]).¹⁰ Cord blood VD status is dependent on prenatal VD status, and its deficiency is associated with early and late childhood asthma and wheeze,¹³⁻¹⁵ suggesting a role for VD sufficiency from the early to late prenatal period and potentially extending throughout childhood. The Vitamin D Antenatal Asthma Reduction Trial (VDAART) was designed to investigate the effect of VD supplementation during pregnancy on the prevention of childhood asthma and wheeze in high-risk pregnancies for childhood asthma and atopy.¹⁶ The data from the VDAART cohort by the age of 3 years is consistent with prior investigations indicating that maternal asthma is a strong predictor of early childhood recurrent wheeze and asthma and that cord blood VD sufficiency could also have a role in the prevention of the early-life respiratory adverse outcomes.^{15,17} However, the combined effects of maternal asthma status and prenatal VD sufficiency status, beginning in early pregnancy, on childhood asthma and wheeze have not been assessed to date.

Here, we have examined the effect of VD status across multiple prenatal time points combined with the effect of maternal asthma status on the development of childhood asthma or recurrent wheeze by age 3 years. We hypothesized that children born to mothers with VD sufficiency during pregnancy have a lower risk of childhood asthma or recurrent wheeze than do children born to mothers with VD insufficiency during early and late pregnancy.

Abbreviations used

aOR:	Adjusted odds ratio
ITT:	Intention-to-treat
25(OH)D:	25-hydroxyvitamin D
VD:	Vitamin D
VDAART:	Vitamin D Antenatal Asthma Reduction Trial
wga:	Weeks of gestational age

METHODS

Study design and participants

The present study is a secondary analysis of the VDAART. The details of VDAART are previously described.^{16,18} In brief, VDAART is a randomized, double-blind, placebo-controlled trial of pregnant women randomized to intervention (4400 IU of VD daily) or placebo (a multivitamin containing 400 IU of VD daily). Maternal-offspring pairs with offspring follow-up data available for the primary trial outcome of asthma or recurrent wheeze by age 3 years were used as the source population for this analysis ($N = 806$ [intention-to-treat (ITT) population]) (Fig 1). The children continued to be followed for assessment of their asthma status at age 6 years. Eligible participants in the VDAART were nonsmoking, pregnant women who were aged 18 to 39 years at 10 to 18 wga, had a personal history of asthma or atopy or a partner (the biologic father of the child) with a history of asthma or atopy, and intended to participate for at least 4 years. All participants provided written consent to participate in the trial. The Methods section of this article's Online Repository materials (available at jacionline.org) provides further details on the eligibility and exclusion criteria of the VDAART participants. VDAART was approved by the institutional review boards of the 3 clinical sites and the data coordinating center (the Channing Division of Network Medicine at Brigham and Women's Hospital).

VD measurement

When discussing VD, we are referring to 25-hydroxyvitamin D (25(OH)D), which is the major circulating form of VD. VD levels in the prenatal period were collected at early pregnancy (10-18 wga) and late pregnancy (32-38 wga). Prenatal VD levels were measured per the VDAART protocol.¹⁶ Maternal prenatal 25(OH)D levels were obtained by using the DiaSorin LIAISON method, which is a validated chemiluminescence assay performed at the Channing Division of Network Medicine, Brigham and Women's Hospital, Boston.¹⁹ The assay is cospecific for 25(OH)D₃ and 25(OH)D₂. The interassay coefficients of variation for 25(OH)D₂ and 25(OH)D₃ 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.

Primary outcome: Offspring asthma or recurrent wheeze

The main outcome of this secondary analysis is the same as the primary outcome of the initial VDAART report, namely, the combined outcome of asthma or recurrent wheeze by age 3 years.¹⁶ Offspring asthma was defined as a parental report of physician-diagnosed asthma. Recurrent wheeze was defined as 1 of the following: (1) parental report of wheeze before and after the child's second birthday; (2) parental report of wheeze before child's second birthday and use of asthma medication (inhaled corticosteroids, leukotriene modifiers, oral steroids) after the second birthday; (3) 2 or more distinct parental reports of wheeze after the child's second birthday; (4) 2 or more distinct reports of asthma medications as already noted after the child's second birthday; and (5) 1 parental report of wheeze and an episode of asthma medication (as already noted) at separate time points after the child's second birthday.¹⁶

Children assessed for the primary outcome were followed up to determine their asthma status at age 6 years. Therefore, we additionally examined the outcome of asthma or recurrent wheeze by age 3 years persisting as active

