ORIGINAL ARTICLE

Epidemiology of Allergic Disease

Maternal and neonatal 25-hydroxyvitamin D concentrations and school-age lung function, asthma and allergy. The Generation R Study

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Summary

Background: Vitamin D deficiency in early life might affect the developing lung and immune system, and subsequently influence the risk of asthma and allergy in later life.

Objective: We examined the associations of 25-hydroxyvitamin D concentrations in mid-gestation and at birth with lung function, asthma, inhalant allergic sensitization and inhalant allergy at school-age.

Methods: This study among 4951 children and their mothers was embedded in a population-based prospective cohort in Rotterdam, the Netherlands. Maternal venous blood samples in mid-gestation and umbilical cord blood samples at birth were used to determine 25-hydroxyvitamin D concentrations. At age 10 years, lung function was measured by spirometry, current asthma and physician-diagnosed inhalant allergy by questionnaire, and inhalant allergic sensitization by skin prick tests. We used multivariable regression models to examine associations.

Results: Higher 25-hydroxyvitamin D concentrations in mid-gestation were associated with a higher forced vital capacity (FVC), but a lower forced expiratory volume in 1 second/FVC (FEV_1/FVC) and a lower forced expiratory flow after exhaling 75% of FVC (FEF_{75}) (Z-score differences [95% CI] 0.02 [0.00, 0.03], −0.02 [−0.03, −0.01] and −0.01 [-0.03, −0.00], respectively, per 10 nmol/L 25-hydroxyvitamin D), but not with asthma. Furthermore, higher 25-hydroxyvitamin D concentrations in mid-gestation were associated with an increased risk of inhalant allergy (Odds Ratio [95% CI] 1.07 [1.02, 1.12]), but not with inhalant allergic sensitization. After additional adjustment for child's 25-hydroxyvitamin D concentrations at the age of 6 years, only the associations of 25-hydroxyvitamin D concentrations in mid-gestation with FEV_1/

FVC and FEF$_{75}$ remained. We did not find consistent associations of 25-hydroxyvitamin D concentrations at birth with respiratory or allergy outcomes.

Conclusion and clinical relevance: Our results suggest that maternal 25-hydroxyvitamin D concentrations in mid-gestation may influence lung development. The clinical implications of the observed associations remain unclear.

1 | INTRODUCTION

Fetal vitamin D deficiency might have adverse effects on the developing lung and immune system, and subsequently influence lung function, and the risk of asthma and allergy in later life.[1,2] Vitamin D deficiency during pregnancy may alter the expression of genes associated with asthma in children,[3] potentially through their roles in lung development and airway branching morphogenesis.[3,4] Vitamin D might also have immunomodulatory effects in utero, including inhibition of fetal Th2 responses, which might affect the development of asthma and allergy in childhood.[5] A combined analysis of two randomized controlled trials showed that high-dose vitamin D supplementation in pregnancy reduced the risk of asthma with 26% in pre-school children.[6] Three meta-analyses of prospective cohort studies on the associations of early life 25-hydroxyvitamin D concentrations with respiratory and allergy outcomes in children found inconsistent results.[7-9] One meta-analysis reported that lower and higher 25-hydroxyvitamin D concentrations during pregnancy were associated with an increased risk of childhood asthma, with the lowest risk at a 25-hydroxyvitamin D concentration of 70 nmol/L,[7] while others did not find consistent associations with asthma,[8,9] lung function[9] or allergic sensitization.[9] However, studies included in these meta-analyses used only one single assessment of 25-hydroxyvitamin D at different time-points during pregnancy or at birth, or were not able to take child's 25-hydroxyvitamin D into account,[10] which may explain the inconsistency. We previously observed that lower 25-hydroxyvitamin D concentrations at birth were associated with a higher airway resistance in children aged 6 years, adjustment for child's 25-hydroxyvitamin D reduced the effect size.[11]

We hypothesized that altered 25-hydroxyvitamin D concentrations in critical periods of early life are associated with adverse childhood respiratory and allergy outcomes. Therefore, we examined among 4951 children and their mothers participating in a population-based prospective cohort study, whether 25-hydroxyvitamin D concentrations in mid-gestation and at birth were associated with lung function, asthma, inhalant allergic sensitization and inhalant allergy in school-age children. Additionally, we examined whether these associations could be explained by child's 25-hydroxyvitamin D concentrations at the age of 6 years.

2 | METHODS

2.1 | Design and cohort

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life until young adulthood in Rotterdam, the Netherlands. A detailed description of the study design has been published previously.[12] The study has been approved by the Medical Ethical Committee of Erasmus MC, University Medical Centre in Rotterdam, the Netherlands (MEC-2012-165-NL40020.078.12). Written informed consent was obtained from the parents or legal representatives of all participating children. A total of 7393 children were participating at the age of 10 years. Twins (n = 185), and children of whom we had no data on 25-hydroxyvitamin D concentrations in mid-gestation or at birth (n = 1171), and children with missing data on lung function, asthma, inhalant allergic sensitization or inhalant allergy at age 10 years (n = 1086) were excluded. This resulted in 4951 mothers and their children for the current analyses (Figure S1).

