

The long-term programming effect of maternal 25-hydroxyvitamin D in pregnancy on allergic airway disease and lung function in offspring after 20 to 25 years of follow-up

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Background: High prenatal vitamin D status has been linked to decreased risk of atopic diseases in early childhood, but whether such relations persist until adulthood has not been explored.

Objective: We sought to examine the association between maternal 25-hydroxyvitamin D (25(OH)D) concentrations and outcomes of allergic airway disease and lung function in offspring with 20 to 25 years of follow-up.

Methods: In a prospective birth cohort with 965 pregnant women enrolled in 1988-1989, maternal 25(OH)D concentrations were quantified in serum from gestational week 30 (n = 850 [88%]). Offspring were followed in nationwide registries with complete follow-up to the age of 25 years (n = 850 [100%]). Additionally, at age 20 years, outcomes of allergic airway disease and lung function were assessed in a subset of offspring by using blood samples and spirometry (n = 410 [45%]) and a questionnaire (n = 641 [70%]).

Results: Exposure to a high maternal 25(OH)D concentration (≥ 125 nmol/L) was associated with an increased risk of asthma hospitalizations in offspring (hazard ratio [HR], 1.81; 95% CI, 0.78-4.16) during 25 years of follow-up compared with the reference group ($75 < 125$ nmol/L). Furthermore, there were lower risks of asthma hospitalizations (HR, 0.29; 95% CI, 0.08-1.02) and asthma medication use (HR, 0.58; 95% CI, 0.35-0.95) in those exposed to a low maternal 25(OH)D concentration

(<50 nmol/L). In a reduced set of participants, we found no associations between maternal 25(OH)D concentrations and offspring allergen-specific IgE, total IgE, and eosinophil cationic protein levels; self-reported doctor's diagnosis of asthma or hay fever; or lung function at 20 years of age.

Conclusions: Our study does not provide support for a protective effect of a high maternal 25(OH)D concentration on outcomes of allergic airway disease and lung function at 20 to 25 years of age. In contrast, a high maternal 25(OH)D concentration might be associated with an increased risk of allergic diseases in offspring. (J Allergy Clin Immunol 2015;■■■■:■■■■-■■■■.)

Key words: Cohort, pregnancy, fetal programming, 25-hydroxyvitamin D, asthma, allergic rhinitis, lung function

Allergic diseases are among the most common chronic conditions worldwide, and they usually appear early in life, suggesting that the *in utero* environment might play an important role in their development. During the last decade, the role of insufficient maternal vitamin D during pregnancy in allergic disease development in offspring has received increasing attention as a potential causal factor.¹ During pregnancy, maternal vitamin D is transported to the fetus through the placenta in the form of 25-hydroxyvitamin D (25(OH)D), the main circulating metabolite of vitamin D.² Results from experimental studies have shown that vitamin D can act as an immunomodulator and affect fetal lung development.³⁻⁵ Therefore suboptimal prenatal vitamin D exposure might result in unfavorable programming and subsequent increased susceptibility for allergic diseases.

Evidence from epidemiologic studies on the association between prenatal vitamin D status and allergic diseases is conflicting. The only randomized controlled trial to date did not report any effect of vitamin D supplementation in pregnancy on a child's allergic outcomes at 3 years of age.⁶ Results from studies that have quantified maternal or cord blood 25(OH)D concentrations have been divergent: inverse associations with respiratory tract infections⁷⁻⁹ and wheeze^{7,10} have been shown in children up to 5 years of age, but high maternal or cord blood 25(OH)D concentrations have also been linked to increased risk of atopic disease in offspring up to 9 years of age.¹¹⁻¹⁴ However, other studies have shown no associations.^{7,8,10,15-17} Therefore uncertainty remains about the role of prenatal 25(OH)D on allergic disease development. Furthermore, the current literature does not provide insight into possible long-term programming

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Abbreviations used

BMI:	Body mass index
DNPR:	Danish National Patient Registry
ECP:	Eosinophil cationic protein
FVC:	Forced vital capacity
HR:	Hazard ratio
ICD:	International Classification of Diseases
25(OH)D:	25-Hydroxyvitamin D
OR:	Odds ratio
RMPS:	Register of Medicinal Product Statistics

effects of prenatal vitamin D on allergic disease outcomes persisting beyond childhood.

In this study we examined the hypothesis of a protective effect of high maternal vitamin D levels in relation to allergic airway disease and lung function in offspring after 20 to 25 years of follow-up.

METHODS

Study sample

The Danish Fetal Origins Cohort recruited 965 women with singleton pregnancies between 1988 and 1989 in Aarhus, Denmark. The cohort has been described in detail elsewhere.¹⁸ Briefly, data collection included a self-administered questionnaire and a face-to-face interview conducted around the 30th week of gestation at a midwife visit. A blood sample was taken, processed, and stored at -20°C .

In 2008, when offspring were 20 years old, we identified 915 (95%) mothers and their offspring from the Danish Central Person Registry and contacted them by mail with an invitation to the offspring to complete a Web-based self-administered questionnaire that, among a variety of health topics, included 4 questions on asthma and hay fever. The offspring were also invited to attend a clinical examination at a hospital in Aarhus.

The Danish Data Protection Agency and the Regional Scientific-Ethical Committee in Aarhus approved the study, and all participants provided written consent before inclusion.

