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

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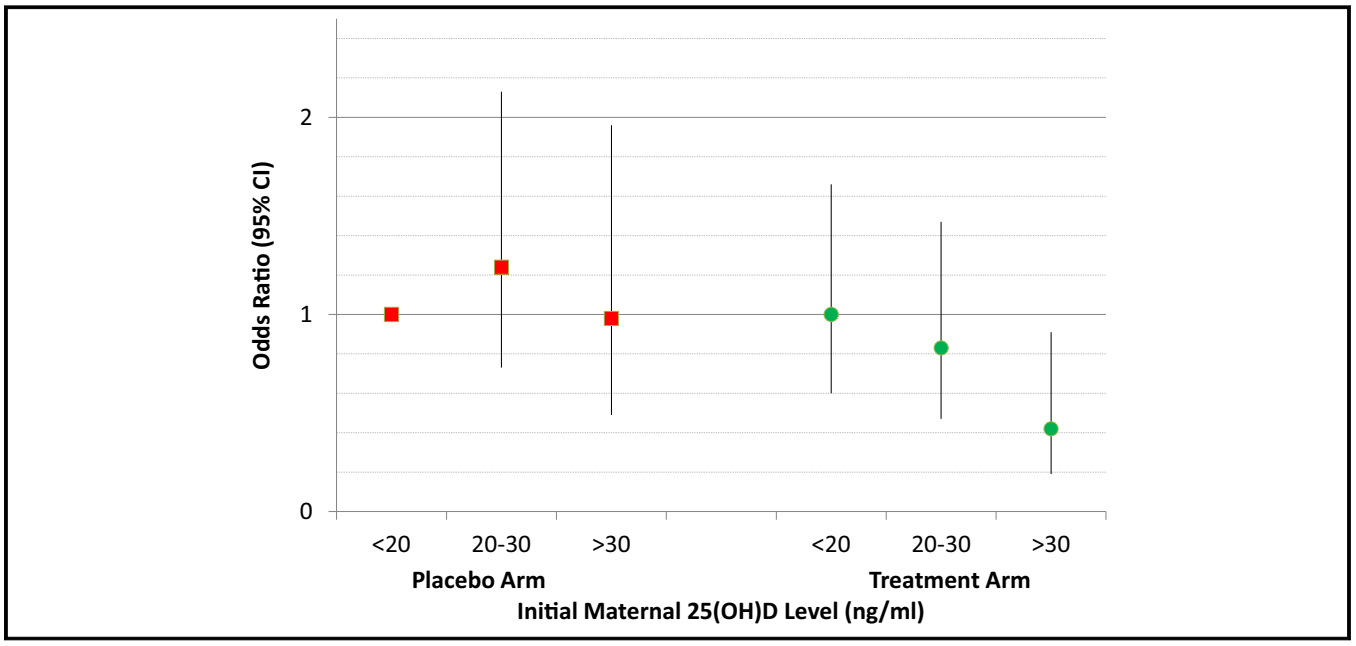
GRAPHICAL ABSTRACT



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Background: Nutrient trials differ from drug trials because participants have varying circulating levels at entry into the trial.
Objective: We sought to study the effect of a vitamin D intervention in pregnancy between subjects of different races and the association between 25-hydroxyvitamin D_3 (25[OH]D) levels in pregnancy and the risk of asthma/recurrent wheeze in offspring.
Methods: The Vitamin D Antenatal Asthma Reduction Trial is a randomized trial of pregnant women at risk of having children with asthma randomized to 4400 international units/d vitamin D or placebo plus 400 international units/d vitamin D. Asthma and recurrent wheezing until age 3 years were recorded.
Results: African American (AA) women (n = 312) had lower initial levels of 25(OH)D (mean [SD], 17.6 ng/mL [8.3 ng/mL]) compared with non-AA women (n = 400; 27.1 ng/mL [9.7 ng/mL], $P < .001$). No racial difference was found from vitamin D supplementation in pregnancy on asthma/recurrent wheezing in offspring (P for interaction = .77). Having an initial level of greater than 30 ng/mL and being randomized to the intervention group was associated with the lowest risk for asthma/recurrent wheeze by age 3 years compared with having an initial level of less than 20 ng/mL and receiving placebo (adjusted odds ratio, 0.42; 95% CI, 0.19-0.91).
Conclusions: We did not find differences between AA and non-AA mothers in the effect of maternal vitamin D supplementation and asthma/recurrent wheeze in offspring at 3 years. Maternal supplementation of vitamin D, particularly in mothers with initial 25(OH)D levels of greater than 30 ng/mL, reduced asthma/recurrent wheeze in the offspring through age 3 years, suggesting that higher vitamin D status beginning in early pregnancy is necessary for asthma/recurrent wheeze prevention in early life. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Vitamin D, asthma, allergy, randomized controlled trial, prenatal*

