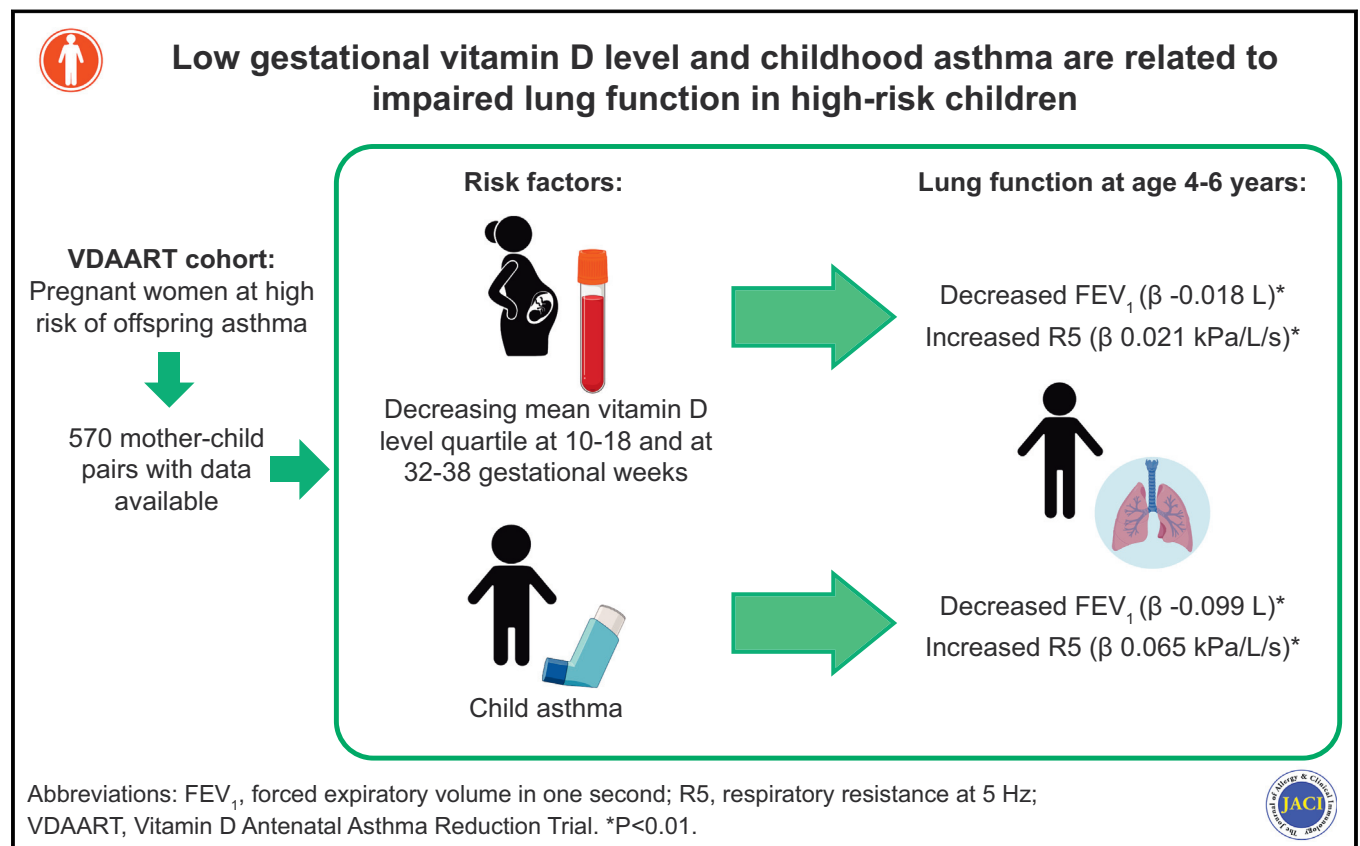


Low gestational vitamin D level and childhood asthma are related to impaired lung function in high-risk children

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GRAPHICAL ABSTRACT



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Background: Lung function impairment in early life often persists into adulthood. Therefore, identifying risk factors for low childhood lung function is crucial.

Objective: We examined the effect of 25-hydroxyvitamin D (25 [OH]D) level and childhood asthma phenotype on childhood lung function in the Vitamin D Antenatal Asthma Reduction Trial (VDAART).

Methods: The 25(OH)D level was measured at set time points in mothers during pregnancy and in children during early life. On the basis of parental reports, children were categorized into 3 clinical phenotypes: asymptomatic/infrequent wheeze, early transient wheeze, and asthma at age 6 years. Lung function was assessed with impulse oscillometry at ages 4, 5, and 6 years and with spirometry at ages 5 and 6 years.

Results: A total of 570 mother-child pairs were included in this post hoc analysis. Mean gestational 25(OH)D-level quartiles were negatively associated with child respiratory resistance at 5 Hz (R5) from age 4 to 6 years (β , -0.021 kPa/L/s; 95% CI, -0.035 to -0.007 ; $P = .003$) and positively associated with FEV₁ (β , 0.018 L; 95% CI, 0.005 - 0.031 ; $P = .008$) and forced vital capacity (β , 0.022 L; 95% CI, 0.009 - 0.036 ; $P = .002$) from age 5 to 6 years. Children with asthma at age 6 years had lower lung function from age 4 to 6 years than the asymptomatic/infrequent wheeze group (β , 0.065 kPa/L/s; 95% CI, 0.028 to 0.102 ; $P < .001$ for R5 and β , -0.063 L; 95% CI, -0.099 to -0.028 ; $P < .001$ for FEV₁).

Conclusions: Low gestational 25(OH)D level and childhood asthma are important risk factors for decreased lung function in early childhood. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: Vitamin D, childhood asthma, lung function, spirometry, impulse oscillometry, VDAART, tobacco smoke

Subjects with childhood asthma often present with lower levels of lung function than their healthy peers. By school age, impairment of lung function is already detectable in a substantial proportion of children with asthma and often persists into adulthood.^{1,2} Furthermore, unselected birth cohorts have demonstrated that in some cases, impairment of lung function may already be detectable in early infancy.^{3,4} These findings suggest that the origins of lung function impairment may be related to environmental or genetic factors influencing fetal lung development. Therefore, identifying and minimizing risk factors for reduced growth in early-life lung function is crucial for later respiratory health.

Vitamin D deficiency (25-hydroxyvitamin D (25[OH]D) level of <20 ng/mL) and insufficiency (25[OH]D level of 20 to <30 ng/mL) are extremely common worldwide, especially in pregnant and lactating women.^{5,6} Vitamin D has been linked to fetal lung and immune system development.⁷ Furthermore, animal studies have demonstrated clear structural and functional lung changes in the offspring of vitamin D-deficient mothers,^{8,9} and clinical trials have suggested that high-dose prenatal vitamin D₃ supplementation may decrease early-life wheezing.^{10,11} However, studies on the association between gestational vitamin D and offspring lung function in humans have yielded conflicting results.¹²⁻¹⁵ We conducted the Vitamin D Antenatal Asthma Reduction Trial (VDAART) to examine the potential of high-dose prenatal vitamin D₃ supplementation for preventing offspring asthma and wheeze. Follow-up data from the VDAART suggested that

Abbreviations used

25(OH)D: 25-hydroxyvitamin D

COPSAC₂₀₁₀: Copenhagen Prospective Studies on Asthma in Childhood 2010

FVC: Forced vital capacity

IOS: Impulse oscillometry

R5: Respiratory resistance at 5 Hz

R5-20: Difference between respiratory resistance at 5 and 20 Hz

VDAART: Vitamin D Antenatal Asthma Reduction Trial

although the supplementation did not reduce the 6-year incidence of childhood asthma and recurrent wheeze, it may have some influence on childhood lung function.¹⁶ However, the Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC₂₀₁₀) trial observed no associations between high-dose prenatal vitamin D₃ supplementation and lung function at age 6 years.¹⁷

In the present *post hoc* study, we investigated the association of (1) 25(OH)D levels during pregnancy and early life, and (2) childhood asthma phenotype with lung function in early childhood obtained via impulse oscillometry (IOS) and spirometry in the VDAART cohort.