Exposure

In 2010, maternal 25(OH)D levels were quantified in serum samples drawn in the third trimester of pregnancy in 1988–1989 by means of isotope dilution liquid chromatography–tandem mass spectrometry (LC-MS/MS vitamin D kit; PerkinElmer, Waltham, Mass), which is considered a valid measure of vitamin D¹⁹ and unaffected by time of storage.²⁰ The limit of detection was 8.5 nmol/L for vitamin D₃ and 5.9 nmol/L for vitamin D₂. Values below these limits were recalculated as $1/2 \times$ the limit of detection for vitamin D₃ and 0 for vitamin D₂. We defined total maternal vitamin D status as the sum of measured 25(OH)D₂ and 25(OH)D₃ concentrations. One outlier with a 25(OH)D₃ value of 367 nmol/L was excluded from the analyses.

Outcomes

Register outcomes (n = 850). We extracted data on asthma prescriptions (Anatomical Therapeutic Chemical classification system codes R03A, R03B, R03C, and R03D) and allergic rhinitis prescriptions (Anatomical Therapeutic Chemical classification system codes R01AC, R01AD, R01AX, R06A, S01GA, and S01GX) given to offspring from the Register of Medicinal Product Statistics (RMPS), which has collected information about prescriptions redeemed at Danish pharmacies since 1995. We used a previously validated algorithm to define *asthma medication use* from the RMPS²¹ as 2 prescriptions of either β_2 -agonists or steroids or 1 prescription of another asthma medication, including leukotriene receptor agonists. We applied a capture time from 1995, when the medication data were

first recorded until the end of 2013. To define cases of *allergic rhinitis medication use*, we used at least 2 prescriptions from 1995 until the end of 2013.

We extracted information about asthma hospitalizations in offspring by using the International Classification of Diseases (ICD) codes from the Danish National Patient Registry (DNPR), which has collected information about all hospital admissions since 1977 and information about all emergency department and outpatient visits since 1995. We defined cases of *asthma hospitalizations* as offspring with one of the following ICD-8 or ICD-10 diagnostic codes from birth to the end of 2013: 493.00, 493.01, 493.02, 493.08, 493.09, J45.0, J45.1, J45.2, J45.8, and J45.9. There were few cases of hospitalizations with an allergic rhinitis ICD code registered in the DNPR (n = 9 [1%]), and therefore this was not included as an outcome in our analyses.

Outcomes from clinical examination (n = 410). From blood samples drawn during the clinical examination in 2008 when the offspring were 20 years old, serum total IgE levels, specific IgE levels against 12 common inhalant allergens, and eosinophil cationic protein (ECP) levels were quantified by means of fluoroimmunoassay with ImmunoCAP (Phadia Laboratory Systems AB, Uppsala, Sweden). *Allergic sensitization* was defined as a positive test result for specific IgE (≥ 0.35 kU_A/L) to at least one of the 12 allergens. During the clinical examination, lung function was measured by using a daily calibrated Vitalograph spirometer (Vitalograph, Clare, Ireland) from which we recorded FEV₁ and forced vital capacity (FVC) values. Offspring were asked not to use any bronchodilators on the day of measurement. Mean values from at least 3 measures that did not vary more than 5% were registered. We estimated the FEV₁/FVC ratio and defined *airway obstruction* as present in offspring with an FEV₁/FVC ratio of less than 75%. The FEV₁/FVC ratio was also analyzed as a continuous variable.

Self-reported outcomes (n = 641). Offspring completed a questionnaire at 20 years of age. Doctor-diagnosed asthma and doctor-diagnosed hay fever were determined from positive answers to the following question: “Has a doctor ever told you that you had asthma/hay fever?” Current asthma medication use was determined from a positive answer to the following question: “Have you used any asthma medication in the previous 12 months?” The presence of current hay fever symptoms was determined from a positive answer to the following question: “Have you had any symptoms of hay fever such as runny eyes or an itchy nose in the previous 12 months?” The questions followed the International Studies on Asthma and Allergies in Childhood core questionnaire.²²

Cohort attrition

A flow chart of the cohort can be seen in Fig 1. Exposure information was available for 850 mothers. At 20 years of age, 410 (45%) of the invited offspring participated in the clinical examination, and 641 (70%) completed the questionnaire. The number of offspring with clinical outcome data varied from 402 to 407, depending on the outcome. The number of offspring with data on self-reported outcomes varied from 618 to 638. The registry-based outcomes (asthma and allergic rhinitis medication use and asthma hospitalizations) were available for 850 (100%) offspring of mothers with exposure information.

Statistical analyses

We defined 4 maternal 25(OH)D exposure groups based partly on guidelines from the Danish National Board of Health²³ and partly from the American Institute of Medicine²⁴ (ie, <50 nmol/L, 50–<75 nmol/L, 75–<125 nmol/L [reference group], and ≥ 125 nmol/L) to be able to account for potential nonlinear relationships. We also analyzed the maternal 25(OH)D concentration as a continuous variable.