Asthma, wheeze, or both are common childhood conditions and impose a great cost on society.[1] Many potential risk factors have been identified for asthma development,[2-4] and several preventive trials have been undertaken,[5-7] but no single intervention has been proved effective. The Vitamin D Antenatal Asthma Reduction Trial (VDAART) is a randomized, double-blind, placebo-controlled trial of 881 pregnant women at risk of having children with asthma randomized to 4000 international units (IU)/d vitamin D plus a prenatal vitamin containing 400 IU of vitamin D or placebo plus 400 IU/d vitamin D. Recently, we reported that maternal vitamin D supplementation with 4400 IU/d in this trial led to an estimated 20% reduction in the incidence of asthma/recurrent wheeze in offspring through age 3 years compared with the control group (hazard ratio, 0.8; 95% CI, 0.6-1.0).[8] The effect did not reach statistical significance ($P = .051$), and several issues might have affected the results. It has been argued that nutrient trials are inherently different from drug trials for several reasons, including that (1) the participants have varying baseline nutrient status before entry into the trial, (2) the intervention needs to be sufficient to produce a change in nutrient status, (3) and the response to the change in nutrient status will depend on initial status on entry into the trial.[9]

Abbreviations used
25(OH)D: 25-hydroxyvitamin D_3
AA: African American
IU: International units
OR: Odds ratio
VDAART: Vitamin D Antenatal Asthma Reduction Trial

VDAART did not select participants based on initial 25-hydroxyvitamin D_3 (25[OH]D) levels, and results might have been affected by variation in these levels.[9] African American (AA) subjects have lower 25(OH)D levels than non-AA subjects.[10,11] Furthermore, AA subjects have a higher incidence of asthma in children compared with subjects of other races,[12] but whether this is explained by the 25(OH)D level is unknown.

We undertook secondary analyses of VDAART data to investigate whether treatment had differential effects by race either on maternal levels of 25(OH)D or on asthma/recurrent wheeze in the offspring and whether the initial and achieved prenatal levels of 25(OH)D were associated with the outcome of asthma/recurrent wheeze in the offspring. We first hypothesized that vitamin D supplementation would elicit a significant increase in 25(OH)D levels in all women, regardless of race/ethnicity. We next hypothesized that the initial and achieved 25(OH)D levels in pregnancy in this vitamin D supplementation trial are associated with a reduction in asthma or recurrent wheeze in offspring.

METHODS

Participants

Pregnant women were recruited from 3 clinical sites across the United States: Boston Medical Center, Boston, Massachusetts; Washington University at St Louis, St Louis, Missouri; and Kaiser Permanente Southern California Region, San Diego, California, as previously described.[13] The Data Coordinating Center was based in the Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, Massachusetts. Eligible participants were pregnant nonsmoking women between the ages of 18 and 39 years, who presented between the estimated gestational ages of 10 and 18 weeks and who had or who conceived the child with a man who had a history of asthma, eczema, or allergic rhinitis. The VDAART protocol was approved by the institutional review boards at each participating institution and at the Brigham and Women's Hospital. All women provided written informed consent.

For this study, we included women with full information about 25(OH)D levels at entry into the trial and at the third trimester. Furthermore, offspring with missing data on asthma/recurrent wheeze through age 3 years were excluded from the analysis, leaving us with a total of 712 participants (Fig 1).

Study design

Details of the study design and the protocol have previously been published.[8,13] We conducted a randomized, double-blind, placebo-controlled study of vitamin D_3 (4000 IU/d vitamin D_3 plus a multivitamin with 400 IU of vitamin D_3) versus placebo (daily placebo pill plus a multivitamin with 400 IU of vitamin D_3). Content of prenatal interval visits and postnatal visits have previously been detailed.[13]

The primary outcome was parental report of a physician's diagnosis of asthma or occurrence of recurrent wheeze in the child's first 3 years of life. Recurrent wheeze was defined by the occurrence of at least 1 of the following 5 conditions: (1) parental report of wheeze after the child's second birthday preceded by at least 1 report of wheeze before the second birthday; (2) report