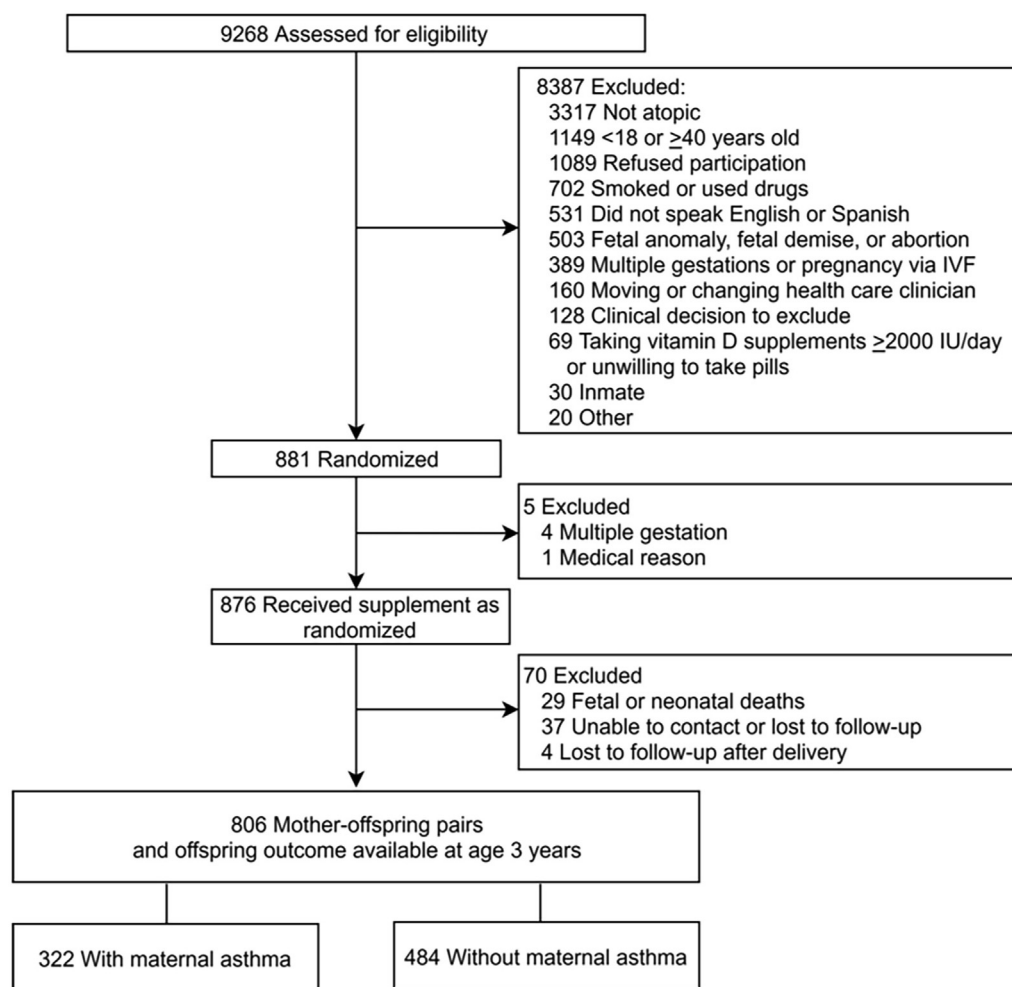


FIG 1. Consolidated Standards of Reporting Trials diagram.

asthma at age 6 years. Active asthma was defined per the VDAART 6-year follow-up report: physician-diagnosed asthma at any time during the first 6 years of life plus a report of the child's use of any asthma medication or report of wheeze after the fifth birthday.²⁰

Main study variables

Maternal asthma status and prenatal VD sufficiency status were the main exposures of interest. Questionnaires completed at enrollment and monthly maternal health questionnaires thereafter provided the information regarding the presence or absence of maternal asthma. A woman was considered to have asthma if she reported physician-diagnosed asthma at any time in her life. VD sufficiency was defined at a 25(OH)D threshold value of 30 ng/mL or higher based on the Endocrine Society's recommendations for maximum health benefits²¹ and prior observation of the relationship of pregnancy VD status and risk of childhood asthma and other adverse pregnancy outcomes^{17,22,23}; otherwise, subjects were considered VD insufficient.

Prenatal VD status (in the following order: early pregnancy at 10-18 wga, late pregnancy at 32-38 wga) was represented by using a composite ordered variable with 3 exposure levels: (1) early *and* late prenatal insufficiency, (2) partial prenatal sufficiency (sufficiency at either the early *or* late pregnancy time point), and (3) early *and* late prenatal sufficiency. To evaluate the effect of the early versus late prenatal time point in a sensitivity analysis, we additionally examined a composite 4-level ordered set of exposure levels: (1) early *and* late prenatal insufficiency, (2) early sufficiency *and* late prenatal

insufficiency, (3) early insufficiency *and* late prenatal sufficiency, and (4) early *and* late prenatal sufficiency.

Additional study variables

Additional study variables were selected as *a priori* determinants or potential confounders in the relationship of the outcome and main variables of interest: maternal age, maternal income, maternal atopy, paternal asthma, trial arm, study site, preterm birth (<37 wga: yes or no), child sex, and child race.