We analyzed registry outcomes by using multivariable Cox regression models and calculated hazard ratios (HRs) and 95% CIs with age as the underlying time scale. We followed offspring from the date of birth in the DNPR or from January 1, 1995, in the RMPS until the age of first asthma hospitalization or asthma or allergic rhinitis medication use, respectively, or the defined end of follow-up on December 31, 2013, whichever came first. No

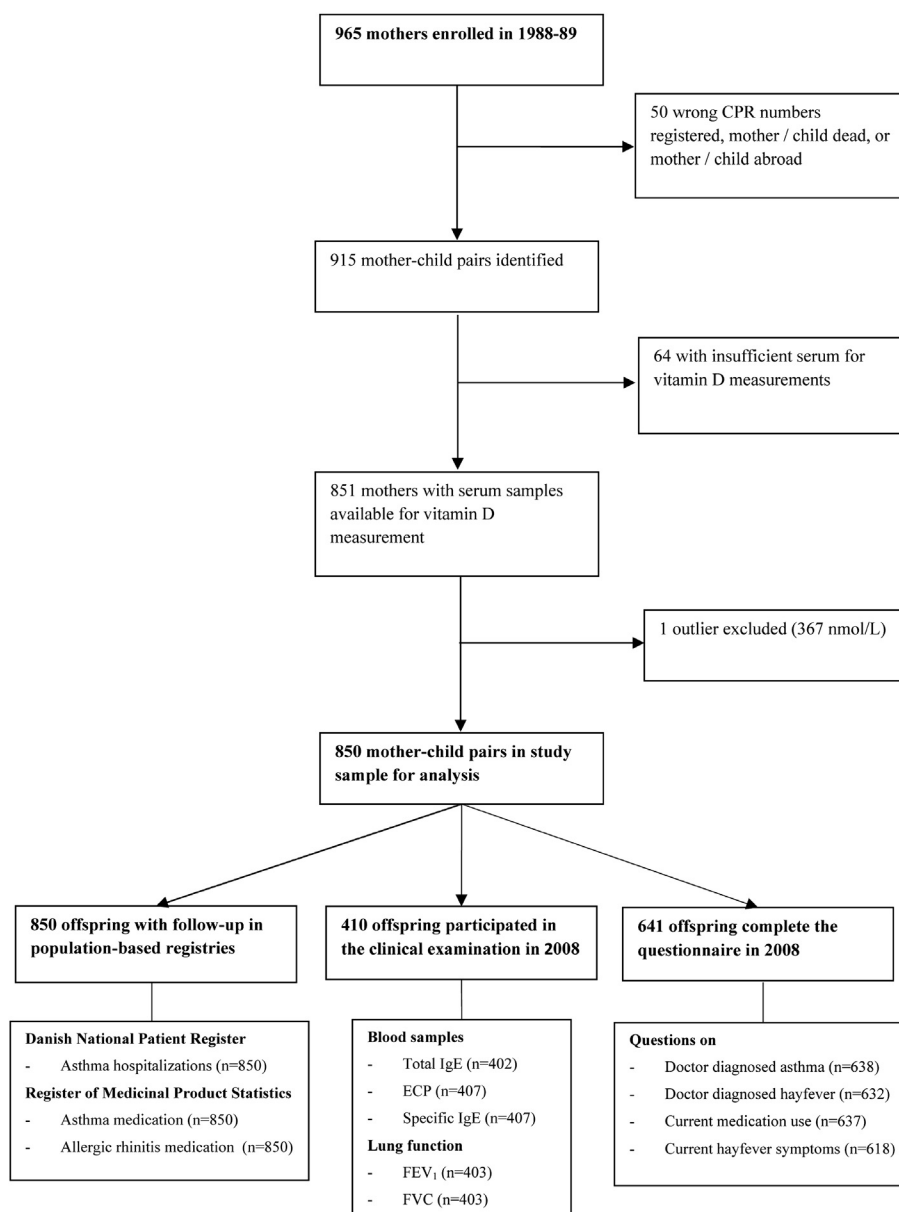


FIG 1. Participation flow in the birth cohort. *CPR*, Central Person Registry.

offspring were censored for other reasons. In the reduced samples we used multivariable logistic regression models to calculate odds ratios (ORs) and 95% CIs to analyze binary outcomes. Continuous outcomes were analyzed by using multivariable linear regression models from which we calculated β estimates for normally distributed variables or ratios between geometric means for log-transformed variables with corresponding 95% CIs. All tests were 2-sided, and we used a threshold *P* value of less than .05 to denote statistical significance. *P* values were based on likelihood ratio tests, and all analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC).

A priori, we selected the following covariates: maternal age (in years), parity (0, 1, and ≥ 2), maternal education (elementary school, high school or technical school, college, higher academic, or other education), prepregnancy body mass index (BMI; in kilograms per square meter), smoking during pregnancy (yes/no), and the offspring's sex. Maternal education, prepregnancy BMI, and smoking were self-reported by the women at study enrollment, whereas information on age, parity, and the offspring's sex was obtained from the Medical Birth Registry. In analyses with FEV₁/FVC ratios, we

additionally adjusted for offspring height measured during the clinical examination. We substituted missing covariate information (maternal education, 5%; smoking, 6%; and prepregnancy BMI, 4%) by using the mode value for education (college) and smoking (nonsmokers) and the median for prepregnancy BMI (20.9 kg/m²). Complete case analysis did not reveal any material changes in effect estimates. Covariates suspected to be on the causal pathway (gestational age and birth weight) were not included in the main models to avoid overadjustment. Adjustment for these variables did not substantially alter study results.

We decided not to adjust for season in our main models to allow for natural variation in 25(OH)D levels. However, in preplanned supplementary analyses we additionally adjusted for season of birth classified according to the strength of the UVB index (winter [December-February], spring [March-May], summer [June-August], and fall [September-November]) to investigate the association between maternal 25(OH)D concentrations and offspring allergic disease independent of the seasonal influence on 25(OH)D concentrations. None of the effect estimates changed materially on additional adjustment for season of birth (data not shown).

