

MS EVELIEN ROSANNE VAN MEEL (Orcid ID : 0000-0002-0826-9931)

DR LIESBETH DUIJTS (Orcid ID : 0000-0002-2068-5524)

Article type : 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.

Sara M. Mensink-Bout, MD^{1,2}, Evelien R. van Meel, MD^{1,2}, Johan C. de Jongste, MD PhD²,
Trudy Voortman, PhD³, Irwin K. Reiss, MD, PhD⁴, Nicolette W. De Jong, PhD⁵, Vincent
W.V. Jaddoe, MD, PhD^{1,6}, Liesbeth Duijts, MD PhD^{2,4}

¹The Generation R Study Group, ²Department of Pediatrics, Division of Respiratory Medicine and Allergology, ³Department of Epidemiology, ⁴Department of Pediatrics, Division of Neonatology, ⁵Department of Internal Medicine, Division of Allergology, and ⁶Department of Pediatrics; Erasmus MC, University Medical Center, Rotterdam, The Netherlands.

Key words: Vitamin D, lung function, asthma, inhalant allergic sensitization and allergy, child, prospective cohort study

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.13384

This article is protected by copyright. All rights reserved.

Corresponding author

Dr. Liesbeth Duijts, MD, PhD.

Erasmus MC - Sophia, University Medical Center Rotterdam, Sp-3435; PO Box 2060, 3000 CB Rotterdam, The Netherlands.

Phone: +31 10 7036263; fax: +31 10 7036811; e-mail: l.duijts@erasmusmc.nl

Contribution of authors to the study

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.

Sources of financial support and conflict of interest

The Generation R Study is made possible by financial support from the Erasmus Medical Centre, Rotterdam, the Erasmus University Rotterdam and The Netherlands Organization for Health Research and Development. Dr Liesbeth Duijts received funding from the European Union's Horizon 2020 co-funded programme ERA-Net on Biomarkers for Nutrition and Health (ERA HDHL) (ALPHABET project (no 696295; 2017), ZonMW The Netherlands (no 529051014; 2017)). The project received funding from the European Union's Horizon 2020 research and innovation programme (LIFECYCLE project, grant agreement no 733206; 2016). The study sponsors had no role in the study design, data analysis, interpretation of data, or writing of this report.

ABSTRACT

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 4,951 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.

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 children³, 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 childhood⁵. 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 children⁶. 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 results⁷⁻⁹. 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/L⁷, while others did not find consistent associations with asthma^{8,9}, lung function⁹ or allergic sensitization⁹. 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 account¹⁰, 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 size¹¹.

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 4,951 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.

METHODS

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 described previously¹². 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 7,393 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 = 1,171), and children with missing data on lung function, asthma, inhalant allergic sensitization or inhalant allergy at age 10 years (n = 1,086) were excluded. This resulted in 4,951 mothers and their children for the current analyses (Figure E-1).

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 analyzed 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 analyzed 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 detail¹⁶. 25-Hydroxyvitamin D status at the age of 6 years was available in a subgroup of 2,809 subjects.

School age lung function, asthma and allergy outcomes Children visited our research center 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 recommendations¹⁷. Lung function measures included Forced Expiratory Volume in 1 second (FEV₁), Forced Vital Capacity (FVC), FEV₁/FVC and Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅), and were converted into sex-, height-, age-, and ethnicity-adjusted z-scores according to the Global Lung Initiative reference data¹⁸. 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 children¹⁹. 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. Contra-indications 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) Questionnaire²⁰. Information on asthma medication use (no; yes) was obtained during the visit at the research center. 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 B.V., Almere, the Netherlands) was measured by skin prick tests using the scanned area method²¹. 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 $\geq 40\%$ of that of the histamine response (i.e. histamine equivalent prick index area ≥ 0.40)²¹. Contra indications 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 h prior to the test or use of corticosteroid ointment ≤ 48 h prior to the test.

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 mold 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 described²⁴. 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 daycare attendance was obtained by multiple questionnaires.

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 χ^2 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

Accepted Article

cord blood 25-hydroxyvitamin D concentrations with lung function, asthma, inhalant allergic sensitization and inhalant allergy. 25-Hydroxyvitamin D concentrations were analyzed 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 mold 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 $\geq 10\%$ 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 FEV₁/FVC, no better fit for a non-

linear model versus 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 daycare 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).