2.2 | 25-Hydroxyvitamin D

Maternal venous blood samples were obtained in mid-gestation (median 20.4 [95% range 18.5-23.4] weeks of gestation) and umbilical cord blood samples at birth (median 40.3 [95% range 36.7-42.3] weeks of gestation).[13] Concentrations of 25-hydroxyvitamin D were analysed from stored frozen samples at −80°C at the Eyles Laboratory at the Queensland Brain Institute, University of Queensland, Australia. Samples were quantified using isotope dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS).[14] 25-Hydroxyvitamin D concentrations were analysed both continuously and in groups based on clinical cut-offs defined as "severely deficient" (<25.0 nmol/L [<10.0 mg/L]), "deficient" (25.0 to <50.0 nmol/L [10.0 to <20.0 mg/L]) and "sufficient" (≥50.0 nmol/L [≥20.0 mg/L]).[15] When the children had a median age of 6.0 years (95% range 5.6-7.6 years), blood samples were collected. Child's 25-hydroxyvitamin D concentrations were measured at the Endocrine Laboratory of the VU University Medical Center, Amsterdam, using isotope dilution online solid phase extraction LC-MS/MS, which has been previously described in detail.[16] 25-Hydroxyvitamin D status at the age of 6 years was available in a subgroup of 2809 subjects.

2.3 | School-age lung function, asthma and allergy outcomes

Children visited our research centre at a median age of 9.7 years (95% range 9.4-10.8 years). Spirometry was performed according to the American Thoracic Society and European Respiratory Society recommendations.[17] Lung function measures included forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flow after exhaling 75% of FVC (FEF_{75}), and were converted into sex-, height-, age- and ethnicity-adjusted z-scores according to the Global Lung Initiative reference data.[18] Only pre-bronchodilation lung function measurements were available in this study. Additionally, we included 342 children with spirometry curves with a >5% deviation, but with at least one blow (according to ATS/ERS criteria) with adequate reach and duration of plateau. We observed no difference in the size or direction of the effect estimates in our analyses when we included these children.[19] Children were asked to stop the use of short-acting bronchodilators 8 hours, and long-acting bronchodilators 48 hours before spirometry, if they did not suffer from asthma symptoms. Contraindications for spirometry were a current acute asthma attack or respiratory tract infection. At age 10 years, information on ever asthma (no; yes), current wheezing (no; yes) and on physician-diagnosed inhalant allergy (no; yes) to pollen (hay fever), house dust mite, cat or dog was obtained from questionnaires based on the International Study on Asthma and Allergy in Childhood (ISAAC) Questionnaire.[20] Information on asthma medication use (no; yes) was obtained during the visit at the research centre. Current asthma (no; yes) was defined as ever diagnosis of asthma with either wheezing or medication use in the past 12 months at age 10 years. At age 10 years, inhalant allergic sensitization (no; yes) to house dust mite, five-grass mixture, birch, cat or dog (ALK-Abelló BV, Almere, the Netherlands) was measured by skin prick tests using the scanned area method.[21] We used two positive controls (histamine dihydrochloride 10 mg/mL) and one negative control (sodium chloride 9 mg/mL). Skin responses were considered positive if the area of the wheal was ≥40% of that of the histamine response (ie histamine equivalent prick index area ≥0.40).[21] Contraindications for a skin prick test were eczema on the volar surface of the left forearm, the use of oral prednisone ≥10 mg daily, antihistamine intake <72 hours prior to the test or use of corticosteroid ointment ≤48 hours prior to the test.

2.4 | Covariates

Information on maternal age, body mass index at enrolment (BMI), educational level, history of asthma or atopy, psychiatric symptoms defined using the Global Severity Index (GSI),[22,23] parity, smoking, pet keeping and damp patches or mould in the house, was obtained from questionnaires during pregnancy. Maternal folate concentrations were measured in early pregnancy (median 13.2 [95% range 9.8-17.4] weeks of gestation) as previously described.[24] Season of blood sampling was recorded at the moment of blood sampling of 25-hydroxyvitamin D. Midwife or hospital records at birth provided

information about child's sex, gestational age at birth and birth weight. Child's ethnic background was categorized on the basis of expected similarities in skin colour into Dutch and Western (Dutch, other European, American and Oceanian), Turkish and Moroccan, African (Cape Verdean, other African, Surinamese-Creole and Dutch Antillean) and Asian (Indonesian, other Asian, Surinamese-Hindu and Surinamese-unspecified).[16] Information on ever breastfeeding and day care attendance was obtained by multiple questionnaires.