Statistical analysis

The main study groups were mothers with and without a personal history of asthma. For the comparison of baseline characteristics between maternal subjects with and without asthma, we used the Student *t* test and chi-square or Fisher exact test, as appropriate. The primary analysis was performed in 3 steps. First, we assessed the risk of offspring asthma or recurrent wheeze by the age of 3 years in high-risk pregnancies with asthma relative to low-risk pregnancies without asthma, regardless of the VD status during pregnancy. Second, we examined how the risk of offspring asthma or recurrent wheeze might differ across the ordered variable of VD status representing the prenatal sufficiency status (at early and late gestation) among pregnant women with and without asthma. Third, to examine whether the postulated protective effect of prenatal VD level extended into later childhood, we examined whether prenatal VD status influenced the risk of offspring asthma or recurrent wheeze by age 3 years progressing into active asthma at age 6 years.

The trend of risk estimates was assessed by using an extended Mantel-Haenszel chi-square test for linear trend with correction for continuity. We used multivariable logistic regression to obtain the adjusted odds of the outcome with inclusion of covariates and potential confounders, and the trend across ordered levels of exposure variable was reexamined. We tested for an interaction between prenatal VD status and maternal asthma. Crude odds ratios and adjusted odds ratios (aORs) were calculated with 95% CIs, and the corresponding *P* values were obtained. The Hosmer-Lemeshow test was used to assess logistic regression model goodness of fit for all models. All statistical analysis was performed by using the R statistical software, version 3.6.2 (the packages effects and performance). All tests were 2 sided, and a *P* value less than a prespecified alpha of .05 was considered statistically significant.

Sensitivity analysis

To assess the effect of early versus late VD sufficiency on the risk of the primary outcome of asthma or recurrent wheeze, we first examined a 4-level composite prenatal VD exposure as defined earlier, as well as the individual effects of baseline and third trimester VD level on a continuous scale. Next, to assess the robustness of our definition of offspring asthma, we examined the outcome of asthma and recurrent wheeze (offspring who had a physician diagnosis of asthma and also recurrent wheeze) by age 3 years.

RESULTS

Baseline characteristics and VD status by study time points

The VDAART ITT population included 806 mother-offspring pairs (Fig 1), of whom 322 mothers (322 of 806 [40.0%]) had a history of physician-diagnosed asthma. Maternal asthma status and offspring asthma or recurrent wheeze outcomes by age 3 years were available for all subjects. Baseline characteristics were balanced between subjects with and without maternal asthma except for younger maternal age at enrollment among mothers with asthma compared with maternal age of those without asthma (mean difference, 0.9 ± 5.5 years [Table II]). The 6-year follow-up data for active asthma status were available for 87.7% of the ITT population (707 of 806), of whom 83.5% (673 of 806) had early and late VD measures.

Overall, 766 subjects (766 of 806 [95.04%]) had VD measurements for both prenatal time points and were included in this analysis. The proportion of subjects with VD sufficiency was higher in the third trimester than at baseline (420 of 766 [54.8%] vs 166 of 766 [21.7%], respectively [$P < .001$]). There was no significant difference in VD sufficiency status between those with and without asthma at either of the prenatal time points.

Maternal asthma and risk of offspring asthma or recurrent wheeze

Of 766 offspring, 202 (26.4%) had physician-diagnosed asthma or recurrent wheeze before age 3 years. The proportion of offspring asthma or recurrent wheeze by age of 3 years was higher among children whose mothers did or did not have asthma (34.2% versus 21.2%, respectively [$P < .001$]). Accordingly, children whose mothers had asthma had almost 2 times higher odds of developing asthma or recurrent wheeze by age of 3 years than those whose mothers did not have asthma, even after adjustment for maternal age, maternal income, maternal atopy, paternal asthma, treatment arm, study site, preterm birth, child sex, and child race (Table II). The Hosmer-Lemeshow test for this and all subsequent models demonstrated adequate goodness of fit ($P \geq .05$).

TABLE I. Baseline characteristics in the VDAART ITT population by maternal asthma status (N = 806)