TABLE I. Maternal and offspring characteristics in 850 mother-offspring pairs from the Danish Fetal Origins Birth Cohort (1988-1989)

Maternal and offspring characteristics	Overall*	Maternal 25(OH)D serum concentration				P value†
		<50 nmol/L, n = 217 (25.5%)	50-<75 nmol/L, n = 199 (23.4%)	75-<125 nmol/L, n = 321 (37.8%)	≥125 nmol/L, n = 113 (13.3%)	
Maternal 25(OH)D (nmol/L)‡	76.2 (57.0)	35.1 (18.3)	62.8 (13.1)	97.6 (22.9)	144.6 (23.4)	
Maternal age at birth (y)	29.0 (4.2)	28.8 (4.1)	29.3 (4.4)	29.1 (4.3)	28.9 (3.8)	.62
Maternal prepregnancy BMI (kg/m ²)§	21.4 (3.1)	21.9 (3.7)	21.2 (3.0)	21.4 (3.0)	21.1 (2.6)	.10
Previous births						.004
0	490 (57.7%)	100 (20.4%)	115 (23.5%)	206 (42.0%)	69 (14.1%)	
1	271 (31.9%)	88 (32.5%)	66 (24.4%)	83 (30.6%)	34 (12.6%)	
≥2	89 (10.5%)	29 (32.6%)	18 (20.2%)	32 (36.0%)	10 (11.2%)	
Smoking during pregnancy						.12
Yes	323 (40.1%)	88 (27.2%)	85 (26.3%)	114 (35.3%)	36 (11.2%)	
No	482 (59.9%)	111 (23.0%)	108 (22.4%)	190 (39.4%)	73 (15.2%)	
Maternal education¶						.37
Elementary school	102 (12.7%)	32 (31.4%)	20 (19.6%)	36 (35.3%)	14 (13.7%)	
High school or technical school	202 (25.1%)	46 (22.8%)	51 (25.3%)	73 (36.1%)	32 (15.8%)	
University	289 (36.0%)	67 (23.2%)	66 (22.8%)	111 (38.4%)	45 (15.6%)	
Higher academic	124 (15.4%)	27 (21.8%)	37 (29.8%)	49 (39.5%)	11 (8.9%)	
Other education	87 (10.8%)	26 (29.9%)	19 (21.8%)	35 (40.2%)	7 (8.1%)	
Season of birth						<.001
Winter	307 (36.1%)	118 (38.4%)	75 (24.4%)	98 (31.9%)	16 (5.2%)	
Spring	132 (15.5%)	38 (28.8%)	48 (36.4%)	41 (31.1%)	5 (3.8%)	
Summer	164 (19.3%)	36 (22.0%)	38 (23.2%)	77 (47.0%)	13 (7.9%)	
Fall	247 (29.1%)	25 (10.1%)	38 (15.4%)	105 (42.5%)	79 (32.0%)	
Birth weight (g)	3500 (532.3)	3454 (575.7)	3510 (489.5)	3485 (529.1)	3612 (517.2)	.07
Gestational age (d)	282.4 (11.7)	281 (12.9)	282 (12.7)	283 (11.0)	283 (8.9)	.38
Sex						.41
Boys	445 (52.4%)	105 (23.6%)	111 (24.9%)	166 (37.3%)	63 (14.2%)	
Girls	405 (47.7%)	112 (27.7%)	88 (21.7%)	155 (38.3%)	50 (12.4%)	

*Values are means (SDs) or numbers (percentages).

†P values are calculated by using the χ^2 test for categorical variables and the F test for continuous variables.

‡Values are medians (interquartile ranges).

§3.7% missing.

||5.3% missing.

¶5.4% missing.

RESULTS

Study population

The median maternal 25(OH)D concentration in our study sample was 76 nmol/L (interquartile range, 57 nmol/L; [Table I](#)). Characteristics of mothers and offspring are presented in [Table I](#), and the distribution of outcomes in offspring are shown in [Table II](#).

We observed few differences between mother-offspring pairs with (n = 850) and without (n = 65) maternal 25(OH)D measures (see [Table E1](#) in this article's Online Repository at www.jacionline.org). Mothers of offspring who participated in the clinical examination or completed the questionnaire were more likely to be nonsmokers and to have higher academic education levels compared with mothers of nonparticipating offspring.

Maternal 25(OH)D concentration and asthma hospitalizations, asthma medication use, and allergic rhinitis medication use in offspring

There was a significant difference in the HRs of asthma hospitalization in offspring across maternal 25(OH)D exposure groups ($P = .03$, [Table III](#)). Compared with the reference group (75-<125 nmol/L), the HR for the lowest exposure group (<50 nmol/L) was 0.29 (95% CI, 0.08-1.02), whereas offspring

TABLE II. Characteristics of offspring outcomes of allergic airway disease and lung function

Offspring outcomes	
Registry-based outcomes (25-y follow-up)	
Asthma medication (n = 850)	126 (14.8%)
Allergic rhinitis medication (n = 850)	205 (24.1%)
Asthma hospitalization (n = 850)	33 (3.9%)
Self-reported outcomes (at 20 y)	
Lifetime doctor's diagnosis of asthma (n = 638)	85 (13.3%)
Current asthma medication use (n = 637)	45 (7.1%)
Lifetime doctor's diagnosis of hay fever (n = 632)	100 (15.8%)
Current hay fever symptoms (n = 618)	198 (32.0%)
Clinical outcomes (at 20 y)	
Allergic sensitization (n = 407)	137 (33.7%)
Total IgE (kU _A /L [n = 402])	27.8 (78.0)*
ECP (μg/L [n = 407])	5.2 (5.0)*
FEV ₁ /FVC ratio (n = 403)	84.7 (7.0)
Airway obstruction (n = 403)	39 (9.7%)

Values are means (SDs) or numbers (percentages), unless otherwise specified.