2.5 | Statistical analysis

A non-response analysis was performed by comparing subject characteristics between children included and not included in the analyses by independent sample t test for continuous normally distributed variables, Mann-Whitney U test for continuous not normally distributed variables, and chi-square test for categorical variables. We calculated Spearman's correlation coefficients for the correlations between 25-hydroxyvitamin D concentrations in mid-gestation, at birth and at the age of 6 years. We used linear or logistic regression models to examine the associations of mid-gestational and cord blood 25-hydroxyvitamin D concentrations with lung function, asthma, inhalant allergic sensitization and inhalant allergy. 25-Hydroxyvitamin D concentrations were analysed as a continuous variable and in categories based on clinical cut-off values, and additionally in categories based on tertiles. Model 1 (main model) was adjusted for the confounders maternal age, BMI at enrolment, educational level, history of asthma or atopy, psychiatric symptoms, parity, smoking during pregnancy, pet keeping, damp patches or mould in the house, folate concentration, and season of blood sampling, and child's sex, gestational age at birth, birth weight, ethnic background, breastfeeding and day care attendance. Potential confounders were included in our models based on literature, if they were related to both 25-hydroxyvitamin D concentrations and at least one of the outcomes, or if the effect estimate changed ≥10% when we included the covariate in the model. Model 2 (child 25-hydroxyvitamin D model) comprised the main model and was additionally adjusted for 25-hydroxyvitamin D concentrations at the age of 6 years. We adjusted childhood 25-hydroxyvitamin D concentrations for the season of blood sampling using the residual method. We performed sensitivity analyses to test the robustness of our results. First, to prevent confounding or effect modification by heterogeneous groups with a non-Dutch ethnic background with different skin types and dietary habits, we repeated the main analyses restricted to children with a Dutch ethnic background. Second, we restricted our analyses on the associations of 25-hydroxyvitamin D as a continuous variable with respiratory and allergy outcomes to mothers and children with severely deficient and deficient concentrations in mid-gestation or at birth. Third, for clinical interpretation we studied the associations of 25-hydroxyvitamin D concentrations with the risk of lung function outcomes below the lower limit of normal defined as lower than the 2.5th centile (z-score −1.96).[18] We tested for multicollinearity using the tolerance statistic. As tolerance was >0.20 for all variables in our models, there were no problems of multicollinearity. We applied natural cubic splines

(3 degrees of freedom) to test for non-linearity of the associations of 25-hydroxyvitamin D concentrations with outcomes in our main models. Except for the associations of 25-hydroxyvitamin D concentrations at birth with FVC and FEV_1/FVC, no better fit for a non-linear model vs a linear model was found. Therefore, we considered linear models to be the most suitable for our main analyses. Missing data for covariates were <20% except for maternal folate concentrations (27.1%) and child's day care attendance (37.4%). To reduce bias and imprecision, we imputed missing data of the covariates with multiple imputations (m = 10) using chained equations. All measures of association are presented as pooled effect estimates with their 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version 24.0 for Windows (IBM Corp., Armonk, NY, USA) and R version 3.5.0 (R Foundation, Vienna, Austria).

3 | RESULTS

3.1 | Subject characteristics

Maternal and child characteristics are shown in Table 1. The median 25-hydroxyvitamin D concentration in mid-gestation was 53.0 nmol/L (95% range 8.1-122.5) and at birth 30.7 nmol/L (95% range 5.3-82.7). Mean FEV_1 Z-score (SD) was 0.17 (0.98), FVC 0.21 (0.94), FEV_1/FVC −0.09 (0.95) and FEF_{75} 0.05 (0.92). The prevalence of current asthma was 5.7% (n = 226), of inhalant allergic sensitization 32.9% (n = 1131) and of physician-diagnosed inhalant allergy 13.3% (n = 369). Non-response analyses showed that children who were not included in the analyses on average had mothers who were younger, had a higher BMI, were lower educated, were less often nulliparous, had less often pets and had lower folate concentrations in early pregnancy, and the children had more often not a Dutch or Western ethnic background compared to children included in the analyses (P-value < 0.05) (Table S1). Non-response analyses comparing children with and without information on 25-hydroxyvitamin D concentrations at the age of 6 years most importantly showed that there was no difference in 25-hydroxyvitamin D concentrations in mid-gestation and at birth between the two groups (Table S2).