Characteristic	Maternal asthma (n = 322)	No maternal asthma (n = 484)	<i>P</i> value
Maternal age (y), mean (SD)	26.7 (5.4)	27.8 (5.5)	<.01
Maternal income, no. (%)			.06
Less than <\$50,000/y	151 (46.9)	190 (39.3)	
≥\$50,000/y	94 (29.2)	175 (36.2)	
Prefer not to answer or do not know	77 (23.9)	119 (24.6)	
Maternal education, no. (%)			.29
Less than college	144 (44.7)	197 (40.7)	
Some college, college graduate, or graduate school	178 (55.3)	287 (59.3)	
Treatment arm: intervention (VD dose of 4400 IU/d), no. (%)	171 (53.1)	234 (48.3)	.21
Site, no. (%)			.89
Boston, Mass	93 (28.9)	147 (30.4)	
San Diego, Calif	112 (34.8)	162 (33.5)	
St Louis, Mo	117 (36.3)	175 (36.2)	
Maternal atopy (yes), no. (%)	252 (78.3)	358 (74.0)	.19
VD sufficiency status*			
Baseline sufficiency at 10-18 wga, no. (%)	70 (21.9)	106 (22.0)	1.00
Missingness (no.)	2	3	
Third trimester sufficiency at 32-38 wga, no. (%)	175 (57.2)	247 (53.2)	.32
Missingness (n)	16	20	
Preterm delivery (<37 wga)	27 (8.4)	44 (9.1)	.81
Child sex (male), no. (%)	178 (55.3)	243 (50.2)	.18
Child race, no. (%)			.39
Black	165 (51.6)	225 (47.0)	
White	98 (30.6)	167 (34.9)	
Other	57 (17.8)	87 (18.2)	

*VD sufficiency defined as a 25(OH)D level of 30 ng/mL or higher. To convert 25(OH)D from ng/mL to nmol/mL, multiply by 2.496.

Effect of early and late pregnancy VD sufficiency status on offspring asthma or recurrent wheeze by maternal asthma status

In examining the effect of early and late prenatal VD status on offspring asthma or recurrent wheeze, a significant interaction was detected between prenatal VD and maternal asthma status ($P_{\text{interaction}} = .03$). Among pregnant women with asthma, a decreasing trend in risk of having a child with asthma or recurrent wheeze across the composite ordered variable representing prenatal VD status indicated a benefit from partial (early or late) prenatal VD sufficiency during pregnancy or maintenance of VD sufficiency in early and late pregnancy ($P_{\text{for trend}} < .001$ [Table II]). In the adjusted model, the trend across the ordered levels of VD exposure remained significant ($P_{\text{for trend}} = .008$), with the highest decrease (~65%) in risk of offspring asthma or recurrent wheeze for mothers with asthma who had early and late prenatal VD sufficiency compared with those with VD insufficiency at both early and late pregnancy (Table II).

In a sensitivity analysis of the effect of the early versus late prenatal time point, we utilized an expanded 4-level prenatal VD exposure. Among with women with asthma, compared with those with early and late VD insufficiency, women with early VD insufficiency but late sufficiency had a 50% reduced risk of

TABLE II. Risk of offspring asthma or recurrent wheeze by age 3 years according to maternal asthma status and prenatal VD status among pregnant women with and without asthma

Exposure status	Offspring asthma or recurrent wheeze			Univariable			Multivariable*		
	Yes (no.)	No (no.)	Total, no. (%)	OR (95% CI)	P value	P for trend	aOR (95% CI)	P value	P for trend
Maternal asthma status									
No maternal asthma	98	364	462 (21.2)	Ref	—	—	Ref	—	—
Maternal asthma	104	200	304 (34.2)	1.93 (1.40-2.68)	<.001	—	2.03 (1.43-2.87)	<.001	—
Total	202	564	766 (26.4)						
Prenatal VD status in mothers <i>without</i> asthma									
Early and late prenatal insufficiency† (insufficient and insufficient)‡	44	151	195 (22.6)	Ref	—	—	Ref	—	—
Partial prenatal sufficiency (sufficient or sufficient)	40	148	188 (21.3)	0.93 (0.57-1.51)	.76	.40	1.23 (0.69-2.17)	.48	.75§
Early and late prenatal sufficiency (sufficient and sufficient)	14	65	79 (17.7)	0.74 (0.37-1.41)	.38	—	1.05 (0.46-0.23)	.91	—
Total	98	364	462 (21.2)						
Prenatal VD status in mothers <i>with</i> asthma									
Early and late prenatal insufficiency (insufficient and insufficient)	54	66	120 (45)	Ref	—	—	Ref	—	—
Partial prenatal sufficiency (sufficient or sufficient)	39	89	128 (30.5)	0.54 (0.32-0.90)	.02	<.001	0.56 (0.31-1.00)	.05	.008
Early and late prenatal sufficiency (sufficient and sufficient)	11	45	56 (19.6)	0.30 (0.14-0.62)	.002	—	0.36 (0.15-0.81)	.02	—
Total	104	200	304 (34.2)						

OR, Odds ratio; Ref, reference.

*Adjusted for maternal age, maternal income, maternal atopy, paternal asthma, treatment arm, study site, preterm birth, child sex, and child race.

†VD sufficiency defined as a 25(OH)D level of 30 ng/mL or higher.

‡In the following order: baseline (10-18 wga), third trimester (32-38 wga).