METHODS

Study population

The VDAART ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00920621) identifier: NCT00920621) is a double-blind randomized controlled trial that randomized pregnant women at 10 to 18 gestational weeks to receive either high-dose vitamin D₃ supplementation (4000 IU/d) or placebo in addition to regular prenatal vitamin D₃ supplementation (400 IU/d).¹⁰ The women and/or the biological fathers of the children had a history of asthma, eczema, or allergic rhinitis. At enrollment, the women filled out a questionnaire,¹⁰ which included information on their demographic characteristics, medical history, active tobacco smoking, and tobacco smoking in the household. Only women who were nonsmokers were included in the trial. Subjects from both the intervention and placebo groups were included in the present *post hoc* analysis.

Asthma phenotypes

The health, respiratory symptoms, and medication use of the children was monitored by telephone every 3 months and in-person annually from birth to age 6 years, and used to categorize the children into 3 clinical phenotypes: (1) asthma at age 6 years was defined as physician-diagnosed asthma at any time from birth to age 6 years and a report of asthma medication use or wheeze after age 5 years,¹⁶ (2) early transient wheeze was defined as physician-diagnosed asthma or recurrent wheeze (≥ 2 episodes based on predefined criteria) at any time from birth to age 3 years¹⁰ but no asthma at age 6 years, and (3) subjects who did not meet the criteria of early transient wheeze or asthma at age 6 years were categorized as asymptomatic/infrequent wheeze.

25(OH)D measurements

Plasma 25(OH)D level was measured in the mothers at baseline (10-18 gestational weeks) and at third trimester (32-38 gestational weeks) and in the children at age 1 and 3 years using the DiaSorin Liaison chemiluminescence immunoassay. Chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine 25(OH)D level from cord blood drawn at the time of delivery.¹⁸

Lung function measurements

Methods for quality control of the lung function measurements have been previously published.¹⁶ IOS measurements were performed at ages 4, 5, and 6 years using the Jaeger MasterScreen IOS System (Vyaire Medical, Inc, Mettawa, Ill). The method uses pulse signals of different frequencies that are superimposed on tidal breathing to determine different measures of lung mechanics, such as respiratory resistance at 5 Hz (R5), which is considered to reflect the resistance of the whole respiratory tract, and the difference between respiratory resistance at 5 and 20 Hz (R5-20), which is thought to reflect mainly peripheral airway properties.^{19,20} IOS was performed with the child sitting and using a nose clip. In total, 4 to 10 trials were recorded and the measurement was regarded as successful if at least 2 acceptable and repeatable recordings with a regular tidal breathing pattern of at least 20 seconds were acquired. The mean value for each IOS index was calculated from the acceptable IOS recordings and used in the analyses.

Spirometry measurements were performed at ages 5 and 6 years according to the American Thoracic Society and European Respiratory Society guidelines²¹ using the Jaeger MasterScreen PFT System (VyaireTM Medical, Inc). The measurements were performed with the child standing and using a nose clip. In total, 4 to 10 trials were recorded and the measurement was regarded as successful if at least 2 acceptable and repeatable recordings were acquired. The mean value for each spirometry index was calculated from the acceptable spirometry recordings and used in the analyses.

Parents were instructed to cease their child's bronchodilator medications at least 8 hours before and inhaled corticosteroids or leukotriene modifiers at least 24 hours before the lung function measurements.

Statistical analyses

Student *t* test and chi-square tests were used to compare demographic data between groups. Because of the lack of validated multiethnic reference values for IOS, the lung function data were analyzed using height-, sex-, and race-adjusted models. Differences in the longitudinal lung function trajectories between groups were evaluated using linear mixed-effects models. Time-dependent covariates included in the models were child height and child age, and fixed covariates were child sex, child race, and study site. To evaluate the association between plasma 25(OH)D level and lung function, children were grouped into quartiles on the basis of 25(OH)D measurement of interest, and the trend between these 25(OH)D-level quartiles (ordinal variable) was evaluated in the linear mixed-effects models. R Version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for the statistical analyses, and a confidence level of 95% was applied.

RESULTS

Baseline characteristics

A flowchart of the study is presented in Fig 1. In total, 570 (71%) of the 806 children in the VDAART cohort performed at least 1 successful lung function measurement, providing data on a total of 1036 IOS measurements and 615 spirometry measurements that were used in the analyses. Of the 570 children, 569 (99.8%) had data on maternal 25(OH)D level from at least 1 time point available and 564 (98.9%) had data on their childhood asthma phenotype. Fig E1 in this article's Online Repository at www.jacionline.org presents the upset diagrams for the repeated lung function measurements. A total of 357 (66%) of the 543 children with data on IOS performed successful measurements on at least 2 separate visits, and 175 (40%) of the 438 children with data on spirometry performed successful measurements on both of the 2 visits. Table I presents the baseline characteristics of the study subjects and those excluded because of missing data or unsuccessful lung function measurements. No differences between these groups were observed except for maternal hay fever (66% for the study subjects vs 59% for the excluded subjects; *P* =

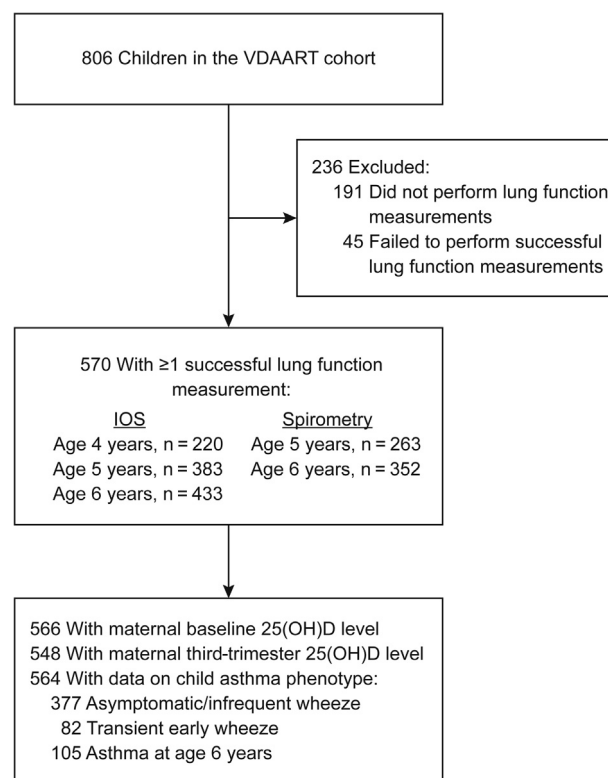


FIG 1. Flowchart of the study.

.042) and study site distribution (study subjects vs excluded subjects: 28% vs 35% for Boston, 33% vs 37% for San Diego, and 40% vs 28% for St Louis; *P* = .004).