3.2 | 25-hydroxyvitamin D concentrations, respiratory and allergy outcomes

Maternal 25-hydroxyvitamin D concentration in mid-gestation moderately correlated with child's 25-hydroxyvitamin D level at birth (ρ = 0.579; P < 0.001) and at the age of 6 years (ρ = 0.300; P < 0.001). A higher maternal 25-hydroxyvitamin D concentration in mid-gestation was associated with a higher FVC (Z-score difference [95% CI] 0.02 [0.00, 0.03] per 10 nmol/L), but a lower FEV_1/FVC (Z-score difference −0.02 [−0.03, −0.01]) and FEF_{75} (Z-score difference −0.01 [−0.03, −0.00]) at school-age (Table 2). After additional adjustment for 25-hydroxyvitamin D concentrations at the age of 6 years, the association of maternal 25-hydroxyvitamin D concentrations in mid-gestation with FVC attenuated into non-significant. Furthermore, a higher 25-hydroxyvitamin D concentration in mid-gestation was

associated with a higher risk of inhalant allergy (Odds Ratio [OR] [95% CI] 1.07 [1.02, 1.12] per 10 nmol/L), but attenuated into non-significant when 25-hydroxyvitamin D concentrations at the age of 6 years were taken into account. We observed similar, but no consistent trends when we studied 25-hydroxyvitamin D in categories based on clinical cut-offs. Maternal 25-hydroxyvitamin D concentrations in mid-gestation were not associated with asthma or inhalant allergic sensitization.

25-Hydroxyvitamin D concentrations at birth were not consistently associated with any lung function measure, asthma, inhalant allergic sensitization or inhalant allergy in our main model (Table 3). We only observed that children with severely deficient 25-hydroxyvitamin D concentrations at birth had a lower FVC (Z-score difference −0.12 [−0.23, −0.02]) compared to children with sufficient concentrations. This attenuated into non-significant after additional adjustment for 25-hydroxyvitamin D concentrations at the age of 6 years. When we studied 25-hydroxyvitamin D concentrations both in mid-gestation and at birth in groups based on tertiles, we observed similar directions of the results compared to the groups based on clinical cut-off values (Tables S3 and S4).

When we repeated our analyses in only children with a Dutch ethnic background, we observed similar directions of the effects of 25-hydroxyvitamin D concentrations in mid-gestation and at birth on respiratory and allergy outcomes (Tables S5 and S6). We observed associations of higher 25-hydroxyvitamin D concentrations in mid-gestation with a higher risk of inhalant allergic sensitization and inhalant allergy (OR 1.06 [1.02, 1.10] and 1.09 [1.03, 1.16]), but both effect estimates attenuated into non-significant when 25-hydroxyvitamin D concentration at the age of 6 years was taken into account. In a sensitivity analysis restricted to mothers or children with severely deficient or deficient levels in mid-gestation or at birth, we observed similar associations as in the total group (data not shown). When we studied the associations of 25-hydroxyvitamin D concentrations with the risk of lung function outcomes below the lower limit of normal, we only observed that a higher 25-hydroxyvitamin D concentration at birth was associated with a lower risk of a z-score below the lower limit of normal for FVC (OR 0.77 [0.61, 0.97]). This association attenuated after adjustment for 25-hydroxyvitamin D at the age of 6 years (data not shown).

4 | DISCUSSION

In this population-based prospective cohort study, we observed that higher maternal 25-hydroxyvitamin D concentrations in mid-gestation were associated with a higher FVC, but a lower FEV_1/FVC and FEF_{75}, and with a higher risk of physician-diagnosed inhalant allergy in children at school-age. Observed associations were partly explained by 25-hydroxyvitamin D concentrations at the age of 6 years, and less prominent when studying 25-hydroxyvitamin D in groups based on clinical cut-off values. We did not find any consistent associations of 25-hydroxyvitamin D concentrations at birth with respiratory or allergy outcomes.

TABLE 1 Maternal and child characteristics

	n = 4951
Maternal characteristics	
Age, y	30.7 (4.9)
Body mass index at enrolment (kg/m^2)^a	23.7 (18.7-35.7)
Educational level, higher (%)	51.0 (2525)
History of asthma or atopy, yes (%)	39.0 (1932)
Psychiatric symptoms (GSI)^a	0.17 (0.0-1.26)
Parity, nullipara (%)	57.9 (2869)
Smoking during pregnancy, yes (%)	30.3 (1502)
Pet keeping, yes (%)	41.9 (2072)
Damp patches or mould in house, yes (%)	16.2 (804)
Folate concentration in early pregnancy, nmol/L^a	18.2 (4.1-51.2)
Mid-gestational blood sampling season (%)	
Spring	31.0 (1537)
Summer	22.2 (1097)
Autumn	23.9 (1182)
Winter	22.9 (1135)
25-Hydroxyvitamin D concentration in mid-gestation (nmol/L)^a	53.0 (8.1-122.5)
Severely deficient (%)	20.1 (905)
Deficient (%)	26.1 (1178)
Sufficient (%)	53.8 (2424)
Child characteristics	
Female sex (%)	50.6 (2504)
Gestational age at birth (weeks)^a	40.1 (35.9-42.3)
Birth weight (grams)	3449 (550)
Ethnic background (%)	
Dutch and Western	70.1 (3471)
Turkish and Moroccan	11.3 (561)
African	10.8 (537)
Asian	7.7 (383)
Ever breastfeeding, yes (%)	88.3 (4372)
Day care attendance first year, yes (%)	61.6 (3049)
At birth blood sampling season (%)	
Spring	24.1 (1192)
Summer	26.7 (1324)
Autumn	27.2 (1349)
Winter	21.9 (1086)
25-Hydroxyvitamin D concentration at birth (nmol/L)^a	30.7 (5.3-82.7)
Severely deficient (%)	40.2 (1320)
Deficient (%)	37.4 (1229)
Sufficient (%)	22.4 (735)
25-Hydroxyvitamin D concentration at the age of 6 y (nmol/L)^a	65.6 (19.1-134.8)
FEV$_1$ (z-score)	0.17 (0.98)