§H-L goodness-of-fit test: $P \geq .05$; McFaddens $R^2 = 0.15$.||H-L goodness-of-fit test: $P \geq .05$; McFadden $R^2 = 0.18$.

offspring asthma or recurrent wheeze (aOR = 0.52; 95% CI = 0.28-0.94; $P = .03$); the greatest risk reduction (~65%) was observed among women with VD sufficiency in early and late pregnancy (aOR = 0.35; 95% CI = 0.15-0.81; $P = .02$). There was no significant effect among women with VD sufficiency only in early pregnancy or among women without asthma.

Among pregnant women without asthma, a trend in risk reduction of offspring asthma or recurrent wheeze was not observed across the ordered variable representing 3 levels of prenatal VD exposure status (P for trend = 0.75 [Table II]). Children born to mothers without asthma who had VD sufficiency at both early and late pregnancy did not show a lower risk of asthma or recurrent wheeze as compared with those born to pregnant women without asthma who were VD insufficient in both early and late pregnancy (Table II). There was no difference in the proportion of offspring asthma or recurrent wheeze in pregnant women with and without asthma who had VD sufficiency in both early and late pregnancy (11 of 56 [19.6%] versus 14 of 79 [17.7%] [$P = .95$]).

Effect of early and late pregnancy VD sufficiency status on offspring asthma and recurrent wheeze

Of 766 offspring, 114 (14.9%) had asthma by age 3 years, of whom 86 also had recurrent wheeze (86 of 766 [11.2%]). Considering offspring asthma and recurrent wheeze as the outcome, we observed a similar trend in risk reduction of the outcome with increasing prenatal VD exposure (P for trend = .01) among offspring of mothers with asthma, with the greatest risk reduction in those with early and late prenatal VD sufficiency (aOR = 0.19; 95% CI = 0.41-0.64 [see Table E1 in the Online Repository at jacionline.org]).

Furthermore, both baseline and third trimester VD levels separately demonstrated an inverse relationship with the risk of offspring asthma and recurrent wheeze among women with asthma (see Fig E1 in the Online Repository at jacionline.org). In the adjusted model, the effect of third trimester, but not baseline, VD level remained significant among women with asthma. Every 10-unit increase in third trimester VD level was associated with a 22% reduction in risk of offspring asthma and recurrent wheeze (aOR_{10-unit} = 0.78; 95% CI = 0.62-0.96).

Progression of early childhood asthma or recurrent wheeze to active asthma at age 6 years

Of the 766 offspring with year 3 outcomes and prenatal VD values available, 673 (87.9%) also had year 6 outcomes ascertained. Among the mother-offspring pairs with early and late prenatal insufficiency, 42 of 211 offspring (24.9%) had the outcome of early asthma or recurrent wheeze by age of 3 years and active asthma at age 6 years. In contrast, 6 of 93 offspring with early and late prenatal sufficiency (6.5%) had progression of asthma or recurrent wheeze to active asthma. In a model adjusted for the same covariates and additionally for maternal asthma status, a linear trend toward reduced risk of the outcome was observed across the ordered variable representing prenatal VD status, with the greatest reduction among those with sufficiency at both prenatal time points (P for trend = .04 [Table III]).

DISCUSSION

This study contributes to the existing literature, as it examines the effects of prenatal VD sufficiency status and maternal asthma status in pregnant women with and without asthma on childhood

TABLE III. Effect of prenatal VD sufficiency status on asthma or recurrent wheeze by age 3 years and active asthma at age 6 years

Prenatal VD status in mothers	Offspring asthma or recurrent wheeze by age 3 years and active asthma by age 6 years			Univariable			Multivariable*		
	Yes	No†	Total, no. (%)	OR (95% CI)	P value	P for trend	aOR (95% CI)	P value	P for trend
Early and late prenatal insufficiency‡ (insufficient and insufficient)§	42	169	211 (24.9)	Ref	—		Ref	—	
Partial prenatal sufficiency (sufficient or sufficient)	32	193	225 (14.2)	0.67 (0.40-1.10)	.12	.003	0.68 (0.37-1.25)	.26	.04
Early and late prenatal sufficiency (sufficient and sufficient)	6	87	93 (6.5)	0.28 (0.10-0.63)	.005		0.37 (0.12-0.97)	.05	
Total	80	449	529 (15.1)¶						

OR, Odds ratio; Ref, reference.

*Adjusted for maternal asthma, maternal age, maternal income, maternal atopy, paternal asthma, maternal asthma, treatment arm, study site, preterm birth, child sex, and child race.

†VD sufficiency defined as a 25(OH)D level of 30 ng/mL or higher.

‡In the following order: baseline (10-18 wga), third trimester (32-38 wga).

§Defined as offspring without asthma or recurrent wheeze by age 3 years and without active asthma at age 6 years.

||Hosmer-Lemeshow goodness-of-fit test: $P \geq .05$; McFadden $R^2 = 0.26$.