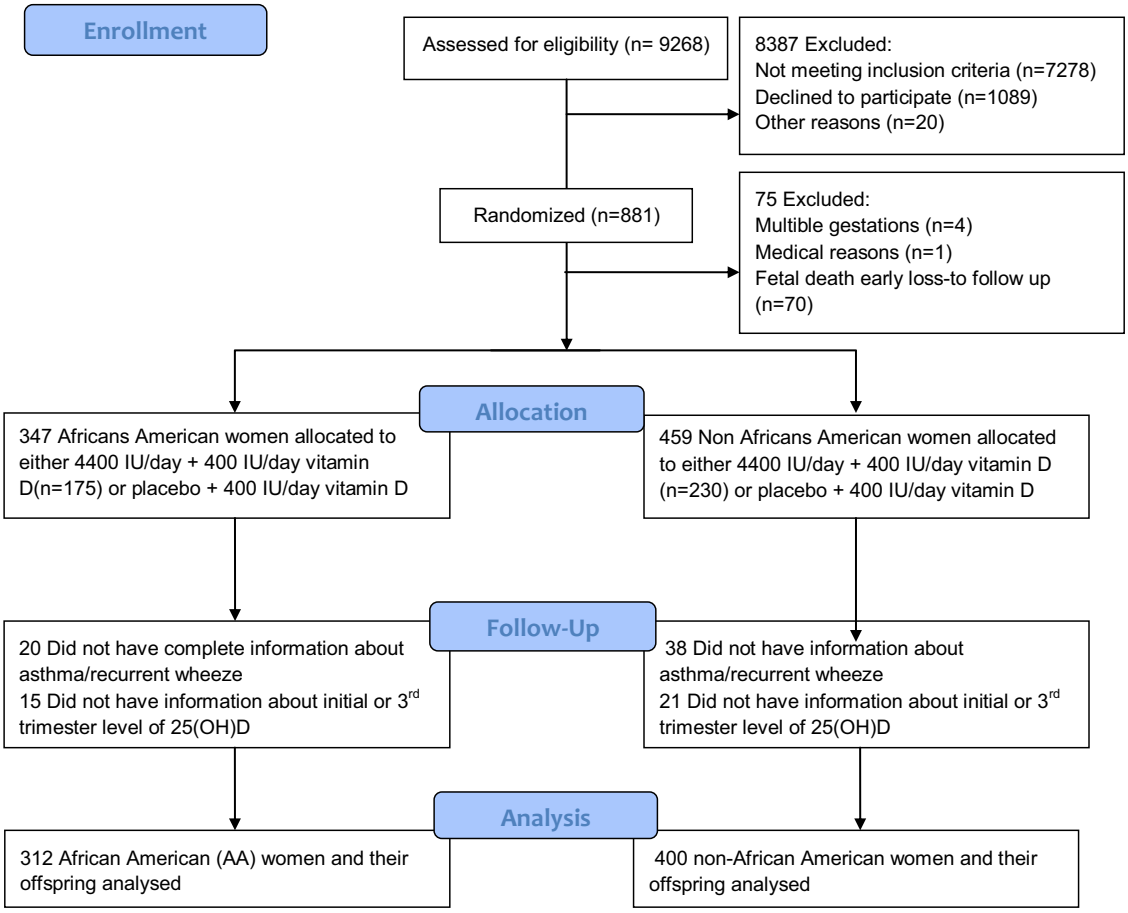

FIG 1. CONSORT 2010 flow diagram.

of child's use of asthma controller medication after the second birthday preceded by a report of wheeze before the second birthday; (3) 2 or more distinct parental reports of wheeze after the second birthday; (4) at least 1 parental report of wheeze and use of asthma controller medications at distinct visits, both subsequent to the second birthday; and (5) 2 distinct reports of use of asthma controller medications after the second birthday. These outcomes were the same as in the initial trial report.

Maternal race was determined by self-reported selection from the categories white (Hispanic/non-Hispanic), black or AA, Asian, Native Hawaiian, or other Pacific Islander, American Indian/Alaskan Native, or other. The categories Asian, Native Hawaiian or Other Pacific Islander, American Indian/Alaskan Native, or other were collapsed into "other."

Blood was drawn at entry into the trial and at the third trimester visit for measurement of circulating levels of 25(OH)D. The 25(OH)D level at trial entry was not an inclusion or exclusion criterion. Circulating levels of 25(OH)D from maternal plasma samples were determined by using the DiaSorin Liaison chemiluminescence immunoassay (DiaSorin, Saluggia, Italy).[14] 25(OH)D levels are reported in nanograms per milliliter (1 ng/mL = 2.496 nmol/L).

Adherence was measured by using Medication Events Monitoring Systems (MEMS) caps (Aardex Ltd, Zug, Switzerland), an electronic cap that records each time a pill bottle is opened, as previously described.[13]

Statistical analysis

25(OH)D levels were measured at entry into the study (weeks 10-18 of gestation) and at weeks 32 to 38 of gestation, and the absolute increase in 25(OH)D levels was calculated. The average 25(OH)D levels between the initial level and the third-trimester level were calculated. Group comparisons of 25(OH)D levels were performed by using the Student *t* test.

Logistic regression models were computed by using the maternal 25(OH)D level/treatment group as the predictor and asthma/recurrent wheeze in children as the outcome. Odds ratios (ORs) were calculated based on a 5 ng/mL difference in the maternal 25(OH)D level, as well as on $\log_2$-transformed 25(OH)D levels, holding all other variables fixed.

Furthermore, women were cross-classified by using clinically interpretable categories of initial 25(OH)D level and treatment assignment; we performed 1 full logistic regression model using an initial level of less than 20 ng/mL and randomized to placebo as the reference. We also used the level of 30 ng/mL to categorize the women into 3 groups: (1) "<30/<30," consisting of women with initial 25(OH)D levels of less than 30 ng/mL and a third-trimester level of less than 30 ng/mL; (2) "<30/>30 or >30/<30," consisting of women with an initial 25(OH)D level of less than 30 ng/mL and a third-trimester level of greater than 30 ng/mL or an initial 25(OH)D level of greater than 30 ng/ml and third-trimester trimester level of less than 30 ng/mL; and (3) ">30/>30," consisting of women with an initial 25(OH)D level of greater than 30 ng/mL and a third-trimester level of greater than 30 ng/mL. Trend tests were performed, with higher 25 (OH)D levels considered as ranking higher.