(Continues)

TABLE 1 (Continued)

	n = 4951
FVC (z-score)	0.21 (0.94)
FEV$_1$/FVC (z-score)	−0.09 (0.95)
FEF$_{75}$ (z-score)	0.05 (0.92)
Current asthma at the age of 10 y, yes (%)	5.7 (226)
Inhalant allergic sensitization at the age of 10 y, yes (%)	32.9 (1131)
Physician-diagnosed inhalant allergy at the age of 10 y, yes (%)	13.3 (369)

Values are means (SD), ^{a}medians (2.5-97.5th percentile) or valid percentages (absolute numbers), based on imputed data. Data on 25-hydroxyvitamin D in mid-gestation (n = 444), at birth (n = 1667), at the age of 6 y (n = 2142), and forced expiratory volume in 1 s (FEV$_1$) (n = 752), forced vital capacity (FVC) (n = 752), FEV$_1$/FVC ratio (n = 752), forced expiratory flow after exhaling 75% of FVC (FEF$_{75}$) (n = 752), current asthma (n = 1001), inhalant allergic sensitization (n = 1512) and physician-diagnosed inhalant allergy (n = 2173) was not imputed.

4.1 | Comparison with previous studies

We found that higher 25-hydroxyvitamin D concentrations in mid-gestation, but not at birth, were associated with a higher FVC but a lower FEV$_1$/FVC and FEF$_{75}$. A recent meta-analysis pooled the results of 4 cohort studies with 292-3784 participants and found no associations of maternal 25-hydroxyvitamin D concentrations during pregnancy with FEV$_1$ or FVC in childhood. However, they combined 25-hydroxyvitamin D concentrations obtained at different time-points in pregnancy or at birth into one exposure variable.[9] One cohort study, which was included in the meta-analysis, measured maternal 25-hydroxyvitamin D concentrations at a similar time-point in pregnancy, mid-gestation, as we did and showed that higher maternal 25-hydroxyvitamin D concentrations in mid-gestation were associated with a higher FVC and a tendency towards a lower FEV$_1$/FVC and FEF$_{25\%-75\%}$ in 260 children aged 6 years.[25] The directions of the effect estimates in our study were similar. Cohort studies within the meta-analysis that examined maternal 25-hydroxyvitamin D concentrations in late pregnancy,[26-28] and one additional high-risk cohort study (n = 257) that examined 25-hydroxyvitamin D concentrations at birth,[29] found no associations with lung function measures, as we did. These findings underline the importance of the differences in effects of 25-hydroxyvitamin D concentrations measured in different periods of pregnancy on fetal lung development.

We observed no consistent associations of maternal 25-hydroxyvitamin D concentrations in mid-gestation and at birth with the risk of asthma in children. These findings are in line with two meta-analyses which pooled the results of population-based or high-risk cohort studies with 186-3177[8] and with 164-20 800 children.[9] However, an additional meta-analysis pooled studies with 164-3177 children and found that lower and higher maternal 25-hydroxyvitamin D concentrations taken at any time in pregnancy were associated

TABLE 2 Associations of 25-hydroxyvitamin D concentrations *in mid-gestation* with respiratory and allergy outcomes at age 10 y