*Medians (interquartile ranges).

of mothers with high 25(OH)D concentrations (≥125 nmol/L) had an HR of 1.80 (95% CI, 0.78-4.14). Furthermore, there was a significant continuous direct trend ($P = .001$). For asthma medication use, we similarly found a decreased HR for the

TABLE III. Multivariable associations between maternal 25(OH)D concentrations in pregnancy and asthma hospitalizations, asthma medication use, and allergic rhinitis medication use in offspring during 25 years of follow-up (n = 850)

	No. of cases/total	HR (95% CI)
Asthma hospitalizations		
<50 nmol/L 25(OH)D	3/217 (1.4%)	0.29 (0.08-1.02)
50-<75 nmol/L 25(OH)D	6/199 (3.0%)	0.62 (0.24-1.59)
75-<125 nmol/L 25(OH)D	15/321 (4.7%)	1
≥125 nmol/L 25(OH)D	9/113 (8.0%)	1.81 (0.78-4.16)
		<i>P</i> = .03*
		<i>P</i> = .001†
Asthma medication use		
<50 nmol/L 25(OH)D	22/217 (10.1%)	0.58 (0.35-0.95)
50-<75 nmol/L 25(OH)D	28/199 (14.1%)	0.83 (0.52-1.31)
75-<125 nmol/L 25(OH)D	56/321 (17.5%)	1
≥125 nmol/L 25(OH)D	20/113 (17.7%)	1.02 (0.61-1.71)
		<i>P</i> = .14*
		<i>P</i> = .09†
Allergic rhinitis medication use		
<50 nmol/L 25(OH)D	42/217 (19.4%)	0.75 (0.51-1.10)
50-<75 nmol/L 25(OH)D	53/199 (26.6%)	1.12 (0.79-1.60)
75-<125 nmol/L 25(OH)D	80/321 (24.9%)	1
≥125 nmol/L 25(OH)D	30/113 (26.6%)	1.08 (0.70-1.64)
		<i>P</i> = .24*
		<i>P</i> = .31†

Cox regression models adjusted for potential confounding by maternal age, parity, smoking during pregnancy, prepregnancy BMI, education, and offspring sex.

**P* value for a homogeneity test by which we test for a different effect of maternal 25(OH)D concentrations on outcomes.

†*P* value for the effect of maternal 25(OH)D concentration as a continuous variable on outcomes.

offspring in the lowest maternal exposure group (HR, 0.57; 95% CI, 0.35-0.95) compared with the reference group, whereas the offspring of mothers with the highest 25(OH)D concentrations had an HR of 1.02 (95% CI, 0.61-1.17). However, the HRs did not differ significantly (*P* = .14), and the continuous trend was also nonsignificant (*P* = .09). No associations were seen between maternal 25(OH)D concentrations and allergic rhinitis medication use.

Maternal 25(OH)D concentrations and allergic sensitization, total IgE levels, ECP levels, and measures of lung function in offspring

In a reduced sample of participants (n = 403-407), we found no significant differences in the effect estimates of allergic sensitization or airway obstruction in offspring at 20 years of age across maternal 25(OH)D exposure groups; evaluating maternal 25(OH)D as a continuous exposure did not change this result (Table IV). Also, we did not find any associations between maternal 25(OH)D concentrations and total IgE levels, ECP levels, or FEV₁/FVC ratios in offspring as a continuous measure at age 20 years (see Table E2 in this article's Online Repository at www.jacionline.org).

Maternal 25(OH)D concentrations and self-reported outcomes of asthma and hay fever in offspring

In a reduced sample of participants (n = 618-638), there were no associations between maternal 25(OH)D concentrations and

TABLE IV. Multivariable association between maternal 25(OH)D concentrations in pregnancy and offspring allergic sensitization and airway obstruction (FEV₁/FVC ratio <75%) at 20 years of age in a reduced sample (n = 403-407)

	No. of cases/total	OR (95% CI)
Allergic sensitization (sIgE ≥0.35 kU_A/L)		
<50 nmol/L 25(OH)D	29/104 (27.9%)	0.72 (0.42-1.27)
50-<75 nmol/L 25(OH)D	34/86 (39.5%)	1.28 (0.73-2.23)
75-<125 nmol/L 25(OH)D	54/157 (34.4%)	1
≥125 nmol/L 25(OH)D	20/60 (33.3%)	0.98 (0.52-1.87)
		<i>P</i> = .37*
		<i>P</i> = .43†
Airway obstruction (FEV₁/FVC ratio <75%)		
<50 nmol/L 25(OH)D	10/102 (9.8%)	0.86 (0.37-2.00)
50-<75 nmol/L 25(OH)D	3/86 (3.5%)	0.31 (0.09-1.09)
75-<125 nmol/L 25(OH)D	19/155 (12.3%)	1
≥125 nmol/L 25(OH)D	7/60 (11.7%)	1.14 (0.44-2.98)
		<i>P</i> = .18*
		<i>P</i> = .13†

Logistic regression models adjusted for potential confounding by maternal age, parity, smoking during pregnancy, prepregnancy BMI, education, and offspring sex. Airway obstruction was further adjusted for height of offspring.