Analyses are shown as unadjusted analyses and adjusted for potential confounders. All adjusted analyses included maternal educational level (college: yes/no), treatment group (vitamin D/placebo), adherence (adherence >80%, as measured by using MEMS cap: yes/no), center (Boston Medical Center/Kaiser Permanente/Washington University), prepregnancy body mass index (>25 kg/m^2: yes/no), and maternal age (>27 years: yes/no). The Akaike information criterion was calculated for the model, showing a better fit without race included as a covariate. This was supported by a likelihood ratio test showing a *P* value of .27 between 2 models with/without race included as a covariate. We also conducted analyses stratified for the race of the mother. Race-adjusted models were also performed.

TABLE I. Baseline characteristics of AA and non-AA women

Baseline characteristics	AA women (n = 312)	Non-AA women (n = 400)	*P* value
Active treatment	49% (154)	51% (202)	.82
Maternal asthma	42% (131)	39% (155)	.43
Child's sex (male)	54% (167)	52% (209)	.79
Cesarean section	33% (102)	27% (108)	.12
Married	15% (46)	70% (279)	<.001
Asthma/wheeze (child)	36% (111)	23% (91)	<.001
BMI >25 kg/m^2	70% (217)	54% (214)	<.001
College	10% (30)	55% (219)	<.001
Adherence >80%	26% (81)	77% (309)	<.001
Maternal age >27 y	31% (98)	71% (285)	<.001
Birth weight >3500 g	21% (66)	47% (190)	<.001
Gestational age <37 wk	10% (30)	5% (20)	.02
Siblings	74% (231)	59% (236)	<.001

BMI, Body mass index.

Statistical analyses were conducted with R software (version 3.2.2; R Foundation for Statistical Computing, Vienna, Austria; packages "ggplot2").

RESULTS

Characteristics of the trial population

Of the 881 women randomized, 5 were excluded because of medical reasons or multiple gestations. Of the remaining 876 pregnant women, 712 mothers and their children had full information required for the study (Fig 1); 312 were AA and 400 were non-AA subjects. A comparison of the 712 included versus the 164 excluded participants (see Table E1 in this article's Online Repository at www.jacionline.org) showed that the excluded mothers had significantly lower adherence to the study drug ($P < .001$) and a lower educational level ($P = .02$). For the included participants, a baseline table of the AA versus non-AA mothers showed that the 2 groups differed significantly in the majority of baseline characteristics (marriage, asthma/recurrent wheeze, body mass index, college, adherence, maternal age, birth weight, gestational age, and siblings; Table I).

Maternal 25(OH)D levels

There was no statistically significant difference in mean 25(OH)D levels between the intervention and placebo groups at trial entry. Mothers in the intervention arm had a significantly larger increase in 25(OH)D levels than mothers in the placebo arm (mean [SD], 16.32 ng/mL [13.61 ng/mL] in the intervention arm and 4.17 ng/mL [9.86 ng/mL] in the placebo arm; $P < .001$).

Stratifying by race of the mother, the racial categories of white and "other" had similar 25(OH)D levels at study entry ($P = .21$, Fig 2), and therefore these were combined into one group, the non-AA group.

Maternal 25(OH)D levels were higher in the non-AA group compared with the AA group (mean [SD] initial level: 27.1 ng/mL [9.73 ng/mL] in the non-AA group vs 17.57 ng/mL [8.25 ng/mL] in the AA group [$P < .001$]; mean third-trimester levels: 36.33 ng/mL [13.84 ng/mL] in the non-AA vs 29.12 ng/mL [14.86 ng/mL] in the AA group [$P < .001$]). The AA women had a greater increase in 25(OH)D levels during the trial (11.55 ng/mL [13.86 ng/mL] vs 9.23 ng/mL [12.85] in the non-AA women; $P = .02$).

Within the intervention group, the increase in 25(OH)D levels were 17.8 ng/mL (14.6 ng/mL) in AA women and 15.2 ng/mL (12.7 ng/mL) in non-AA women ($P = .07$), whereas in the placebo group the change in 25(OH)D level was 5.4 ng/mL (9.9 ng/mL) in AA women and 3.2 ng/mL (9.8 ng/mL) in non-AA women ($P = .03$).

Association among race, vitamin D supplementation, and development of asthma/recurrent wheeze by age 3 years

A total of 202 (28%) children had asthma/recurrent wheezing by age 3 years: 111 (36%) from AA mothers and 91 (23%) from non-AA mothers ($P < .001$). The risk of asthma/recurrent wheeze by treatment group stratified by race showed a similar effect estimate in the 2 strata that was not statistically significant; there was no interaction between race and treatment group ($P = .77$; see Table E2 in this article's Online Repository at www.jacionline.org).

Association between 25(OH)D levels throughout pregnancy and asthma/recurrent wheeze by age 3 years

We next investigated the initial vitamin D level in the mothers. For each 5 ng/mL increase in the initial level of maternal 25(OH)D, there was a significantly decreased risk of asthma/recurrent wheeze at age 3 years (adjusted OR, 0.92; 95% CI, 0.85-0.99; Table II). This was not significant in a stratified analysis for race. For average initial and third-trimester 25(OH)D levels, a significant reduction in the risk of asthma/recurrent wheeze was found for a 5 ng/mL increase in the average 25(OH)D level (adjusted OR, 0.89; 95% CI, 0.80-0.99), and when stratifying for race, we saw a significant effect in the non-AA women (adjusted OR, 0.85; 95% CI, 0.73-0.995). Adjusting the data for race did not alter our results significantly (see Table E3 in this article's Online Repository at www.jacionline.org). In Table E4 in this article's Online Repository at www.jacionline.org, we present the data $\log_2$-transformed, and overall, we see similar effects when compared with the untransformed model (Table II). We see that a doubling of the average 25(OH)D level is associated with an approximate 33% reduction in the risk of asthma/recurrent wheeze (adjusted OR, 0.67; 95% CI, 0.48-0.93); this is also significant in the non-AA group (adjusted OR, 0.53; 95% CI, 0.28-0.98).