	FEV_1 Z-score change (95% CI) n = 4199	FVC Z-score change (95% CI) n = 4199	FEV_1/FVC Z-score change (95% CI) n = 4199	FEF_{75} Z-score change (95% CI) n = 4199	Current asthma OR (95% CI) n = 3950	Inhalant allergic sensitization OR (95% CI) n = 3439	Inhalant allergy OR (95% CI) n = 2778
Model 1: Main model							
25-hydroxyvitamin D per 10 nmol/L (n = 4507)	0.00 (−0.01, 0.02)	**0.02 (0.00, 0.03)**	**−0.02 (−0.03, −0.01)**	**−0.01 (−0.03, −0.00)**	1.04 (0.98, 1.10)	1.01 (0.98, 1.04)	**1.07 (1.02, 1.12)**
Clinical cut-offs							
Severely deficient (n = 905)	−0.01 (−0.11, 0.09)	−0.07 (−0.17, 0.02)	0.10 (−0.00, 0.20)	0.09 (−0.01, 0.18)	0.98 (0.62, 1.54)	1.02 (0.79, 1.31)	0.70 (0.47, 1.05)
Deficient (n = 1178)	−0.03 (−0.11, 0.04)	−0.05 (−0.12, 0.02)	0.02 (−0.06, 0.09)	0.03 (−0.04, 0.10)	0.94 (0.66, 1.35)	0.89 (0.73, 1.07)	0.90 (0.61, 1.33)
Sufficient (n = 2424)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
25-hydroxyvitamin D per 10 nmol/L (n = 2580)	−0.00 (−0.02, 0.01)	0.01 (−0.00, 0.03)	**−0.02 (−0.03, −0.00)**	**−0.02 (−0.03, −0.00)**	1.07 (0.99, 1.15)	1.01 (0.97, 1.05)	1.04 (0.98, 1.12)
Clinical cut-offs							
Severely deficient (n = 508)	0.03 (−0.09, 0.16)	−0.04 (−0.16, 0.08)	0.10 (−0.03, 0.23)	0.08 (−0.04, 0.20)	0.95 (0.52, 1.75)	0.95 (0.69, 1.32)	0.93 (0.54, 1.58)
Deficient (n = 678)	0.01 (−0.09, 0.10)	−0.02 (−0.11, 0.07)	0.03 (−0.07, 0.12)	0.05 (−0.04, 0.14)	0.78 (0.48, 1.27)	0.91 (0.71, 1.16)	0.87 (0.58, 1.31)
Sufficient (n = 1394)	Reference	Reference	Reference	Reference	Reference	Reference	Reference

Values are change in Z-scores or odds ratios (OR) with 95% confidence interval (95% CI), derived from linear or logistic regression models, respectively. Bold indicates *P*-value < 0.05. Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), forced expiratory flow after exhaling 75% of FVC (FEF_{75}). Concentrations of 25-hydroxyvitamin D are categorized as "severely deficient" (<25.0 nmol/L [<10.0 mg/L]), "deficient" (25.0 to <50.0 nmol/L [10.0 to <20.0 mg/L]), "sufficient" (≥50.0 nmol/L [≥20.0 mg/L]). Model 1 (main model) was adjusted for the confounders maternal age, BMI at enrolment, educational level, history of asthma or atopy, psychiatric symptoms (GSI), parity, smoking during pregnancy, pet keeping, damp patches or mould in the house, folate concentration and season of blood sampling, and child's sex, gestational age at birth, birthweight, ethnic background, breastfeeding and day care attendance. Model 2 (Child 25-hydroxyvitamin D model) comprised the main model and was additionally adjusted for season-adjusted 25-hydroxyvitamin D concentrations at the age of 6 y.

TABLE 3 Associations of 25-hydroxyvitamin D concentrations *at birth* with respiratory and allergy outcomes at age 10 y

	FEV$_1$ Z-score change (95% CI) n = 4199	FVC Z-score change (95% CI) n = 4199	FEV$_1$/FVC Z-score change (95% CI) n = 4199	FEF$_{75}$ Z-score change (95% CI) n = 4199	Current asthma OR (95% CI) n = 3950	Inhalant allergic sensitization OR (95% CI) n = 3439	Inhalant allergy OR (95% CI) n = 2778
Model 1: Main model							
25-hydroxyvitamin D per 10 nmol/L (n = 3284)	0.00 (−0.02, 0.02)	0.01 (−0.01, 0.03)	−0.01 (−0.03, 0.01)	−0.00 (−0.02, 0.02)	1.03 (0.94, 1.14)	0.97 (0.92, 1.02)	1.04 (0.97, 1.12)
Clinical cut-offs							
Severely deficient (n = 1320)	−0.06 (−0.17, 0.06)	**−0.12 (−0.23, −0.02)**	0.10 (−0.01, 0.21)	0.03 (−0.07, 0.13)	0.72 (0.43, 1.23)	1.13 (0.84, 1.51)	0.90 (0.59, 1.38)
Deficient (n = 1229)	−0.03 (−0.13, 0.06)	−0.03 (−0.12, 0.06)	−0.01 (−0.11, 0.08)	−0.04 (−0.13, 0.05)	0.87 (0.55, 1.37)	1.23 (0.96, 1.59)	1.01 (0.70, 1.44)
Sufficient (n = 735)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
25-hydroxyvitamin D per 10 nmol/L (n = 1859)	−0.01 (−0.03, 0.02)	−0.00 (−0.03, 0.02)	0.00 (−0.03, 0.03)	−0.00 (−0.03, 0.02)	1.05 (0.92, 1.20)	0.96 (0.89, 1.03)	1.04 (0.93, 1.15)
Clinical cut-offs							
Severely deficient (n = 752)	−0.05 (−0.19, 0.10)	−0.07 (−0.21, 0.07)	0.02 (−0.13, 0.16)	−0.03 (−0.16, 0.11)	0.59 (0.29, 1.20)	1.20 (0.82, 1.77)	1.07 (0.59, 1.94)
Deficient (n = 697)	−0.02 (−0.15, 0.11)	0.05 (−0.07, 0.17)	**−0.13 (−0.26, −0.01)**	−0.09 (−0.21, 0.03)	0.64 (0.35, 1.18)	1.34 (0.96, 1.87)	0.95 (0.58, 1.56)
Sufficient (n = 410)	Reference	Reference	Reference	Reference	Reference	Reference	Reference