25(OH)D and lung function

Mean gestational 25(OH)D-level quartiles showed a positive trend with offspring lung function at ages 4, 5, and 6 years (Fig 2). In the linear mixed-effects models, baseline, third-trimester, and mean gestational 25(OH)D-level quartiles showed significant negative associations with the longitudinal trajectories of child R5 and R5-20 and positive associations with FEV₁ and forced vital capacity (FVC), all indicating an increasing level of lung function with increasing 25(OH)D-level quartile (Table II). At the age of 6 years, offspring of mothers in the highest mean gestational 25(OH)D-level quartile had 12 mL higher FEV₁ (10.7%; 1.24 L vs 1.12 L) and 0.08 kPa/L/s lower R5 (8.7%; 0.84 kPa/L/s vs 0.92 kPa/L/s) levels than those with mothers in the lowest quartile. However, no association between maternal 25(OH)D-level quartiles and child FEV₁/FVC ratio was observed. All associations between maternal 25(OH)D-level quartiles and child lung function remained significant after adjusting for maternal age, education, asthma, and household tobacco smoking at enrollment, except for the association between baseline 25(OH)D level and R5-20 (see Table E1 in this article's Online Repository at www.jacionline.org). Similarly, all associations remained significant after adjusting for allergic sensitization, except for the associations of baseline 25(OH)D-level quartiles with R5-20 and FEV₁ (see Table E2 in this article's Online Repository at www.jacionline.org). No consistent differences in the association between gestational 25(OH)D-level quartiles and child lung

TABLE I. Baseline characteristics of subjects in the VDAART cohort (n = 806) based on inclusion in the present study

Characteristic	Study subjects (n = 570)	Excluded (n = 236)	P value	Missing data (%)
Maternal age, mean $\pm$ SD	27.46 $\pm$ 5.51	27.08 $\pm$ 5.49	.367	0
Maternal education, n (%)			.335	0
<High school	76 (13.3)	24 (10.2)		
High school or technical school	164 (28.8)	77 (32.6)		
College or graduate school	330 (57.9)	135 (57.2)		
Household income, n (%)			.687	0
<\$30,000	175 (30.7)	61 (25.8)		
\$30,000-\$49,999	73 (12.8)	32 (13.6)		
\$50,000-\$74,999	65 (11.4)	32 (13.6)		
>\$75,000	119 (20.9)	53 (22.5)		
Refused to say or unknown	138 (24.2)	58 (24.6)		
Environmental tobacco smoke exposure,* n (%)	93 (16.3)	29 (12.3)	.179	0
Medical history of mother, n (%)				
Asthma	239 (41.9)	83 (35.2)	.088	0
Hay fever	378 (66.3)	138 (58.5)	.042	0
Eczema	178 (31.2)	79 (33.5)	.589	0
Medical history of father, n (%)				
Asthma	140 (24.6)	47 (20.0)	.193	0.2
Hay fever	243 (42.6)	101 (43.2)	.952	0.2
Eczema	95 (16.7)	38 (16.3)	.985	0.4
Vitamin D ₃ intervention, n (%)	285 (50.0)	120 (50.8)	.887	0
25(OH)D level (ng/mL), mean $\pm$ SD				
Mother at 10-18 gestational weeks	22.8 $\pm$ 10.2	23.5 $\pm$ 10.4	.355	0.6
Mother at 32-38 gestational weeks	32.6 $\pm$ 14.4	34.0 $\pm$ 15.2	.230	4.5
Cord blood at birth	23.6 $\pm$ 11.9	23.5 $\pm$ 12.3	.904	23.6
Child at age 1 y	29.6 $\pm$ 9.7	30.0 $\pm$ 10.8	.622	21.7
Child at age 3 y	21.6 $\pm$ 9.8	20.2 $\pm$ 7.3	.201	30.5
Child sex: male, n (%)	295 (51.8)	126 (53.4)	.730	0
Child race, n (%)			.381	0
Black	284 (50.4)	106 (45.1)		
White	180 (31.9)	85 (36.2)		
Other	100 (17.7)	44 (18.7)		
Preterm birth,† n (%)	50 (8.8)	21 (9.0)	>.999	0.2
Child allergic sensitization,‡ n (%)	340 (65.1)	46 (58.2)	.286	25.4
Child asthma phenotype, n (%)			.247	13.4
Asymptomatic/infrequent wheeze	377 (66.8)	80 (59.7)		
Early transient wheeze	82 (14.5)	26 (19.4)		
Asthma at age 6 y	105 (18.6)	28 (20.9)		
Study site, n (%)			.004	0
Boston	157 (27.5)	83 (35.2)		
San Diego	186 (32.6)	88 (37.3)		
St Louis	227 (39.8)	65 (27.5)		

P values less than .05 are in boldface.

*Exposure was defined as household tobacco smoking reported by the mother at study enrollment (10-18 gestational weeks). All women enrolled in the study were nonsmokers at study enrollment.

†Gestational age <37 wk.

‡Serum specific IgE positive (≥ 0.35 kU/L) to ≥ 1 food or aeroallergen and/or total serum IgE level of ≥ 60 kU/L at the age of 3 and/or 6 y.

function were observed when stratified by child sex or race (see [Tables E3](#) and [E4](#) in this article's Online Repository at www.jacionline.org). Child 25(OH)D-level quartiles at birth, age 1 year, and age 3 years did not show significant associations with lung function except for cord blood 25(OH)D-level quartiles, which were negatively associated with R5 and R5-20 (see [Table E5](#) in this article's Online Repository at www.jacionline.org).

We performed a separate analysis to compare the lung function of children whose mothers had either deficient (<20 ng/mL, n = 91), insufficient (20-29 ng/mL, n = 61), or sufficient (≥ 30 ng/mL, n = 96) 25(OH)D level at both baseline and third trimester. Similar to the 25(OH)D-level quartiles, these gestational 25(OH)D-level groups showed a positive trend with offspring lung function from age 4 to 6 years ([Fig 3](#)). In the linear mixed-effects models, the gestational 25(OH)D-level groups showed significant

positive associations with the longitudinal trajectories of child R5 and R5-20, and negative associations with FEV₁ and FVC (see [Table E6](#) in this article's Online Repository at www.jacionline.org).

Childhood asthma phenotype and lung function

Children with asthma at age 6 years had lower levels of lung function from age 4 to 6 years than the asymptomatic/infrequent wheeze group, and differences between the lung function of the transient early wheeze group and the asymptomatic/infrequent wheeze group were consistently smaller than what was seen between the asthma group and the asymptomatic/infrequent wheeze group ([Fig 4](#)). In the mixed-effects models, all IOS and spirometry indices demonstrated significantly lower longitudinal