¶A total of 529 of 673 subjects with prenatal VD and year 3 and year 6 outcome met the definition for the selective phenotype,§ thus excluding 144 offspring with only asthma or recurrent wheeze by age 3 years or active asthma at age 6 years.

asthma or recurrent wheeze. Mothers with asthma had a 2 times higher risk of having a child with asthma or recurrent wheeze before the age of 3 years than did mothers without asthma. However, this risk among mothers with asthma was substantially attenuated if they were VD sufficient (VD level ≥ 30 ng/mL) at early and late pregnancy. Therefore, women with asthma who start their pregnancies with high levels of VD and remain VD sufficient throughout pregnancy are likely to experience a reduced risk of asthma or recurrent wheeze in their children before age 3 years. Yet, on the basis of our sensitivity analysis with a 4-level prenatal VD exposure to evaluate the weight of the early versus late time point, pregnant women with asthma and VD insufficiency at their early pregnancy might also benefit from improvement of their VD status during pregnancy.

The optimal dosage of VD supplementation and the definition of VD sufficiency have generated much debate. At present, there is no consensus as to what constitutes VD sufficiency for non-bone-related outcomes. Indeed, sufficient levels may vary depending on the health outcome of interest, with VD exerting differential effects on skeletal and extraskeletal health, and between the pregnant versus nonpregnant state.²³ Our investigation of prenatal VD sufficiency at a threshold VD level of 30 ng/mL demonstrated the greatest reduction of risk of offspring asthma or recurrent wheeze in mothers with asthma, although there was a role for partial prenatal VD sufficiency. Considering the pleiotropic effects of VD, it is also possible that there may be distinct or interacting mechanisms by which it influences fetal lung programming depending on baseline VD levels and in a dose-response fashion, as we have observed. Our study suggests that a threshold VD sufficiency of 30 ng/mL in early pregnancy appears to be a minimum threshold with regard to the risk of early childhood asthma or recurrent wheeze development. Given the observed dose-response effects of VD at early and particularly late pregnancy, higher levels may be more protective in pregnancies with a high risk for early childhood asthma. Nevertheless, this observation is a matter for further investigation of effectiveness and safety.

A regulatory role for VD has been implicated in as early as the second week of gestation in murine experiments examining fetal lung development and as early as in the pseudoglandular stage of

lung development (5-17 weeks) in human fetal lung programming, potentially affecting developing lung transcriptomes more so among those with asthma.^{10,24} What has been unclear, however, is the temporal contribution of VD to lung development in early versus late pregnancy. Collectively with our results, these findings highlight a role for ensuring early prenatal VD sufficiency status and, additionally, the maintenance of sufficient VD levels through pregnancy in providing the greatest reduction of childhood adverse respiratory outcome development.

The literature supports a role for VD supplementation, most probably among those with prenatal VD deficiency, which is common in pregnancy.^{25,26} It should be also noted that observational studies following 25(OH)D levels across pregnancy have shown that 25(OH)D levels either remain unchanged or decrease over time.^{27,28} A randomized controlled trial of VD supplementation in pregnant women found considerable VD deficiency at the time of delivery as well, with median serum 25(OH)D levels of 24 nmol/L (10 ng/mL), which is consistent with the deficiency in the control arm, versus 65 nmol/L (26 ng/mL) in the intervention arm, which was supplemented with VD beginning at 20 weeks of gestation with dosing based on the serum 25(OH)D levels at the time of study entry.²⁵

Notably, in our study, the protective effect of VD sufficiency in pregnancy on offspring outcomes by age 3 years was seen only among mothers with asthma. This observation implicates a distinct role for VD sufficiency throughout pregnancy and maternal asthma beyond the heritability due to maternal asthma status alone, which has not previously been reported. Specifically, variation in genetic pathways of immune response and lung development may modulate the effect of VD sufficiency in pregnancy. However, in a sensitivity analysis and with consideration of the outcome of asthma or recurrent wheeze by age 3 years and active asthma at age 6 years, we observed a linear trend in prenatal VD status effect independent of maternal asthma status.

VD has been associated with reductions in asthma exacerbations and respiratory infections.²⁹⁻³¹ This effect may be of particular importance in pregnant women with asthma, as impairment in asthma control and an increase in asthma severity during pregnancy are associated with perinatal morbidities, including preterm labor, preeclampsia, and low birth weight.^{32,33} These

observations, along with our findings, warrant further studies to investigate the underlying mechanisms of our observed effect of VD in risk reduction of offspring asthma or recurrent wheeze, particularly among children born to mothers who had asthma.