**P* value for a homogeneity test by which we test for a different effect of maternal 25(OH)D concentrations on outcomes.

†*P* value for effect of maternal 25(OH)D concentration as a continuous variable on outcomes.

self-reported doctor-diagnosed asthma, doctor-diagnosed hay fever, or current hay fever symptoms in offspring (Table V). In contrast, we found a significant difference in the ORs of self-reported current asthma medication use at age 20 years (*P* = .04) across maternal 25(OH)D exposure groups. Offspring of mothers with the lowest 25(OH)D levels (<50 nmol/L) had a lower OR for using current asthma medication (OR, 0.39; 95% CI, 0.14-1.08) compared with the reference group (75-<125 nmol/L), whereas offspring of mothers with the highest 25(OH)D concentrations (≥125 nmol/L) had an increased OR of 1.82 (95% CI, 0.81-4.04), and there was a significant continuous direct trend (*P* = .01). However, we could not replicate this finding in the RMPS. When we extracted information about asthma prescriptions redeemed in 2007-2008 corresponding to the same time period as the questionnaire was completed, we saw suggestions toward similar effect estimates as for the self-reported outcome, but the association was not statistically significant (*P* for trend = .17, data not shown).

DISCUSSION

In this prospective study we found no evidence of a protective programming effect of a high maternal 25(OH)D concentration in the third trimester of pregnancy and outcomes of allergic airway disease and lung function in offspring after 20 to 25 years of follow-up. In contrast, there was a direct association for maternal 25(OH)D concentrations with asthma hospitalizations during a 25-year follow-up period and with self-reported current asthma medication use at 20 years of age, with the highest risks in those exposed to the highest maternal 25(OH)D concentrations (≥125 nmol/L). In a reduced sample of participants, we found no associations between maternal 25(OH)D concentrations and allergic sensitization, total IgE levels, ECP levels, self-reported doctor's diagnosis of asthma or hay fever, or measures of lung function in offspring at 20 years of age. Our results were

TABLE V. Multivariable association between maternal 25(OH)D concentration in pregnancy and self-reported outcomes of asthma and hay fever in offspring at 20 years of age (n = 618-638)

	No. of cases/total	OR (95% CI)
Doctor-diagnosed asthma		
<50 nmol/L 25(OH)D	17/163 (10.4%)	0.80 (0.42-1.53)
50-<75 nmol/L 25(OH)D	24/145 (16.6%)	1.34 (0.75-2.40)
75-<125 nmol/L 25(OH)D	31/244 (12.7%)	1 (ref.)
≥125 nmol/L 25(OH)D	13/86 (15.1%)	1.22 (0.60-2.48)
		P = .48*
		P = .71†
Doctor-diagnosed hay fever		
<50 nmol/L 25(OH)D	25/161 (15.5%)	0.82 (0.46-1.43)
50-<75 nmol/L 25(OH)D	22/145 (15.2%)	0.86 (0.48-1.52)
75-<125 nmol/L 25(OH)D	42/242 (17.4%)	1 (ref.)
≥125 nmol/L 25(OH)D	11/84 (13.1%)	0.69 (0.33-1.43)
		P = .74*
		P = .99†
Current asthma medication use		
<50 nmol/L 25(OH)D	5/162 (3.1%)	0.39 (0.14-1.08)
50-<75 nmol/L 25(OH)D	10/145 (6.9%)	0.87 (0.39-1.94)
75-<125 nmol/L 25(OH)D	19/244 (7.8%)	1 (ref.)
≥125 nmol/L 25(OH)D	11/86 (12.8%)	1.82 (0.81-4.04)
		P = .04*
		P = .01†
Current hay fever symptoms		
<50 nmol/L 25(OH)D	47/155 (30.3%)	0.86 (0.54-1.35)
50-<75 nmol/L 25(OH)D	44/139 (31.7%)	0.92 (0.58-1.46)
75-<125 nmol/L 25(OH)D	78/237 (32.9%)	1 (ref.)
≥125 nmol/L 25(OH)D	29/87 (33.3%)	0.94 (0.55-1.61)
		P = .93*
		P = .76†

Logistic regression models adjusted for potential confounding by maternal age, parity, smoking during pregnancy, prepregnancy BMI, education, and offspring sex.

*P value for a homogeneity test by which we test for a different effect of maternal 25(OH)D concentrations on outcomes.

†P value for the effect of maternal 25(OH)D concentration as a continuous variable on outcomes.

consistent regardless of adjustment for a range of possible confounding variables and also after including season of birth.

The strongest associations in our study were found for 2 register outcomes that included a longitudinal follow-up over a 25-year period (n = 850), whereas the follow-up measures of allergic airway disease and lung function in the reduced sample (n = 410) at 20 years of age did not suggest any relation with maternal 25(OH)D status. We can only speculate whether any associations between maternal 25(OH)D concentrations and measures of allergic airway disease or lung function in offspring from the clinical examination would have been present earlier in childhood. In the Australian Raine cohort 25(OH)D concentrations in midpregnancy were positively associated with lung function measures at 6 years of age but not at age 14 years.²⁵ Furthermore, although we asked the offspring not to use any asthma or hay fever medication on the day of the examination, it cannot be ruled out that the effect of long-acting medications has prevented us from detecting any associations with outcomes from the clinical examination in well-medicated offspring, a circumstance that would not affect the registry-based outcomes.