RESULTS

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 FEV₁ Z-score (SD) was 0.17 (0.98), FVC 0.21 (0.94), FEV₁/FVC -0.09 (0.95) and FEF₇₅ 0.05 (0.92). The prevalence of current asthma was 5.7% (n = 226), of inhalant allergic sensitization 32.9% (n = 1,131) 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 E-1). 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 E-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 ($p=0.579$; $p<0.001$), and at the age of 6 years ($p=0.300$;

Accepted Article

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 FEV₁/FVC (Z-score difference -0.02 (-0.03, -0.01)) and FEF₇₅ (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 (Table E-3) (Table E-4).

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 (Table E-5) (Table E-6). 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).

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 FEV₁/FVC and FEF₇₅, 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.

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 FEV₁/FVC and FEF₇₅. A recent meta-analysis pooled the results of 4 cohort studies with 292 to 3,784 participants and found no associations of maternal 25-hydroxyvitamin D concentrations during pregnancy with FEV₁ or FVC in childhood. However, they combined 25-hydroxyvitamin D concentrations obtained at different time points in pregnancy or at birth into one exposure variable⁹. 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

Accepted Article

a lower FEV₁/FVC and FEF_{25-75%} in 260 children aged 6 years²⁵. The directions of effects estimates of our study were similar. Cohort studies within the meta-analysis that examined maternal 25-hydroxyvitamin D concentrations in late pregnancy²⁶⁻²⁸, and one additional high-risk cohort study (n = 257) that examined 25-hydroxyvitamin D concentrations at birth²⁹, 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 to 3,177⁸ and with 164 to 20,800 children⁹. However, an additional meta-analysis pooled studies with 164 to 3,177 children and found that lower and higher maternal 25-hydroxyvitamin D concentrations taken at any time in pregnancy were associated with a higher risk of asthma in children⁷. A combined analysis of two randomized controlled trials showed that women who received daily a high dose of vitamin D supplementation (4,000 IU/d or 2,400 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 years⁶. The meta-analyses and randomized controlled trials mostly assessed asthma at a young age, which more likely could reflect viral induced wheezing³⁰. 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 childhood²⁵⁻²⁷, 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.

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 development³². 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 pathogenesis³. Furthermore, in vivo mice studies reported that in utero 25-hydroxyvitamin D deficiency can result in a reduced lung volume³³, a diminished tracheal diameter and alveolar simplification³⁴, as well as an altered lung function³³ with increased airway resistance and decreased pulmonary compliance³⁴. 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 FEV₁, which could explain the associations of higher 25-hydroxyvitamin D concentrations with a lower FEV₁/FVC ratio. FEF₇₅ might be used as predictor of small airways disease, but the importance is subject of debate³⁵. We need to be careful with a biological interpretation of the association of higher mid-gestational 25-hydroxyvitamin D concentrations with lower FEF₇₅ 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

Accepted Article

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 D₃, 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 D³⁹. In human cord blood cells, 1,25-dihydroxyvitamin D has been shown to suppress IL-4 and IL-4 induced IL-13 expression⁵, and in an in vivo mice study, perinatal 25-hydroxyvitamin D deficiency resulted in Th2 skewing and increased airway eosinophilia⁴⁰. In five-week-old mice results seemed different⁴¹. 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.

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

Accepted Article

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 placenta⁴², 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 concentrations⁴³, they are on average 75% of maternal serum concentrations⁴³, meaning that we need to be careful to interchange maternal and fetal 25-hydroxyvitamin D values⁴⁴. 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 women⁴⁵, so 25-hydroxyvitamin D concentrations might not fully represent 1,25-dihydroxyvitamin D activity⁴⁵. 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 daily⁴⁶. 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}.

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 FEV₁/FVC and FEF₇₅, 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 preconceptional on long-term respiratory or allergy outcomes might be subject for further study.

ACKNOWLEDGMENTS

The Generation R Study is conducted by the Erasmus Medical Centre in close collaboration with the School of Law and the Faculty of Social Sciences at the Erasmus University, Rotterdam, the Municipal Health Service, Rotterdam area, and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond (Star-MDC), Rotterdam. We gratefully acknowledge the contribution of children and their parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam.

REFERENCES

1. Duijts L, Reiss IK, Brusselle G, de Jongste JC, Early origins of chronic obstructive lung diseases across the life course. *Eur J Epidemiol* 2014;29: 871-85.
2. Litonjua AA, Weiss ST, Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120: 1031-5.
3. Kho AT, Sharma S, Qiu W, Gaedigk R, Klanderman B, Niu S, Anderson C, Leeder JS, Weiss ST, Tantisira KG, Vitamin D related genes in lung development and asthma pathogenesis. *BMC Med Genomics* 2013;6: 47.