Association between initial and final levels of vitamin D and risk of asthma/recurrent wheeze at age 3 years

To study the effect of the initial level of 25(OH)D by treatment, we constructed groups based on the 25(OH)D level at trial entry (<20 ng/mL, ≥20 to <30 ng/mL, and ≥30 ng/mL) and the treatment group (active/placebo; Table III and see Graphical Abstract). In a confounder-adjusted model we found that with an initial 25(OH)D level of 30 ng/mL or greater, a significant reduction in offspring asthma/recurrent wheeze was found in the mothers randomized to the intervention group when compared with women with an initial 25(OH)D level of less than 20 ng/mL and randomized to the placebo group (adjusted OR, 0.42; 95% CI, 0.19-0.91). However, women with lower initial levels randomized to the intervention group and women with higher initial levels randomized to the control

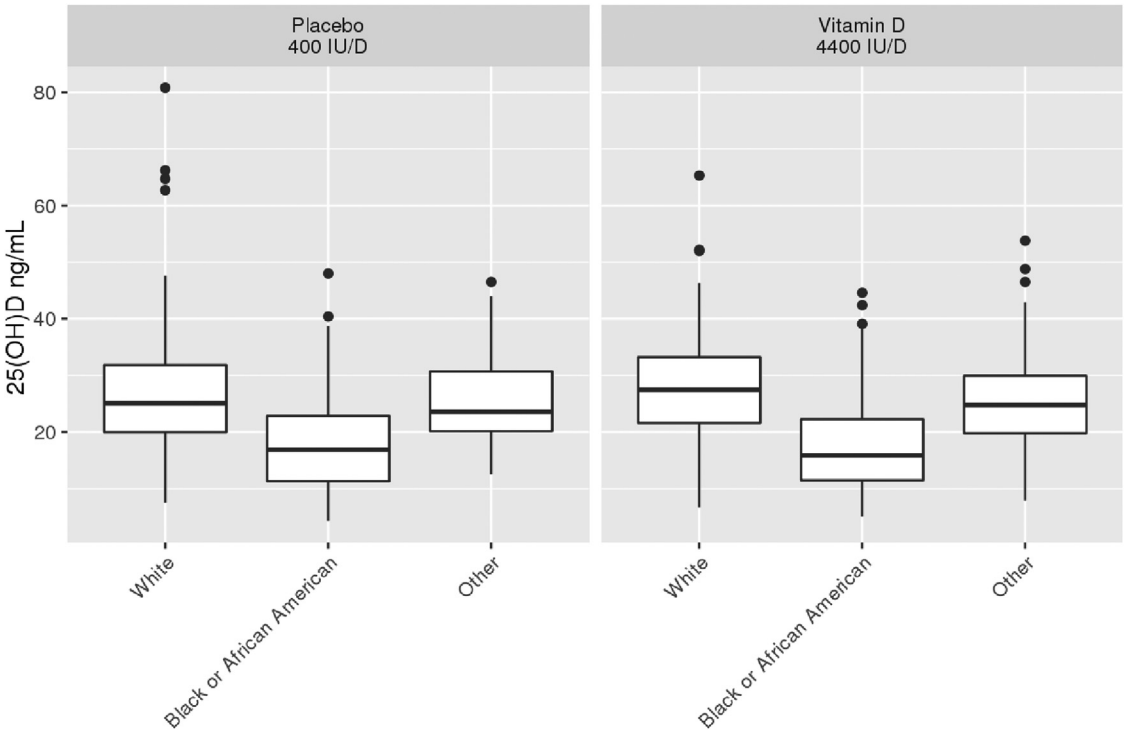

FIG 2. Plot of 25(OH)D levels at entry into the trial divided by race and treatment group. *Other* refers to Asian, Native Hawaiian or other Pacific Islander, American Indian/Alaskan Native, or others.