We are not aware of any other prospective studies that have examined the relation of maternal 25(OH)D concentrations in pregnancy with allergic airway disease or lung function in

offspring with follow-up until adulthood. One previous study reported a direct association between vitamin D supplementation in the first year of life and current asthma or allergic rhinitis at 31 years of age in a Finnish cohort of children born in 1966.²⁶ However, different timing of exposure (prenatal vs postnatal), as well as different exposure measurements (maternal report of vitamin D supplementation vs quantification of 25[OH]D) complicates comparison with our results. Our results are partly in line with those of previous studies that reported no associations between late pregnancy or cord blood 25(OH)D levels and offspring lung function^{27,28} and allergic sensitization^{16,17,29} in children up to 9 years of age. Furthermore, evidence of a direct association has also been found between maternal or cord blood 25(OH)D concentrations and food allergy in 2-year-old offspring in a German birth cohort¹⁴ and with asthma at 9 years of age in a cohort study from the United Kingdom.¹¹ High predicted maternal vitamin status (>100 nmol/L) was recently linked to increased risk of asthma hospitalization until 7 years of age in a different Danish study population.¹²

Results from experimental studies have pointed toward a complicated immune response to vitamin D, but the exact mechanisms remain to be elucidated. One explanation is that the biologically active form of vitamin D, 1,25(OH)D, affects development of T_H cell responses. Consistent with the direct associations with register-based asthma outcomes in our data, vitamin D has been shown to inhibit T_H1 and upregulate proallergic T_H2 responses.^{30,31} However, the effect of vitamin D on T_H cell development has also been subject to opposite results.^{32,33} Furthermore, the immune relationship might be mediated by genetic variability that affects individual susceptibility to and effects of vitamin D exposure.³⁴ This might explain the inconsistent results across not only epidemiologic but also experimental studies.

Strengths of the present study include the long follow-up period, which enabled us to examine the fetal programming effect of maternal 25(OH)D concentrations on the risk of outcomes of allergic airway disease and lung function with a long-term perspective. We had objective measures of both the exposure (maternal 25(OH)D concentration) and some of our outcomes, including register data, which have previously been shown to be of high validity,³⁵ as well as clinical measures at age 20 years in a subset of offspring. Compared with earlier studies that have for the most part relied on maternal report of either the exposure (vitamin D intake) or the outcome (offspring allergic airway disease) or both, we believe our use of objective measures to be a considerable strength, since we hereby minimize the risk of misclassification. By using register data with complete follow-up through 25 years, we limited the possibility of selection bias; in the reduced samples with outcomes based on the self-reported (n = 641) and clinical examination (n = 410), data selection bias constitutes a potential risk. However, we did not see any differences either with respect to maternal 25(OH)D concentrations or registry outcomes across participation status in the self-reported and clinical examination compared with the full study sample (data not shown).

There are also some limitations to our study that require consideration. Regarding exposure, we relied on a single 25(OH)D measurement, which might not reflect maternal vitamin D status during the entire pregnancy, which usually spans over 3 seasons. Any misclassification that might have resulted from this we expect to be random and at the most would have attenuated our

results toward the null. Furthermore, we had detailed information about a range of potential confounders from questionnaires and registries, but these did not include parental atopic history, which could potentially confound the associations under study. With respect to the outcomes, it can be seen as a limitation that medication use in the offspring was not registered until 1995. This compromises our ability to examine the relation of prenatal 25(OH)D status on early transient wheezing and asthma symptoms early in life, which is a common outcome in other studies. Furthermore, some of the self-reported outcomes covered diagnoses given through the entire lifetime, which might not be valid for nonpersistent outcomes in early childhood because of inaccurate recall or lack thereof.

In summary, in this prospective observational cohort with 20 to 25 years of follow-up, we found no evidence of a protective programming effect of high maternal 25(OH)D concentrations on allergic airway disease and lung function in offspring; rather, we saw a direct association for some of our outcomes with increasing risks of asthma hospitalizations and asthma medication use in offspring with increasing maternal 25(OH)D concentration. Therefore our results do not lend support to recommending pregnant women to take supplements with vitamin D to achieve high 25(OH)D serum concentrations for the purpose of preventing allergic airway disease or improving lung function in their offspring.

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Key messages

- Exposure to high prenatal vitamin D levels might be associated with a decreased risk of allergic respiratory disease; however, there is limited knowledge about long-term programming effects.
- In this prospective study with 20 to 25 years of follow-up, we found no evidence of a protective association between high maternal 25(OH)D concentrations and allergic airway disease or lung function in offspring. In contrast, there was a significant increased risk of asthma hospitalization in offspring exposed to a high maternal 25(OH)D concentration.
- This result does not advocate for vitamin D supplementation in pregnancy to obtain high 25(OH)D serum concentrations to prevent allergic airway disease in offspring.