Values are change in Z-scores or odds ratios (OR) with 95% confidence interval (95% CI), derived from linear or logistic regression models, respectively. Bold indicates *P*-value < 0.05. Forced expiratory volume in 1 s (FEV$_1$), forced vital capacity (FVC), forced expiratory flow after exhaling 75% of FVC (FEF$_{75}$). Concentrations of 25-hydroxyvitamin D are categorized as "severely deficient" (<25.0 nmol/L [<10.0 mg/L]), "deficient" (25.0 to <50.0 nmol/L [10.0 to <20.0 mg/L]), "sufficient" (≥50.0 nmol/L [≥20.0 mg/L]). Model 1 (main model) was adjusted for the confounders maternal age, BMI at enrolment, educational level, history of asthma or atopy, psychiatric symptoms (GSI), parity, smoking during pregnancy, pet keeping, damp patches or mould in the house and folate concentration, and child's sex, gestational age at birth, birthweight, ethnic background, breastfeeding, day care attendance and season of blood sampling. Model 2 (Child 25-hydroxyvitamin D model) comprised the main model and was additionally adjusted for season-adjusted 25-hydroxyvitamin D concentrations at the age of 6 y.

with a higher risk of asthma in children.[7] A combined analysis of two randomized controlled trials showed that women who received daily a high dose of vitamin D supplementation (4000 IU/d or 2400 IU/d) from a gestational age ranging from 10 to 26 weeks onwards had, compared to the control group of women who received placebo, a 26% reduced risk of a child with asthma/recurrent wheeze at the age of 3 years.[6] The meta-analyses and randomized controlled trials mostly assessed asthma at a young age, which more likely could reflect viral induced wheezing.[30] Our study additionally shows the long-term effects of maternal 25-hydroxyvitamin D concentrations in mid-gestation and at birth on asthma in children, which was not present.

We found an association of higher 25-hydroxyvitamin D concentrations in mid-gestation with a higher risk of physician-diagnosed inhalant allergy, and in only children with a Dutch ethnic background, a higher risk of inhalant allergic sensitization. However, these findings were explained by 25-hydroxyvitamin D concentrations at the age of 6 years. Previous studies observed no associations of 25-hydroxyvitamin D concentrations in pregnancy with inhalant allergic sensitization or allergy in childhood,[25-27] but high 25-hydroxyvitamin D concentrations (≥100 nmol/L) at birth were associated with an increased risk of skin prick test positivity for inhalant allergens (Odds Ratio 3.4 [1.0-11.4]), compared to the reference group (75-99.9 nmol/L).[31] Child's 25-hydroxyvitamin D concentrations were not taken into account. Effects of 25-hydroxyvitamin D concentrations on allergic outcomes remain inconclusive.

4.2 | Interpretation of the results

The associations of maternal 25-hydroxyvitamin D concentrations with lung function outcomes could be partly explained by the role of vitamin D in lung development. Our maternal venous blood samples were taken in mid-gestation, which might reflect the pseudoglandular and canalicular stage of lung development.[32] Genomic studies showed that 1,25-dihydroxyvitamin D, the biologically active form, already in these stages of lung development influences the expression of genes, which have previously been associated with lung development and asthma pathogenesis.[3] Furthermore, in vivo mice studies reported that in utero 25-hydroxyvitamin D deficiency can result in a reduced lung volume,[33] a diminished tracheal diameter and alveolar simplification,[34] as well an altered lung function[33] with increased airway resistance and decreased pulmonary compliance.[34] Our observations that higher 25-hydroxyvitamin D concentrations are associated with a higher FVC are in line with these studies. We did not find an association with FEV_1, which could explain the associations of higher 25-hydroxyvitamin D concentrations with a lower FEV_1/FVC ratio. FEF_{75} might be used as predictor of small airways disease, but the importance is subject of debate.[35] We need to be careful with a biological interpretation of the association of higher mid-gestational 25-hydroxyvitamin D concentrations with lower FEF_{75} values, since development of smaller airways mostly occurs in later stages of pregnancy. The observed effects of 25-hydroxyvitamin D concentrations per 10 nmol/L