Suggested roles for prenatal VD supplementation include an effect on transient early childhood wheeze through the reduction of viral lower respiratory tract infections rather than a reduction in childhood asthma.³⁴ Indeed, the diagnosis of asthma in preschool children is difficult and often made in retrospect, making it difficult to predict which children will develop later childhood asthma on the basis of early-life wheezing. The recently published results of the VDAART 6-year ITT follow-up indicate that although there was no significant effect of VD supplementation on the development of school-age asthma or recurrent wheeze by age 6 years, ITT analysis revealed an effect in airway resistance measures on impulse oscillometry in offspring of mothers receiving high-dose VD supplementation. Here, we report a trend toward a reduced risk of early-life asthma or recurrent wheeze progressing into school-age active asthma, with improvement and maintenance of VD sufficiency during pregnancy. This observation suggests that prenatal VD supplementation may yet have an effect on childhood asthma outcomes, at least among those with low VD status. Measures such as level-based assessment of VD effect, early VD supplementation, and assessment in populations at highest risk of offspring asthma may help further define the role of VD in supplementation trials for prevention of childhood asthma.

There were several strengths and limitations to this study. This analysis was performed by using data collected from a clinical trial of women and infants who were closely followed longitudinally. All asthma diagnoses are based on the reported physician diagnosis of asthma, which is used most commonly. We further increased the precision of this diagnosis by examining the outcome of asthma and recurrent wheeze. Notably, by following the effect of prenatal VD out to age 6 years, we observed a trend in risk reduction of early-life asthma or recurrent wheeze to later childhood asthma influenced by early and late VD sufficiency on the progression of this phenotype. This implies a role for prenatal VD in prevention of childhood asthma and wheezing phenotypes beyond transient wheeze. VD exposure was measured directly instead of indirectly estimated by using surrogate measures such as dietary intake, and the measurements were collected at multiple time points. We did not observe the same pattern of risk reduction among women without asthma, potentially because a larger sample size might be required to capture a prenatal effect of VD exposure, if any, throughout pregnancy. This is a limitation, particularly given the lower baseline risk of offspring asthma among pregnant women without asthma than among those with maternal asthma, which is the strongest risk factor for childhood asthma. It is noted that the VDAART was designed for evaluating prenatal VD supplementation and its effect on the maternal VD levels and trial outcome of asthma or recurrent wheeze by age of 3 years. Therefore, we considered the prenatal VD status in early and late pregnancy as the main exposure for this study. The question of how prenatal and postnatal VD supplementation, as well as induced status in mother and child, affect childhood asthma is a matter for further investigation. We also acknowledge that maternal asthma status did not differentiate between current or prior asthma, although our estimates of the effect of maternal asthma status on offspring asthma or recurrent wheeze are in line with what has been reported in the literature.^{8,35} Finally, some studies suggest a weaker effect of paternal asthma on the risk of

childhood asthma compared with the effect of maternal asthma.³⁵ Although we did examine the effect of paternal asthma on our results, the results for maternal asthma and VD did not change as a function of paternal asthma, as was also previously shown in the VDAART.¹⁰

In conclusion, this study indicates a protective effect of prenatal VD sufficiency throughout pregnancy on the development of childhood asthma or recurrent wheeze by age 3 years, particularly in attenuating the increased baseline risk conferred by maternal asthma. Further studies may shed light on the underlying mechanisms, as this effect appears to interact with the genetic contribution from maternal asthma risk. Furthermore, the protective trend of early and late prenatal VD sufficiency appears to extend from the progression of early-life asthma or recurrent wheeze to asthma at age 6 years. These investigations have implications for identifying populations of pregnant women at greater risk for offspring asthma and recurrent wheeze outcomes owing to combined VD insufficiency and a history of maternal asthma.

We thank the participants of the VDAART for their contributions and support.

Clinical implications: Early identification and correction of vitamin D insufficiency in pregnant women with asthma have implications for the prevention of early offspring asthma or recurrent wheeze and the persistence of offspring asthma to later childhood.

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ELIGIBILITY CRITERIA

The subjects included in this analysis are from a subset of enrolled pregnant women in the VDAART. Accordingly, the inclusion and exclusion criteria were consistent with the trial protocol.

Inclusion criteria

The inclusion criteria were as follows: the pregnant woman or her partner (the biologic father or the child) had a history of asthma, eczema, or allergic rhinitis (the pregnant women were considered to have asthma or allergy if they provided a positive answer to the direct question regarding a history of these diseases on the enrollment questionnaire or on any monthly subsequent questionnaire); the gestational age of the fetus was between 10 and 18 weeks at the time of randomization; the pregnant woman was between 18 and 39 years of age; she was not a current smoker (defined as not having smoked for at least 1 month before enrollment) and not a user of other nicotine products (eg, nicotine patch) for at least 1 month before enrollment; she spoke English or Spanish; and she intended to participate in the trial through her pregnancy.

Exclusion criteria

The exclusion criteria were as follows: the gestational age of the fetus was more 18 weeks; the pregnant woman had any of 6 chronic medical conditions (hypertension requiring medications, diabetes mellitus, parathyroid disease, uncontrolled thyroid disease, kidney stones, and sarcoidosis); she was taking VD supplements containing more than 2,000 IU of vitamin D₃ per day; she had a multiple-gestation pregnancy; her pregnancy was achieved by assisted reproduction techniques (eg, intrauterine insemination, *in vitro* fertilization); she was currently using illicit drugs (defined as any use in the 6 months before enrollment); she was previously enrolled in the VDAART for a prior pregnancy; a major fetal anomaly was detected before delivery; the woman had a score of 15 or higher on the depression scale of the Patient Health Questionnaire-9; and she had any condition that, in the opinion of the clinical center principal investigator, would inhibit compliance with the study medications or prohibit long-term participation in the trial.