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TABLE E1. Characteristics among mother-child pairs (n = 915) based on exposure information availability, completion of follow-up questionnaires, and participation in clinical examination in the Danish Fetal of Origins Cohort 1988-1989

	No serum sample	Serum sample	P value
Maternal characteristics	n = 65	n = 850	
Mean 25(OH)D (nmol/L)	—	80.4 (40.5)	—
Maternal age at birth (y)	29.0 (4.7)	29.0 (4.2)	.97
Prepregnancy BMI (kg/m ²)	21.5 (2.8)	21.4 (3.1)	.83
Previous births (% nulliparous)	41 (64.1%)	490 (57.7%)	.37
Maternal smoking during pregnancy (% smokers)	21 (38.9%)	323 (40.1%)	.86
Maternal education (% higher academic)	7 (10.9%)	124 (14.6%)	.95
Birth season (% winter)	10 (15.6%)	307 (36.2%)	<.001
Offspring characteristics			
Birth weight (g)	3321.9 (605.3)	3500.0 (532.3)	.01
Gestational age (d)	282.4 (12.5)	282.3 (11.7)	.99
Sex (% boys)	31 (48.4%)	445 (52.4%)	.55
	Nonparticipants, questionnaire	Participants, questionnaire	P value
Maternal characteristics	n = 225	n = 690	
Mean 25(OH)D (nmol/L)	78.6 (40.0)	81.0 (40.9)	.46
Maternal age at birth (y)	29.7 (4.5)	29.2 (4.1)	.16
Prepregnancy BMI (kg/m ²)	21.7 (3.7)	21.3 (2.9)	.14
Previous births (% nulliparous)	125 (55.6%)	406 (58.9%)	.30
Maternal smoking during pregnancy (% smokers)	105 (50%)	239 (36.8%)	.01
Maternal education (% higher academic)	15 (7.1%)	116 (17.9%)	<.001
Birth season (% winter)	69 (30.7%)	248 (36.0%)	.05
Offspring characteristics			
Birth weight (g)	3384.4 (559.9)	3521.0 (528.6)	.001
Gestational age (d)	281.8 (12.2)	282.6 (11.6)	.39
Sex (% boys)	151 (67.1%)	325 (47.2%)	<.001
	Nonparticipants, clinical examination	Participants, clinical examination	P value
Maternal characteristics	n = 472	n = 443	
Mean 25(OH)D (nmol/L)	78.4 (39.9)	82.6 (41.1)	.13
Maternal age at birth (y)	28.9 (4.3)	29.2 (4.1)	.32
Prepregnancy BMI (kg/m ²)	21.4 (3.2)	21.4 (3.0)	.97
Previous births (% nulliparous)	265 (56.1%)	266 (60.2%)	.46
Maternal smoking during pregnancy (% smokers)	188 (42.9%)	156 (37.1%)	.06
Maternal education (% higher academic)	51 (10.8%)	80 (18.1%)	<.001
Birth season (% winter)	153 (32.4%)	164 (37.1%)	.52
Offspring characteristics			
Birth weight (g)	3467.6 (558.1)	3508.7 (518.4)	.25
Gestational age (d)	282.1 (12.0)	282.6 (11.4)	.59
Sex (% boys)	300 (63.6%)	176 (39.8%)	<.001

Missing values for 25(OH)D, prepregnancy BMI, maternal smoking during pregnancy, and maternal education so numbers and % do not add up to total n. Values are means (SDs) or numbers (percentages). *P* values were calculated by using χ^2 or *t* tests.

TABLE E2. Multivariable associations between maternal 25(OH)D concentrations in pregnancy and total IgE levels, ECP levels, and FEV₁/FVC ratios in offspring at 20 years of age (n = 403-407)

	Median (IQR)	Ratio between geometric means (95% CI)
Total IgE (kU _A /L)		
<50 nmol/L 25(OH)D	19.0 (56.2)	0.71 (0.48 to 1.08)
50-<75 nmol/L 25(OH)D	33.2 (86.0)	0.99 (0.64 to 1.52)
75-<125 nmol/L 25(OH)D	31.9 (78.4)	1
≥125 nmol/L 25(OH)D	17.8 (99.2)	0.71 (0.43 to 1.16)
		<i>P</i> = .26*
		<i>P</i> = .85†
ECP (μg/L)		
<50 nmol/L 25(OH)D	4.9 (5.2)	0.93 (0.79 to 1.10)
50-<75 nmol/L 25(OH)D	5.0 (3.8)	0.90 (0.75 to 1.07)
75-<125 nmol/L 25(OH)D	5.7 (6.2)	1
≥125 nmol/L 25(OH)D	4.9 (4.1)	0.85 (0.69 to 1.04)
		<i>P</i> = .38*
		<i>P</i> = .97†
FEV ₁ /FVC ratio (%)		
	Mean (SD)	β (95% CI)
<50 nmol/L 25(OH)D	84.0 (7.2)	0.11 (−1.63 to 1.86)
50-<75 nmol/L 25(OH)D	85.7 (6.7)	1.50 (−0.34 to 3.35)
75-<125 nmol/L 25(OH)D	84.0 (6.8)	0
≥125 nmol/L 25(OH)D	84.3 (7.4)	0.15 (−1.93 to 2.23)
		<i>P</i> = .41*
		<i>P</i> = .32†

Linear regression models adjusted for potential confounding by maternal age, parity, smoking during pregnancy, prepregnancy BMI, education, and offspring sex. FEV₁/FVC ratio was further adjusted for offspring height.

IQR, Interquartile range.

**P* value for a homogeneity test by which we test for a different effect of maternal 25(OH)D concentrations on outcomes.

†*P* value for the effect of maternal 25(OH)D concentration as a continuous variable on outcomes.