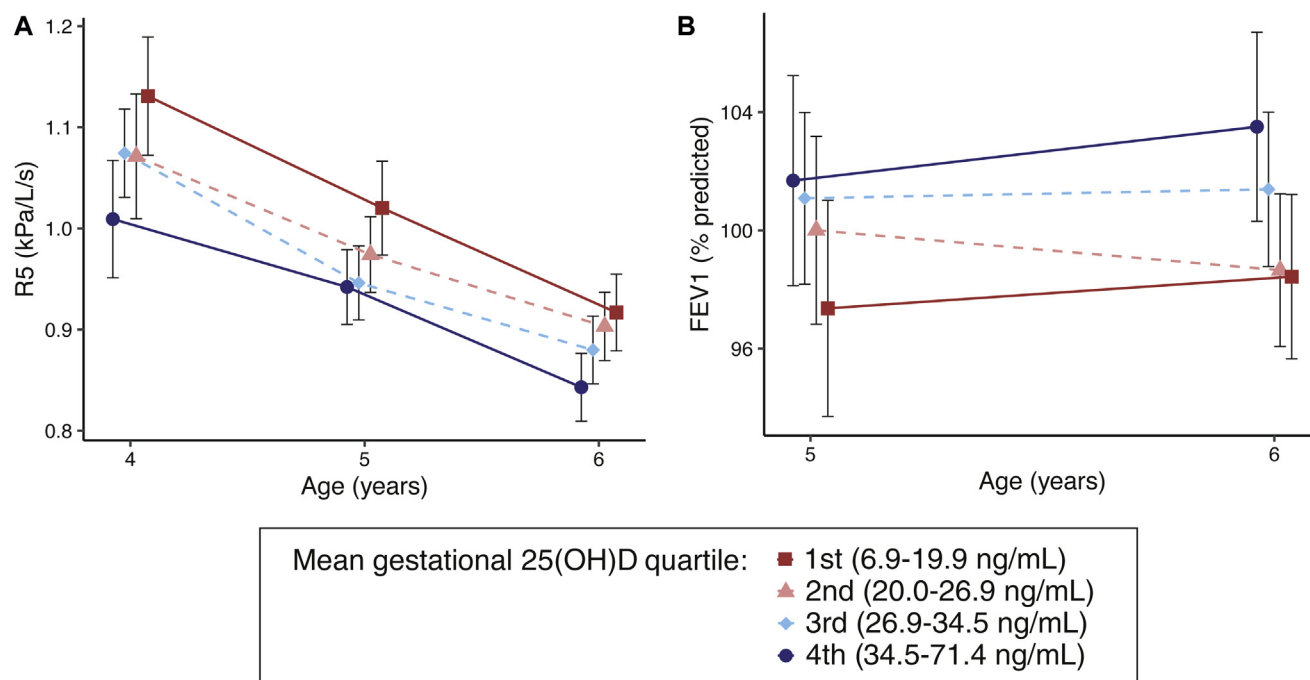


FIG 2. Mean values for child lung function at ages 4, 5, and 6 years stratified by maternal 25(OH)D-level quartiles during pregnancy (mean value of the levels at 10-18 gestational weeks and at 32-38 gestational weeks). **A**, R5. **B**, FEV₁.²² Data points illustrate the mean values of the lung function indices for each group, and the whiskers illustrate the 95% CI.

TABLE II. Maternal 25(OH)D-level quartiles at different time points and offspring lung function trajectories from age 4 to 6 y

Gestational 25(OH)D	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β (95% CI)	P value	β (95% CI)	P value
Baseline* 25(OH)D-level quartiles	-0.017 (-0.032 to -0.003)	.019	-0.011 (-0.021 to -0.001)	.041
Third-trimester† 25(OH)D-level quartiles	-0.028 (-0.041 to -0.015)	<.001	-0.019 (-0.029 to -0.010)	<.001
Mean‡ 25(OH)D-level quartiles	-0.021 (-0.035 to -0.007)	.003	-0.015 (-0.025 to -0.005)	.004

	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
Baseline* 25(OH)D-level quartiles	0.015 (0.001 to 0.028)	.040	0.024 (0.010 to 0.038)	<.001	-0.518 (-1.080 to 0.428)	.073
Third-trimester† 25(OH)D-level quartiles	0.016 (0.004 to 0.029)	.013	0.018 (0.006 to 0.031)	.006	-0.038 (-0.550 to 0.473)	.884
Mean‡ 25(OH)D-level quartiles	0.018 (0.005 to 0.031)	.008	0.022 (0.009 to 0.036)	.002	-0.122 (-0.664 to 0.419)	.660

Data presented as β estimates (95% CIs) and P values for the trend between the 25(OH)D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, sex, race, and study site were included as fixed covariates. P values less than .05 are in boldface.

*At 10-18 gestational weeks.

†At 32-38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.

lung function levels in children with asthma at age 6 years than in the asymptomatic/infrequent wheeze group, whereas the early transient wheeze group differed from the asymptomatic/infrequent wheeze group only in relation to R5-20 (Table III). All associations between the asthma phenotypes and lung function remained significant after adjusting for allergic sensitization (see Table E7 in this article's Online Repository at www.jacionline.org). No significant interactions between child asthma status at age 6 years and gestational 25(OH)D-level quartiles were observed for the longitudinal lung function trajectories (see Table E8 in this article's Online Repository at www.jacionline.org).

Multivariable model of different risk factors for decreased lung function

Multivariable models evaluating the effects of mean gestational 25(OH)D-level quartiles, childhood asthma phenotype, preterm birth, and household tobacco smoking during pregnancy on childhood longitudinal lung function trajectories are presented in Table IV. In these models, mean gestational 25(OH)D quartiles showed significant negative associations with child R5 and R5-20 and positive associations with FEV₁ and FVC. Asthma at age 6 years was associated with increased R5 and R5-20 and decreased FEV₁ and FEV₁/FVC and a