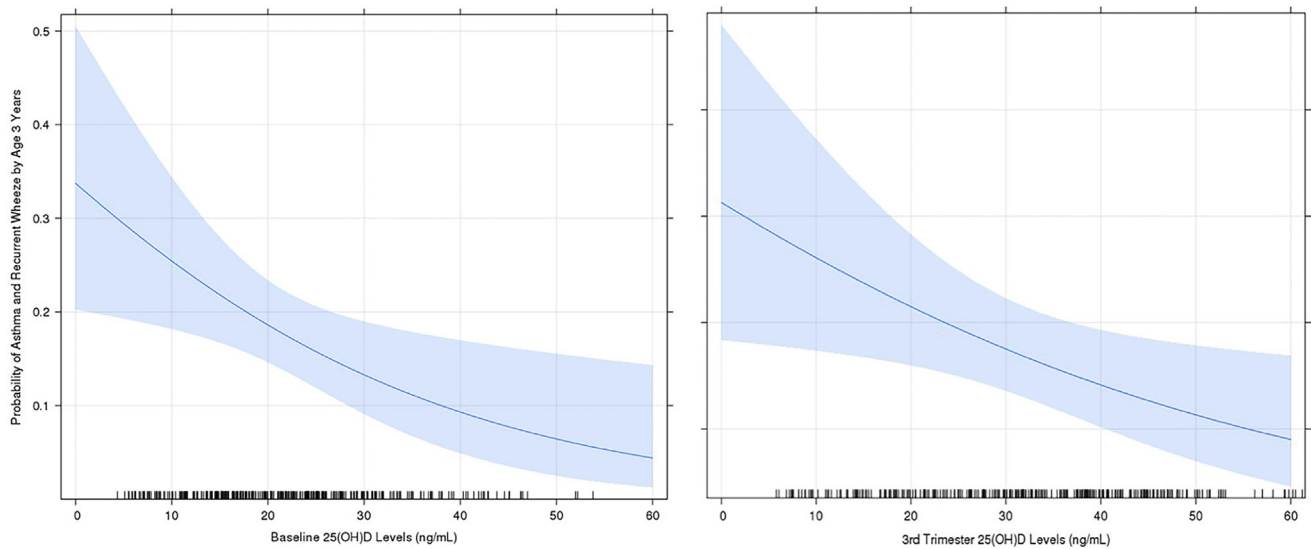


FIG E1. Dose-response relationships between baseline (10-18 weeks) and third trimester (32-38 weeks) 25(OH)D levels among women with asthma and the risk of their offspring developing asthma or recurrent wheeze by age 3 years. The shaded areas displayed indicate the 95% CIs around the regression line. All 25(OH)D values are in ng/mL.

TABLE EI. Risk of offspring asthma *and* recurrent wheeze by age 3 years according to maternal asthma status and prenatal VD status among women with and without asthma

Prenatal VD status	Offspring asthma and recurrent wheeze			Univariable			Multivariable*		
	Yes	No	Total	OR (95% CI)	P	P for	aOR (95% CI)	P	P for
					value	trend		value	trend
In mothers without asthma									
Early and late prenatal insufficiency [†] (insufficient and insufficient) [‡]	18	177	195	Ref	—		Ref	—	
Partial prenatal sufficiency (sufficient or sufficient)	14	174	188	0.79 (0.38-1.64)	.53	.14	0.86 (0.36-2.00)	.73	.33
Early and late prenatal sufficiency (sufficient and sufficient)	3	76	79	0.39 (0.09-1.19)	.14		0.48 (0.10-1.72)	.30	
Total	35	427	462						
In mothers with asthma									
Early and late prenatal insufficiency (insufficient and insufficient)	28	92	120	Ref	—		Ref	—	
Partial prenatal sufficiency (sufficient or sufficient)	19	109	128	0.57 (0.30-1.09)	.09	.006	0.61 (0.30-1.25)	.18	.01
Early and late prenatal sufficiency (sufficient and sufficient)	4	52	56	0.25 (0.07-0.69)	.01		0.19 (0.41-0.64)	.02	
Total	51	253	304						

OR, Odds ratio; Ref, reference.

*Adjusted for maternal age, maternal income, maternal atopy, paternal asthma, treatment arm, study site, preterm birth, child sex, and child race.

[†]VD sufficiency defined as a 25(OH)D level of 30 ng/mL or higher.[‡]In the following order: baseline (10-18 wga), third trimester (32-38 wga).