increase on lung function outcomes are minimal. Therefore, we cannot draw any conclusions other than that 25-hydroxyvitamin D concentrations in mid-gestation may influence lung development, but seem not related to the risk of clinically manifest lung function changes or childhood asthma. Umbilical cord blood samples at birth might reflect the third trimester of pregnancy, in which the alveolar stage of lung development and surfactant synthesis occurs.[32,36] However, our study showed no effect of maternal 25-hydroxyvitamin D concentrations at birth on child's respiratory outcomes. Aside from its role in lung development, vitamin D might influence the immune system. The placenta may synthesize 1,25-dihydroxyvitamin D_3, which suggest it could act as an autocrine or paracrine regulator of the developing fetal immune system already in the first trimester of pregnancy.[37,38] Furthermore, 1,25-dihydroxyvitamin D might have both stimulating and inhibitory effects on Th2 responses, which effect might probably depend on the time period of exposure to 1,25-dihydroxyvitamin D.[39] In human cord blood cells, 1,25-dihydroxyvitamin D has been shown to suppress IL-4 and IL-4 induced IL-13 expression,[5] and in an in vivo mice study, perinatal 25-hydroxyvitamin D deficiency resulted in Th2 skewing and increased airway eosinophilia.[40] In five-week-old mice, results seemed different.[41] The association of higher 25-hydroxyvitamin D concentrations in mid-gestation with physician-diagnosed inhalant allergies attenuated after adjustment for 25-hydroxyvitamin D concentrations at the age of 6 years. Additional research to the underlying mechanism at different time-points during immune system development is needed.

4.3 | Strengths and limitations

The strengths of our study include that it was embedded in a prospective population-based cohort study with a large sample-size, and with detailed measurements on 25-hydroxyvitamin D concentrations at several time-points in early life, and on respiratory and allergy outcomes. We were able to adjust our models for multiple confounders, and additionally for 25-hydroxyvitamin D levels at the age of 6 years. The adjustment for folate concentration and maternal psychiatric symptoms might be tenuous. However, omitting these two confounders from our statistical models did not change our results (data not shown). We also need to mention some limitations. Non-response analyses showed a selection towards a relatively more healthy and economically advanced population. This selective non-response could have led to biased effect estimates if the examined associations would differ between children included and not included in the analyses. Second, 25-hydroxyvitamin D concentrations were used to quantify vitamin D status, which might reflect both diet and sun exposure. Currently, no cut-off values are available to define vitamin D deficiency in pregnancy. For clinical interpretation and to make this study comparable to previous studies on vitamin D and asthma, we choose to define the groups based on current guidelines for clinical cut-off values in the general population. A different approach, when we studied 25-hydroxyvitamin D in groups based

on tertiles showed similar results. 25-Hydroxyvitamin D crosses the placenta,[42] so the use of maternal 25-hydroxyvitamin D concentrations might be a good proxy for fetal 25-hydroxyvitamin D status. However, although cord blood 25-hydroxyvitamin D concentrations correlate with maternal serum concentrations,[43] they are on average 75% of maternal serum concentrations,[43] meaning that we need to be careful to interchange maternal and fetal 25-hydroxyvitamin D values.[44] Furthermore, the biologically active 1,25-dihydroxyvitamin D concentration rises during pregnancy, and the ratio of 1,25-dihydroxyvitamin D/25-hydroxyvitamin D might become twice as high as in non-pregnant women,[45] so 25-hydroxyvitamin D concentrations might not fully represent 1,25-dihydroxyvitamin D activity.[45] Information on asthma and inhalant allergy was obtained from validated questions, adapted from the ISAAC Core Questionnaires. Nevertheless, reporting bias cannot be excluded which might lead to under- or overestimations of the observed associations. Last, residual confounding due to unmeasured factors including the intake of other nutrients or supplements might have influenced or even explain our results. During the recruitment period for this study (2002-2006), the guidelines from the Health Council of the Netherlands suggested that all pregnant women should be advised to take 10 μg of vitamin D daily.[46] Unfortunately, we had no detailed information on the intake of vitamin D from supplements for the current analyses and therefore cannot examine this potential effect on the observed associations. However, the observed 25-hydroxyvitamin D concentrations in mid-gestation and at birth are consistent with previous Dutch studies.[47,48]

5 | CONCLUSION

Our results suggest that, after additional adjustment for 25-hydroxyvitamin D at the age of 6 years, higher maternal 25-hydroxyvitamin D concentrations in mid-gestation are associated with a lower FEV_1/FVC and FEF_{75}, but not with asthma. We did not find any consistent associations of 25-hydroxyvitamin D concentrations at birth with respiratory or allergy outcomes. In line with the inconsistent results of previous observational studies, our study shows the complexity of the associations of early life vitamin D with respiratory and allergy outcomes. Further understanding of the mechanisms underlying the associations at different time-points in early life is needed. Randomized controlled trials focusing on the effect of vitamin D supplementation in pregnancy or even pre-conceptional on long-term respiratory or allergy outcomes might be subject for further study.

AUTHOR CONTRIBUTION

SMB, EM and LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content and gave final approval of the version to be published. JJ, TV, IR, NJ and VJ contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content and gave final approval of the version to be published.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.