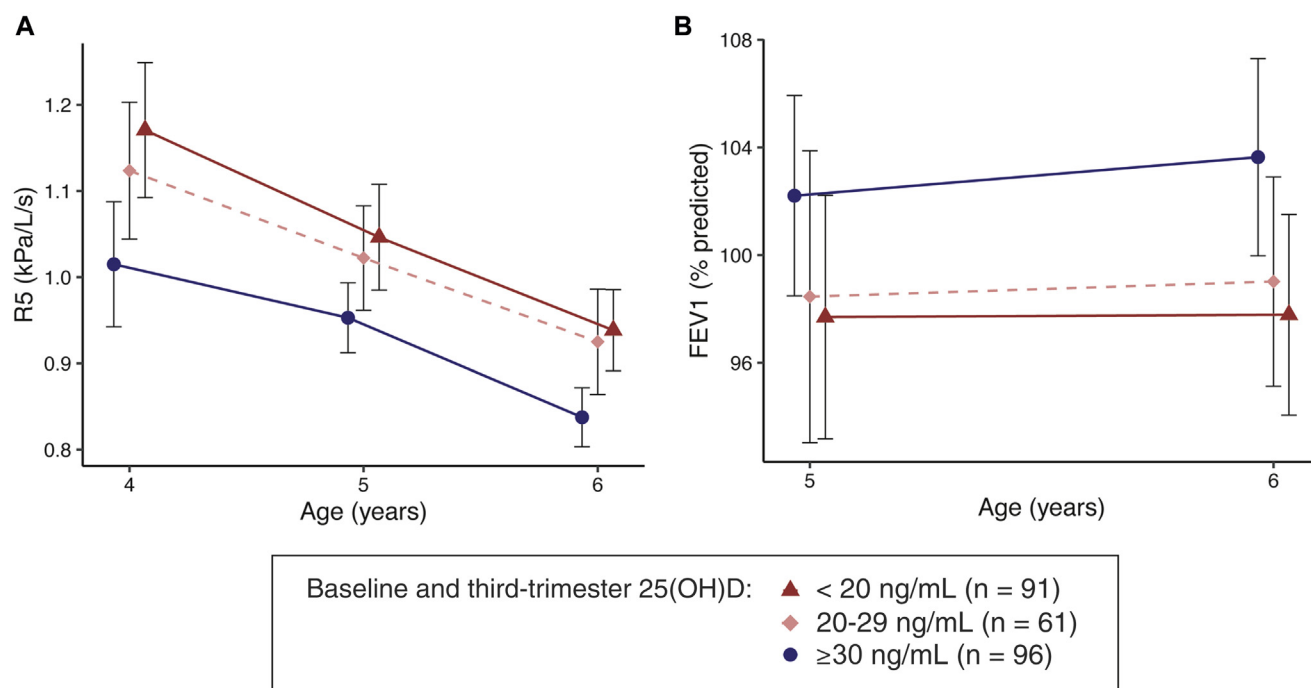


FIG 3. Mean values for child lung function at ages 4, 5, and 6 years among the subset of children whose mothers had either deficient (<20 ng/mL, n = 91), insufficient (20-29 ng/mL, n = 61), or sufficient (≥30 ng/mL, n = 96) 25(OH)D level at both baseline (10-18 gestational weeks) and third trimester (32-38 gestational weeks). **A**, R5. **B**, FEV₁.²² Data points illustrate the mean values of the lung function indices for each group, and the whiskers illustrate the 95% CI.

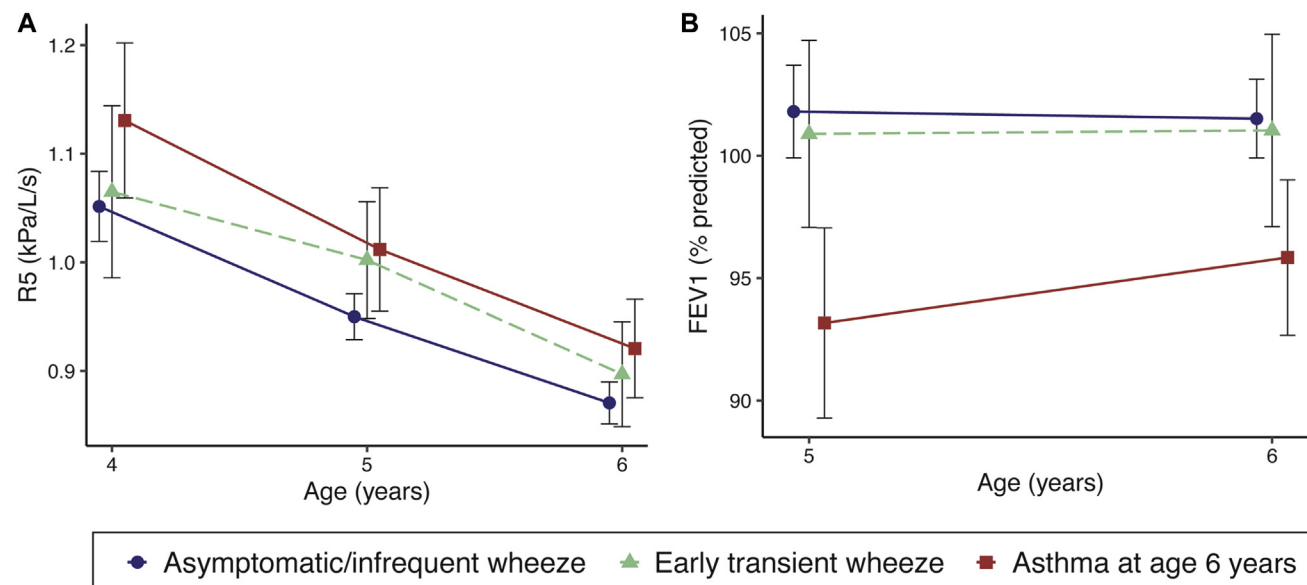


FIG 4. Mean values for child lung function at ages 4, 5, and 6 years stratified by childhood asthma phenotype. **A**, R5. **B**, FEV₁.²² Data points illustrate the mean values of the lung function indices for each group, and the whiskers illustrate the 95% CI.

borderline significant decrease in FVC, whereas the early transient wheeze phenotype showed increased FVC only when compared with the asymptomatic/infrequent wheeze group. Household tobacco smoking at study enrollment was associated

with increased R5 and R5-20, but not with significant changes in the spirometry indices. Preterm birth was associated with increased R5-20 and decreased FEV₁, as well as borderline significant changes in R5 and FVC.

TABLE III. Childhood asthma phenotypes and longitudinal lung function trajectories from age 4 to 6 y

Asthma phenotype	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value
Asymptomatic/infrequent wheeze	Reference		Reference	
Early transient wheeze	0.036 (−0.004 to 0.077)	.081	0.035 (0.006 to 0.065)	.021
Asthma at age 6 y	0.065 (0.028 to 0.102)	<.001	0.058 (0.031 to 0.085)	<.001

	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value
Asymptomatic/infrequent wheeze	Reference		Reference		Reference	
Early transient wheeze	0.012 (−0.024 to −0.049)	.513	0.030 (−0.008 to 0.068)	.123	−1.292 (−2.802 to 0.216)	.097
Asthma at age 6 y	−0.063 (−0.099 to −0.028)	<.001	−0.046 (−0.083 to −0.010)	.015	−2.004 (−3.467 to −0.540)	.008

Data presented as β estimates (95% CIs) and *P* values for the differences between the childhood asthma phenotypes when compared with asymptomatic children in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). Childhood asthma phenotype, sex, race, and study site were included as fixed covariates. *P* values less than .05 are in boldface.

TABLE IV. Multivariable mixed-effects model for the longitudinal lung function trajectories

Risk factor	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value
Mean* gestational 25(OH)D-level quartile	−0.019 (−0.032 to −0.005)	.008	−0.012 (−0.022 to −0.002)	.020
Asthma phenotype	Reference		Reference	
Asymptomatic/infrequent wheeze	Reference		Reference	
Early transient wheeze	0.025 (−0.016 to 0.066)	.232	0.027 (−0.003 to 0.057)	.077
Asthma at age 6 y	0.055 (0.018 to 0.093)	.005	0.051 (0.024 to 0.079)	<.001
Preterm birth†	0.049 (−0.008 to 0.106)	.095	0.052 (0.011 to 0.094)	.015
ETS exposure‡	0.064 (0.023 to 0.104)	.003	0.044 (0.014 to 0.074)	.004

	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value
Mean* gestational 25(OH)D-level quartile	0.016 (0.003 to 0.029)	.015	0.022 (0.008 to 0.035)	.002	−0.202 (−0.743 to 0.337)	.468
Asthma phenotype	Reference		Reference		Reference	
Asymptomatic/infrequent wheeze	Reference		Reference		Reference	
Early transient wheeze	0.025 (−0.013 to 0.062)	.209	0.045 (0.006 to 0.084)	.026	−1.368 (−2.934 to 0.194)	.091
Asthma at age 6 y	−0.053 (−0.089 to −0.017)	.005	−0.037 (−0.075 to 0.000)	.056	−1.800 (−3.304 to −0.293)	.021
Preterm birth†	−0.063 (−0.116 to −0.009)	.024	−0.053 (−0.109 to 0.002)	.063	−1.198 (−3.436 to 1.041)	.301
ETS exposure‡	−0.017 (−0.055 to 0.020)	.372	−0.026 (−0.065 to 0.013)	.195	0.096 (−1.476 to 1.665)	.906

ETS, Environmental tobacco smoke.

Data presented as β estimates (95% CIs) and *P* values for all risk factors in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, childhood asthma phenotype, preterm birth, ETS exposure, sex, race, and study site were included as fixed covariates. *P* values less than .05 are in boldface.