TABLE II. Level-based analysis: Logistic regression of 25(OH)D levels and asthma/wheeze for a 5 ng/mL difference

	Crude		Adjusted*	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
All women (n = 712)				
Baseline 25(OH)D	**0.89 (0.82-0.97)**	**.006**	0.93 (0.84-1.03)	.15
Third-trimester 25(OH)D	**0.91 (0.86-0.96)**	**.002**	**0.92 (0.85-0.99)**	**.02**
Average 25(OH)D	0.87 (0.80-0.94)	**<.001**	0.89 (0.80-0.99)	**.03**
AA women (n = 312)				
Baseline 25(OH)D	1.03 (0.89-1.18)	.68	1.08 (0.92-1.28)	.50
Third-trimester 25(OH)D	**0.92 (0.85-1.00)**	**.05**	0.93 (0.84-1.03)	.17
Average 25(OH)D	**0.92 (0.82-1.04)**	**.19**	0.96 (0.83-1.11)	.59
Non-AA women (n = 400)				
Baseline 25(OH)D	0.88 (0.77-1.00)	.06	0.86 (0.74-1.00)	.057
Third-trimester 25(OH)D	0.94 (0.86-1.03)	.17	0.91 (0.81-1.02)	.096
Average 25(OH)D	0.89 (0.79-1.01)	.06	0.85 (0.73-0.995)	.04

P values of less than .05 and corresponding ORs (95% CIs) are shown in boldface.
*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), treatment group, and maternal body mass index.

group did not manifest a statistically significant reduction in offspring asthma/recurrent wheeze compared with the reference group (Table III and Graphical Abstract). We performed a trend test of the 3 groups constructed from the initial 25(OH)D level. Here we saw a significant trend for a reduced risk of asthma/wheeze at age 3 years in offspring in the intervention arm (adjusted $P = .03$) but not the placebo arm (adjusted $P = .34$). The largest protective effect from asthma/recurrent wheeze in offspring was found in women with initial 25(OH)D levels of greater than 40 ng/mL and randomized to the intervention group (adjusted OR, 0.13; 95% CI, 0.02-0.99), although the numbers for this analysis were very small (placebo group, n = 17; active treatment group, n = 23; see Table E5 in this article's Online Repository at www.jacionline.org).

Constructing categories of vitamin D based on initial and third-trimester levels (Table IV) showed that having 25(OH)D levels of greater than 30 ng/mL at initiation of the study and at the third trimester (>30/>30) was associated with reduction in asthma/recurrent wheeze in the offspring, although it was not significant in the adjusted model (adjusted OR, 0.56; 95% CI, 0.31-1.01; *P* for trend = .057) compared with having 25(OH)D levels of less than 30 ng/mL at initiation of the study and in the third trimester (<30/<30).

DISCUSSION

In secondary analyses of data from the randomized trial VDAART of high-dose vitamin D intervention, we found that

TABLE III. ORs (95% CIs) of asthma/recurrent wheeze stratified for initial 25(OH)D level and placebo/active treatment

Treatment group	Maternal inclusion level of 25(OH)D	Adjusted* OR (95% CI)
Placebo	<20 ng/mL	1
	≥20 to <30 ng/mL	1.24 (0.73-2.13)
	≥30 ng/mL	0.98 (0.49-1.96)
Active treatment	<20 ng/mL	1.00 (0.6-1.66)
	≥20 to <30 ng/mL	0.83 (0.47-1.47)
	≥30 ng/mL	**0.42 (0.19-0.91)**

P values of less than .05 are shown in boldface. All analyses are adjusted for center, maternal education, body mass index, maternal age, and adherence. Trend test for placebo (<20 as reference): adjusted $P = .34$; trend test for active treatment (<20 as reference), adjusted $P = .03$.
*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), and maternal body mass index. Risk estimates are ORs for each category compared with baseline (placebo group and <20 ng/mL).

TABLE IV. Logistic regression of risk of asthma/recurrent wheeze by vitamin D categories

	Crude OR (95% CI)	Adjusted* OR (95% CI)	*P* value for trend, crude/adjusted*
All (n = 712)			**.002/.057**
<30/<30 (n = 288)	1	1	
<30/>30 or >30/<30 (n = 299)	**0.70 (0.49-0.99)**	0.82 (0.55-1.24)	
>30/>30 (n = 125)	**0.48 (0.29-0.80)**	0.56 (0.31-1.01)	

P values of less than .05 are shown in boldface.
<30/<30, Initial 25(OH)D levels of less than 30 ng/mL and third-trimester levels of less than 30 ng/mL; *<30/>30 or >30/<30*, initial 25(OH)D level of less than 30 ng/mL and third-trimester level of greater than 30 ng/mL or initial 25(OH)D levels of greater than 30 ng/mL and third-trimester level of less than 30 ng/mL; *>30/>30*, initial 25(OH)D level of greater than 30 ng/mL and third-trimester level of greater than 30 ng/mL.
*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), treatment group, and maternal body mass index.

pregnant AA women had significantly lower initial 25(OH)D levels and a higher absolute increase during the trial. A higher average maternal 25(OH)D level during pregnancy was associated with a confounder-adjusted significant reduction in asthma/recurrent wheeze in the children of AA and non-AA mothers. Next, we found that initial 25(OH)D levels in early pregnancy modified the effects of maternal vitamin D supplementation, such that in a confounder-adjusted model, we saw that children born to mothers who had levels of greater than 30 ng/mL at inclusion of the study and were randomized to the intervention arm had the lowest risk of asthma/recurrent wheeze compared with those who had levels of less than 20 ng/mL and were randomized to the placebo arm (Table III and Graphical Abstract). There did not seem to be a plateau in the effect, hence having an initial level of greater than 40 ng/mL and being randomized to the intervention arm were associated with the largest reduction in asthma/recurrent wheeze (see Table E2), but this finding will need to be confirmed in studies with larger numbers.