*Mean of maternal baseline (10–18 gestational weeks) and third-trimester (32–38 gestational weeks) 25[OH]D levels.

†Gestational age <37 wk.

‡ETS exposure defined as tobacco smoking in the household reported by the mother at the time of study enrollment (10–18 gestational weeks). All mothers enrolled in the study were nonsmokers at enrollment.

DISCUSSION

The present study provides evidence that low gestational 25(OH)D level and childhood asthma phenotype are important and independent risk factors for decreased lung function from age 4 to 6 years. Gestational 25(OH)D level at baseline (10–18 gestational weeks) and third trimester (32–38 gestational weeks) were both significantly associated with offspring lung function, whereas 25(OH)D levels during early childhood were not associated with later lung function, suggesting that the main

long-term effects of vitamin D on lung function might be mediated via alterations in fetal lung development.

Vitamin D influences fetal lung growth and development, particularly during saccular and alveolar stages in late pregnancy.^{23–26} 25(OH)D is thought to readily cross the placenta in humans as it does in rats.²⁷ Furthermore, vitamin D receptors are expressed in lung and immune cells, and *in vitro* studies have demonstrated that stimulation with 1,25-hydroxyvitamin D affects several key functions of fetal lung cells.^{24,28} However, it is

unclear to what extent the effects of maternal 25(OH)D level on fetal lung development are attributed to the direct effects of vitamin D and whether there are other potential mediators such as differences in the plasma metabolites²⁹ of the mothers. Animal studies have demonstrated clear structural and functional lung changes in the offspring of vitamin D-deficient mothers.^{8,9} However, few studies have investigated the association between gestational 25(OH)D and offspring lung function in humans, and the results have been conflicting. Although most studies have found no significant associations between gestational 25(OH)D level and child lung function,¹³⁻¹⁵ a weak positive association between maternal 25(OH)D level at 16 to 20 gestational weeks and child FVC at age 6 years was observed in the Australian Raine cohort.¹² We found that maternal 25(OH)D level at 10 to 18 gestational weeks and at third trimester were both significantly associated with offspring lung function. Interestingly, 25(OH)D level at 10 to 18 gestational weeks, during proximal airway branching, had the strongest associations with FVC and FEV₁, which mainly reflect the function of larger airways, whereas 25(OH)D level at third trimester, during the development of the peripheral lung zone, showed the strongest associations with R5 and R5-20, which are more sensitive to changes in the peripheral airways.^{19,20} In contrast, child 25(OH)D level at age 1 or 3 years was not associated with later lung function.

Because of the lack of data on the optimal 25(OH)D level for respiratory health, we analyzed the effects of vitamin D on lung function by grouping subjects into quartiles on the basis of 25(OH)D level. A step-wise increase in child lung function was observed with increasing maternal 25(OH)D-level quartiles. The difference in FEV₁, which is considered clinically as the most important lung function index, between the offspring of the lowest and highest maternal 25(OH)D-level quartiles was more than 10%. This exceeds the minimal clinically important difference (5%-10%) based on adult studies,³⁰ but because data on clinically significant differences in childhood lung function are lacking, further follow-up is needed to clarify the clinical significance of the observed lung function differences in later life and whether subjects who are exposed to low 25(OH)D levels *in utero* are at risk for altered longitudinal lung function trajectories.³¹ The highest baseline gestational 25(OH)D-level quartile was greater than or equal to 28.5 ng/mL, and both the third and fourth quartiles of third-trimester 25(OH)D level were greater than or equal to 30 ng/mL, suggesting that a gestational 25(OH)D level of at least 30 ng/mL might be optimal for offspring respiratory health in contrast to 20 ng/mL, which has traditionally been used to define vitamin D sufficiency on the basis of studies on skeletal health.³² This was further supported by our secondary analyses, where the offspring of mothers with a 25(OH)D level of greater than or equal to 30 ng/mL at baseline and at third trimester had significantly higher levels of lung function than those with lower maternal 25(OH)D levels, which is in line with results from the Raine cohort.¹²

Although the intention-to-treat analyses of the VDAART and COPSAC₂₀₁₀ trials resulted in weak or null associations between high-dose prenatal vitamin D₃ supplementation and childhood lung function,^{16,17} we observed that maternal 25(OH)D levels at baseline and third trimester were significantly associated with offspring lung function. In addition to possible differences in adherence to the intervention and unaccounted vitamin D sources affecting the acquirement of vitamin D, genetic polymorphism may influence the increase in serum 25(OH)D level in response

to supplementation.^{33,34} Therefore, plasma 25(OH)D level may provide a more precise measure of an individual's vitamin D status, explaining why the association with lung function was more evident with maternal 25(OH)D levels as opposed to the intention-to-treat analysis in VDAART. Furthermore, the vitamin D₃ supplementation may have been started too late (10-18 gestational weeks in VDAART and 22-26 gestational weeks in COPSAC₂₀₁₀) or at a suboptimal dose (4400 IU/d in VDAART and 2800 IU/d in COPSAC₂₀₁₀) for most beneficial effects on offspring lung function, because we observed that maternal 25(OH)D level at 10 to 18 gestational weeks was significantly associated with offspring lung function and all subjects in the supplementation groups did not reach a 25(OH)D level of greater than or equal to 30 ng/mL at third trimester.^{10,11} The associations between gestational 25(OH)D level and offspring lung function remained significant in the multivariable model, which included other potential risk factors for lung function impairment such as childhood asthma phenotype, suggesting that gestational vitamin D has an independent effect on child lung function that is not mediated via changes in the risk of asthma. Furthermore, no significant interactions between gestational 25(OH)D level and child asthma were observed. Because of the notion of a "vitamin D paradox" with regard to blacks, vitamin D, and osteoporosis,³⁵ we also conducted race-stratified analyses. We found similar effects of gestational 25(OH)D levels on offspring lung function between black and nonblack participants in our cohort. Thus, higher 25(OH)D levels were associated with lower airway resistance and higher spirometry indices in both race categories. Organ system-specific effects of vitamin D require further study.

Lung function deficits in asthma are often detectable by school age, when spirometry can be reliably performed, suggesting that impairment of lung function occurs earlier in life.^{2,36,37} However, few studies have evaluated the lung function trajectories of different asthma phenotypes during preschool years.³⁸ We observed that children with asthma at age 6 years in the VDAART cohort had significantly lower levels of lung function from age 4 to 6 years than those in the asymptomatic/infrequent wheeze group, highlighting the importance of applicable lung function measurements for preschool age children to detect early lung function deficits. This is in line with previous studies that have reported an association between reduced infant lung function and asthma at school age³⁹ as well as persistent wheezing,¹ because the vast majority of the children with asthma at age 6 years in our study (91 of 105 [87%]) had asthmatic symptoms already during their first 3 years of life. Previous studies on the lung function of the early transient wheezing phenotype have been inconsistent.^{1,2,38,40} In our study, the early transient wheeze group showed subtle differences from the asymptomatic/infrequent wheeze group only in the IOS indices, particularly R5-20. Unlike spirometry, IOS requires only passive patient cooperation and can be reliably performed in preschool children.⁴¹ Our findings support a role for IOS in detecting early lung function deficits related to asthma, but validated multiethnic reference values are required for wider clinical implementation of IOS. Even though asthma control medications do not appear to affect the natural course of lung function in subjects with asthma,⁴²⁻⁴⁴ they can have beneficial effects on asthma control and short-term lung function.^{45,46} Therefore, minimizing the effects of medications on lung function by selecting medication-naïve subjects with asthma or subjects with a longer medication cessation could be hypothesized to result in larger differences in the lung function of subjects with

asthma when compared with asymptomatic controls than what was observed in the present study.

Environmental tobacco smoke exposure during pregnancy has been linked to different adverse birth outcomes such as stillbirth, low birth weight, and preterm birth,^{47,48} but its effects on offspring lung function have not been fully elucidated. We found that even in the absence of active smoking of the mother, household tobacco smoking at study enrollment was associated with increased respiratory resistance in the children, suggesting that minimizing not only active smoking but also secondhand smoke exposure during pregnancy could be important for offspring respiratory health. Further studies with objective measures of tobacco smoke exposure during pregnancy and early childhood could clarify the extent of exposure in subjects with detectable lung function deficits. In line with previous reports identifying preterm birth as an important risk factor for decreased lung function,^{49,50} we observed significant associations between preterm birth and lung function. Only 8 of the preterm children (16%) were born very preterm (<32 gestational weeks) when complications of prematurity are most often seen,⁵¹ highlighting that even moderate to late preterm birth might have significant effects on later lung function.⁵⁰

This study has several limitations. It should be acknowledged that loss to follow-up and missing data can lead to bias in longitudinal studies. However, comparison of child and parental baseline characteristics did not reveal any significant differences between the study subjects and those excluded except for a higher prevalence of maternal hay fever among the study subjects and differences in study site distribution. Furthermore, although our analyses were adjusted for different baseline characteristics of the mothers and children and the multivariable model included different risk factors for lung function impairment, the possibility of residual confounding should be acknowledged. Another limitation is that VDAART is a high-risk cohort for offspring asthma and half of the participating women received high-dose vitamin D₃ supplementation during pregnancy, which might limit the generalizability of the results and therefore, further studies in unselected populations are required to confirm the optimal gestational 25(OH)D level for offspring respiratory health in the general population. Finally, approximately half of the VDAART participants were black, which is more than in the general US population.⁵² This is due to the baseline demographic characteristics of the patient populations of the 3 study sites,⁵³ especially at St Louis and Boston (54% and 69% of the study subjects were black, respectively) and the higher prevalence of atopic diseases among black subjects than in the general US population,⁵⁴ because only women with a history of atopic diseases in themselves or their partners were recruited for VDAART.

Conclusions

We found that low gestational 25(OH)D level and childhood asthma were independently associated with decreased lung function from age 4 to 6 years. Both early and late pregnancy 25(OH)D levels were significantly associated with offspring lung function and suggested that gestational 25(OH)D level of greater than or equal to 30 ng/mL might be optimal for offspring respiratory health in subjects who are at high risk of asthma, but further studies are needed to define the optimal gestational 25(OH)D level in the general population.

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Clinical implications: Low gestational vitamin D level appears as an important modifiable risk factor for impaired lung function in early childhood.

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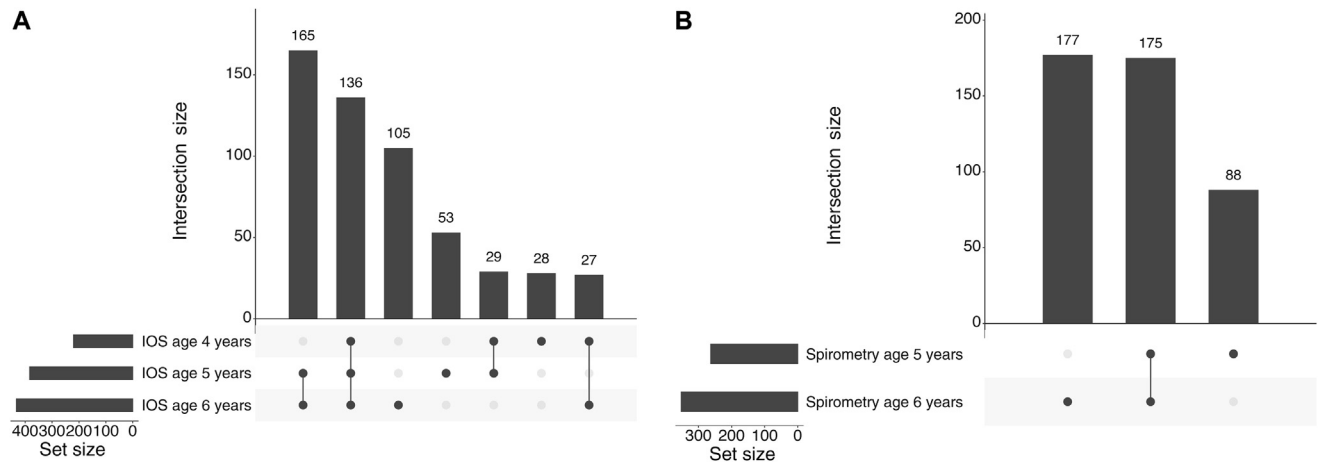


FIG E1. Upset diagrams illustrating the number of subjects who performed successful (A) IOS and (B) spirometry measurements at each visit.

TABLE E1. Maternal 25(OH)D-level quartiles at different time points and offspring lung function trajectories from age 4 to 6 y

Gestational 25(OH)D	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D-level quartiles	−0.016	.033	−0.010	.073
Third-trimester† 25(OH)D-level quartiles	−0.028	<.001	−0.019	<.001
Mean‡ 25(OH)D-level quartiles	−0.021	.002	−0.015	.004

	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β	<i>P</i> value	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D-level quartiles	0.015	.038	0.024	.001	−0.458	.116
Third-trimester† 25(OH)D-level quartiles	0.016	.012	0.018	.006	−0.006	.982
Mean‡ 25(OH)D-level quartiles	0.019	.007	0.022	.002	−0.074	.791

Data presented as β estimates (95% CIs) and *P* values for the trend between the 25(OH)D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, sex, race, study site, maternal age, maternal education level, maternal asthma, and household tobacco smoking at enrollment were included as fixed covariates. *P* values less than .05 are in boldface.

*At 10–18 gestational weeks.

†At 32–38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.

TABLE E2. Maternal 25(OH)D-level quartiles at different time points and offspring lung function trajectories from age 4 to 6 y among subjects with data on allergic sensitization (n = 522)

Maternal 25(OH)D status	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D-level quartiles	−0.016	.032	−0.010	.080
Third-trimester† 25(OH)D-level quartiles	−0.028	<.001	−0.019	<.001
Mean‡ 25(OH)D-level quartiles	−0.020	.006	−0.015	.008

Maternal 25(OH)D status	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β	<i>P</i> value	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D-level quartiles	0.013	.083	0.023	.003	−0.571	.059
Third-trimester† 25(OH)D-level quartiles	0.015	.028	0.017	.016	−0.030	.914
Mean‡ 25(OH)D-level quartiles	0.016	.028	0.020	.008	−0.114	.699

Data presented as β estimates (95% CIs) and *P* values for the trend between the 25(OH)D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, sex, race, study site, and allergic sensitization were included as fixed covariates. *P* values less than .05 are in boldface.

*At 10-18 gestational weeks.

†At 32-38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.