The strength of this study is that it included a large proportion of AA pregnant women, allowing for the investigation of potential racial differences. The baseline characteristics of AA and non-AA women showed that there were fundamental differences between the 2 groups, and this is the rationale for reporting race-stratified analyses.

We excluded women with missing information about 25(OH)D levels, as well as offspring with missing data on asthma/recurrent wheeze through age 3 years. The excluded mothers had significantly lower adherence to the study drug ($P < .001$) and a lower educational level ($P = .02$); hence the characteristics of the excluded participants are related to low 25(OH)D levels and increased asthma risk. Excluding them would likely bias our findings to the null because they would have strengthened the association between low 25(OH)D levels and asthma occurrence.

Interpretation

Pregnant women metabolize vitamin D differently than nonpregnant women,[15] and AA subjects in general have lower levels of vitamin D than non-AA subjects.[16,17] This likely explains why we did not find an effect of baseline level on outcomes among AA participants. There have been concerns that AA subjects are not able to increase their 25(OH)D levels as effectively as non-AA subjects, but recent studies have shown that AA subjects increase their serum 25(OH)D levels similarly to subjects of other races,[18,19] and our findings show that this also applies to pregnant AA women. The pregnant AA women included in this study increased their vitamin D levels more than the non-AA women, despite a lower overall adherence to the study medication. This might be due to a lower initial 25(OH)D level in the AA women. Similarly, a recent randomized vitamin D intervention trial in nonpregnant young women showed that AA women had lower baseline levels and a greater absolute increase in and similar final 25(OH)D levels when compared with their non-AA peers.[18]

Fetal lung development starts in the first trimester of pregnancy and is affected by vitamin D status[20,21]; furthermore, vitamin D has an effect on the developing immune system throughout pregnancy.[22] This could be a causal link as to why we found that high 25(OH)D levels at the 2 measured time points (ie, late in the first trimester and in the third trimester) were both associated with a reduction in asthma/recurrent wheeze in the offspring. In a confounder-adjusted analysis accounting for the initial 25(OH)D level and treatment group, we observed that the combination of a high level at trial entry and active treatment conferred the greatest protective effect against asthma/recurrent wheeze, with an 80% reduced risk in offspring born to women with an initial level of greater than 40 ng/mL and randomized to vitamin D compared with women with an initial level of less than 20 ng/mL and randomized to the placebo group. This effect persisted when adjusting for all potential confounders. This was supported by a confounder-adjusted analysis of the average level of the 2 measured levels of 25(OH)D. Here we saw a significant reduction in asthma/recurrent wheeze for a 5 ng/mL increase in the average 25(OH)D level.

To further explore this, we constructed 3 groups of women based on their initial and third-trimester vitamin D levels; we used a cutoff of 30 ng/mL because this is the widely used clinical limit for vitamin D sufficiency/insufficiency. We saw that women with a level of greater than 30 ng/mL at both time points had an adjusted 44% reduced risk of asthma/recurrent wheeze in offspring compared with women with 25(OH)D levels of less than 30 ng/mL at both time points, although the trend test for this analysis was nonsignificant at a *P* value of .057. Although an adjusted 18% reduction in asthma/recurrent wheeze was found

in women with 25(OH)D levels of greater than 30 ng/mL at just 1 time point.

One often overlooked issue in nutrient trials is that participants are entering the trial with different circulating levels of the nutrient.[9] This might affect the response to nutrient supplementation, and a concern would be that we would see effects only in those who had the lowest 25(OH)D levels at entry into the trial, whereas those with high levels would benefit less or even exhibit adverse effects. This does not appear to be the case for vitamin D supplementation because we show that the children who derived the most benefit were those who were born to mothers with high initial levels and were in the intervention arm, and models did not show any plateau in effect. This finding suggests that for lung outcomes in early life, exposure of the fetus to a 25(OH)D level of 30 ng/mL or even greater in pregnancy is needed for maximal benefit against wheezing illnesses. Future studies should include more frequent measures to explore the optimal protective level.

Conclusion

In VDAART, a randomized trial of high-dose vitamin D intervention in pregnant women, we found that pregnant AA women had significantly lower 25(OH)D levels at entry into the trial and a higher absolute increase during the trial. There was no difference in the response to vitamin D in pregnancy between the races with regard to asthma/recurrent wheeze in offspring. The greatest protective effect of maternal 25(OH)D levels on asthma/recurrent wheeze in offspring was found from having high levels of 25(OH)D in the first trimester and being randomized to the intervention arm. Therefore prenatal care strategies for asthma and wheeze prevention should target increasing vitamin D levels early in pregnancy. Future studies will need to determine the optimal level for asthma/recurrent wheeze prevention in young children.

We thank VDAART participants for their participation and contribution to the trial. Also, we thank the supporters of the VDAART cohort.