TABLE E3. Sex-stratified mixed-effects models for the longitudinal lung function trajectories of children categorized by maternal 25(OH)D-level quartiles at different time points

Child 25(OH)D	Males (n = 295)							
	IOS				Spirometry			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)		FEV ₁ (L)		FVC (L)	
	β	P value	β	P value	β	P value	β	P value
Baseline* 25(OH)D-level quartiles	−0.011	.302	−0.003	.749	0.014	.192	0.027	.013
Third- trimester† 25(OH) D-level quartiles	−0.027	.005	−0.016	.035	0.020	.051	0.022	.032
Mean‡ 25(OH)D-level quartiles	−0.019	.064	−0.009	.262	0.019	.066	0.025	.018
	Females (n = 275)							
	IOS				Spirometry			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)		FEV ₁ (L)		FVC (L)	
	β	P value	β	P value	β	P value	β	P value
Baseline* 25(OH)D-level quartiles	−0.023	.026	−0.018	.011	0.017	.058	0.023	.014
Third- trimester† 25(OH) D-level quartiles	−0.026	.005	−0.021	.001	0.013	.114	0.018	.046
Mean‡ 25(OH)D-level quartiles	−0.020	.042	−0.020	.004	0.018	.040	0.022	.018

Data presented as β estimates and *P* values for the trend between the 25(OH)D-level quartiles in mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, race, and study site were included as fixed covariates. *P* values less than .05 are in boldface.

*At 10-18 gestational weeks.

†At 32-38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.

TABLE E4. Race-stratified mixed-effects models for the longitudinal lung function trajectories of children categorized by maternal 25(OH)D level at different time points

Maternal 25(OH)D status	Blacks (n = 284)							
	IOLS				Spirometry			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)		FEV ₁ (L)		FVC (L)	
	β	P value	β	P value	β	P value	β	P value
Baseline* 25(OH)D-level quartiles	−0.015	.150	−0.005	.508	0.019	.053	0.025	.012
Third-trimester† 25(OH)D-level quartiles	−0.021	.021	−0.016	.018	0.018	.031	0.015	.091
Mean‡ 25(OH)D-level quartiles	−0.016	.113	−0.011	.119	0.018	.046	0.015	.104
	Other races (n = 286)							
	IOS				Spirometry			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)		FEV ₁ (L)		FVC (L)	
	β	P value	β	P value	β	P value	β	P value
Baseline* 25(OH)D-level quartiles	−0.020	.065	−0.015	.061	0.011	.315	0.020	.077
Third-trimester† 25(OH)D-level quartiles	−0.035	<.001	−0.022	.003	0.012	.216	0.020	.047
Mean‡ 25(OH)D-level quartiles	−0.026	.011	−0.017	.027	0.016	.119	0.025	.017

Data presented as β estimates and *P* values for the trend between the 25(OH)D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, sex, and study site were included as fixed covariates. *P* values less than .05 are in boldface.

*At 10–18 gestational weeks.

†At 32–38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.

TABLE E5. 25(OH)D-level quartiles during early life and longitudinal lung function trajectories from age 4 to 6 y

25(OH)D status	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
Cord blood 25(OH)D-level quartiles	−0.015	.045	−0.012	.024
Age 1 y 25(OH)D-level quartiles	-8.8×10^{-5}	.990	0.001	.785
Age 3 y 25(OH)D-level quartiles	−0.009	.206	−0.007	.215

	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β	<i>P</i> value	β	<i>P</i> value	β	<i>P</i> value
Cord blood 25(OH)D-level quartiles	0.007	.298	0.011	.127	−0.284	.333
Age 1 y 25(OH)D-level quartiles	0.002	.809	0.002	.776	−0.009	.973
Age 3 y 25(OH)D-level quartiles	0.005	.529	0.007	.347	−0.063	.834

Data presented as β estimates and *P* values for the trend between the 25(OH)D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, sex, race, and study site were included as fixed covariates. *P* values less than .05 are in boldface.

TABLE E6. Gestational 25(OH)D-level groups and offspring longitudinal lung function trajectories from age 4 to 6 y

Gestational 25(OH)D level (ng/mL)	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
<20	0.094	.003	0.063	.006
20-29	0.078	.007	0.042	.046
≥30	Reference		Reference	
Trend	0.049	.002	0.032	.005

Gestational 25(OH)D level (ng/mL)	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β	<i>P</i> value	β	<i>P</i> value	β	<i>P</i> value
<20	−0.081	.007	−0.098	.001	0.378	.761
20-29	−0.041	.138	−0.035	.215	−0.851	.465
≥30	Reference		Reference		Reference	
Trend	−0.041	.007	−0.048	.001	0.123	.842

Only those subjects who fulfilled the 25(OH)D-level criteria both at baseline (10-18 gestational weeks) and at third trimester (32-38 gestational weeks) were included in the analysis. Number of subjects in each subgroup: n = 91 for <20 ng/mL, n = 61 for 20-29 ng/mL, and n = 96 for ≥30 ng/mL. *P* values less than .05 are in boldface. Data presented as β estimates and *P* values for each group and for the trend between the groups in the mixed-effect models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level group, sex, race, and study site were included as fixed covariates.

TABLE E7. Childhood asthma phenotypes and longitudinal lung function trajectories from age 4 to 6 y among subjects with data on allergic sensitization (n = 522)

Asthma phenotype	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
Asymptomatic/infrequent wheeze	Reference		Reference	
Early transient wheeze	0.044	.038	0.039	.013
Asthma at age 6 y	0.063	.002	0.056	<.001

Asthma phenotype	Spirometry					
	FEV ₁ (L)		FVC (L)		FEV ₁ /FVC ratio	
	β	<i>P</i> value	β	<i>P</i> value	β	<i>P</i> value
Asymptomatic/infrequent wheeze	Reference		Reference		Reference	
Early transient wheeze	0.011	.574	0.026	.194	−1.119	.161
Asthma at age 6 y	−0.059	.002	−0.046	.021	−1.604	.044

Data presented as β estimates (95% CIs) and *P* values for the trend between the 25[OH]D-level quartiles in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). Childhood asthma phenotype, sex, race, study site, and allergic sensitization were included as fixed covariates. *P* values less than .05 are in boldface.

TABLE E8. Interaction between maternal 25(OH)D-level quartiles and child asthma status at age 6 y for the longitudinal lung function trajectories

	IOS			
	R5 (kPa/L/s)		R5-20 (kPa/L/s)	
	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D quartiles $\times$ Asthma	0.002	.924	0.008	.536
Third-trimester† 25(OH)D quartiles $\times$ Asthma	0.014	.423	0.007	.574
Mean‡ 25(OH)D quartiles $\times$ Asthma	0.011	.528	0.009	.491

	Spirometry			
	FEV ₁ (L)		FVC (L)	
	β	<i>P</i> value	β	<i>P</i> value
Baseline* 25(OH)D quartiles $\times$ Asthma	−0.010	.538	0.003	.842
Third-trimester† 25(OH)D quartiles $\times$ Asthma	−0.014	.388	0.020	.248
Mean‡ 25(OH)D quartiles $\times$ Asthma	2.6×10^{-5}	.999	−0.004	.799

Data presented as β estimates and *P* values for the interaction between 25(OH)D-level quartiles and child asthma status at age 6 y (yes/no) in the mixed-effects models. Time-dependent covariates included in the model were child age (years) and height (cm). 25(OH)D-level quartiles, child asthma status at age 6 y, sex, race, and study site were included as fixed covariates.

*At 10-18 gestational weeks.

†At 32-38 gestational weeks.

‡Mean of maternal baseline and third-trimester 25(OH)D levels.