Key messages

- **Nutrient trials differ from drug trials because all participants have some level of exposure to the nutrient both before randomization and during the trial.**
- **For the first time, we show that the level at inclusion in the study and the level achieved are important to take into consideration when analyzing data from randomized trials of prenatal vitamin D supplementation.**
- **Supplementation of vitamin D in pregnancy in mothers with initial 25(OH)D levels of at least 30 ng/mL reduced asthma and recurrent wheeze in the offspring through age 3 years; these findings are of major public health interest because of the widespread nature of vitamin D deficiency.**

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TABLE E1. Dropout table of the 712 included versus 164 excluded participants

	Included (n = 712)	Excluded (n = 164)	*P* value	Missing data
Treatment arm	50% (356)	51% (84)	.85	0
Maternal asthma	40% (286)	44% (72)	.43	0
Child's sex (male)	53% (376)	47% (44)	.32	70
Cesarean section	29% (210)	32% (29)	.73	73
Married	46% (325)	42% (69)	.46	0
Asthma/recurrent wheeze (child)	28% (202)	44% (16)	.06	128
Maternal BMI >25 kg/m^2	61% (431)	68% (55)	.48	83
College	35% (249)	25% (41)	**.02**	0
Adherence >80%	55% (390)	40% (64)	**<.001**	2
Maternal age >27 y	54% (383)	52% (85)	.71	0
Birth weight >3500 g	36% (256)	32% (29)	.52	73
Siblings	66% (467)	63% (104)	.66	0

P values of less than .05 are shown in boldface.
BMI, Body mass index.

TABLE E2. Logistic regression of treatment group and risk of asthma/wheeze in offspring stratified for race of the mother

	Crude		Adjusted*	
	OR	*P* value	OR	*P* value
All subjects (n = 712)				
Placebo	1.0	.097	1.0	.09
Active treatment	0.76 (0.55-1.05)		0.74 (0.53-1.05)	
Stratified analyses†				
AA women (n = 312)				
Placebo	1.0		1.0	
Active treatment	0.72 (0.45-1.15)	.17	0.72 (0.44-1.17)	.18
Non-AA women (n = 400)				
Placebo	1.0	.34	1.0	.23
Active treatment	0.80 (0.50-1.28)		0.74 (0.45-1.21)	

*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), and maternal body mass index.
†No interaction between race and treatment ($P = .77$).

TABLE E3. Level-based analysis with additional adjustment for race: Logistic regression of 25(OH)D levels and asthma/wheeze

All women (n = 712)	Adjusted* OR (95% CI)
Baseline 25(OH)D	0.94 (0.85-1.04)
Third-trimester 25(OH)D	**0.92 (0.86-0.99)**
Average 25(OH)D	**0.90 (0.81-0.99)**

*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), treatment group, and maternal BMI and race.

TABLE E4. Level-based analysis: Logistic regression of 25(OH)D levels and asthma/wheeze

	Crude		Adjusted*	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
All women (n = 712)				
Baseline 25(OH)D	**0.73 (0.58-0.92)**	**.007**	0.83 (0.63-1.11)	.23
Third-trimester 25(OH)D	**0.66 (0.53-0.83)**	**<.001**	**0.71 (0.54-0.93)**	**.01**
Average 25(OH)D	**0.60 (0.46-0.78)**	**<.001**	**0.67 (0.48-0.93)**	**.02**
AA women (n = 312)				
Baseline 25(OH)D	1.08 (0.78-1.5)	.64	1.18 (0.81-1.72)	.50
Third-trimester 25(OH)D	**0.68 (0.51-0.91)**	**.01**	0.71 (0.5-1.007)	.05
Average 25(OH)D	**0.74 (0.52-1.05)**	**.1**	0.82 (0.54-1.24)	.35
Non-AA women (n = 400)				
Baseline 25(OH)D	0.59 (0.37-0.93)	.06	**0.57 (0.34-0.95)**	**.03**
Third-trimester 25(OH)D	**0.81 (0.55-1.21)**	**.01**	0.76 (0.47-1.23)	.26
Average 25(OH)D	0.61 (0.37-1.02)	.06	**0.53 (0.28-0.98)**	**.04**

25(OH)D levels are $\log_2$-transformed. *P* values of less than .05 and corresponding ORs (95% CIs) are shown in boldface.
*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), treatment group, and maternal body mass index.

TABLE E5. Risk of asthma/wheeze stratified by inclusion 25(OH)D level and treatment arm

Inclusion 25(OH)D level (ng/mL)	No.	No. of cases (asthma/recurrent wheeze)	Crude OR (95% CI)	Adjusted* OR (95% CI)
<20 AND placebo	150	49	1	1
≥20, <30 AND placebo	134	42	0.94 (0.57-1.55)	1.24 (0.73-2.12)
≥30 <40 AND placebo	55	15	0.77 (0.42-1.18)	0.94 (0.45-1.97)
≥40 AND placebo	17	5	0.86 (0.28-2.57)	1.14 (0.32-3.99)
<20 AND active treatment	144	45	0.94 (0.57-1.53)	0.98 (0.59-1.62)
≥20, <30 AND active treatment	133	34	0.71 (0.42-1.19)	0.83 (0.47-1.46)
≥30, <40 AND active treatment	56	10	0.45 (0.21-0.96)	0.56 (0.24-1.28)
≥40 AND active treatment	23	2	0.20 (0.04-0.87)	0.13 (0.02-0.99)

*Adjusted for center, maternal education, maternal age, adherence to study drug (>80%), and maternal body mass index.