4. Foong RE, Bosco A, Jones AC, Gout A, Gorman S, Hart PH, Zosky GR, The effects of in utero vitamin D deficiency on airway smooth muscle mass and lung function. *Am J Respir Cell Mol Biol* 2015;53: 664-75.
5. Pichler J, Gerstmayr M, Szepfalusi Z, Urbanek R, Peterlik M, Willheim M, 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. *Pediatr Res* 2002;52: 12-8.
6. Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, Stokholm J, Bonnelykke K, Bisgaard H, Weiss ST, Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. *PLoS One* 2017;12: e0186657.
7. Song H, Yang L, Jia C, Maternal vitamin D status during pregnancy and risk of childhood asthma: A meta-analysis of prospective studies. *Mol Nutr Food Res* 2017;61.
8. Feng H, Xun P, Pike K, Wills AK, Chawes BL, Bisgaard H, Cai W, Wan Y, He K, In utero exposure to 25-hydroxyvitamin D and risk of childhood asthma, wheeze, and respiratory tract infections: A meta-analysis of birth cohort studies. *J Allergy Clin Immunol* 2017;139: 1508-17.
9. Pacheco-Gonzalez RM, Garcia-Marcos L, Morales E, Prenatal vitamin D status and respiratory and allergic outcomes in childhood: A meta-analysis of observational studies. *Pediatr Allergy Immunol* 2018;29: 243-53.
10. Tromp IIM, Franco OH, van den Hooven EH, Heijboer AC, Jaddoe VWV, Duijts L, de Jongste JC, Moll HA, Kiefte-de Jong JC, 25-Hydroxyvitamin D concentrations, asthma and eczema in childhood: The generation R study. *Clin Nutr* 2018;37: 169-76.
11. Gazibara T, den Dekker HT, de Jongste JC, McGrath JJ, Eyles DW, Burne TH, Reiss IK, Franco OH, Tiemeier H, Jaddoe VW, Duijts L, Associations of maternal and fetal 25-hydroxyvitamin D levels with childhood lung function and asthma: the Generation R Study. *Clin Exp Allergy* 2016;46: 337-46.
12. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, de Jongste JC, Klaver CC, van der Lugt A, Mackenbach JP, Moll HA, Peeters RP, Raat H, Rings EH,

Rivadeneira F, van der Schroeff MP, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius E, Felix JF, Jaddoe VW, The Generation R Study: design and cohort update 2017. *Eur J Epidemiol* 2016;31: 1243-64.

13. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW, The Generation R Study: Biobank update 2015. *Eur J Epidemiol* 2014;29: 911-27.

14. Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T, Mortensen PB, Norgaard-Pedersen B, Hougaard DM, McGrath J, A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots. *Clin Chim Acta* 2009;403: 145-51.

15. Holick MF, Vitamin D deficiency. *N Engl J Med* 2007;357: 266-81.

16. Voortman T, van den Hooven EH, Heijboer AC, Hofman A, Jaddoe VW, Franco OH, Vitamin D deficiency in school-age children is associated with sociodemographic and lifestyle factors. *J Nutr* 2015;145: 791-8.

17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, Force AET, Standardisation of spirometry. *Eur Respir J* 2005;26: 319-38.

18. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J, Initiative ERSGLF, Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40: 1324-43.

19. den Dekker HT, Jaddoe VWV, Reiss IK, de Jongste JC, Duijts L, Fetal and Infant Growth Patterns and Risk of Lower Lung Function and Asthma. The Generation R Study. *Am J Respir Crit Care Med* 2018;197: 183-92.

20. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al., International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8: 483-91.

21. van der Valk JPM, Gerth van Wijk R, Hoorn E, Groenendijk L, Groenendijk IM, de Jong NW, Measurement and interpretation of skin prick test results. *Clinical and Translational Allergy* 2015;6: 8.
22. Guxens M, Sonnenschein-van der Voort AM, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, Jaddoe VW, Duijts L, Parental psychological distress during pregnancy and wheezing in preschool children: the Generation R Study. *J Allergy Clin Immunol* 2014;133: 59-67 e1-12.
23. De Beurs E, Brief Symptom Inventory, handleiding [Dutch manual]. *PITS BV, Leiden (The Netherlands)* 2004.
24. Steenweg-de Graaff J, Ghassabian A, Jaddoe VW, Tiemeier H, Roza SJ, Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. *Eur J Public Health* 2015;25: 431-3.
25. Zosky GR, Hart PH, Whitehouse AJ, Kusel MM, Ang W, Foong RE, Chen L, Holt PG, Sly PD, Hall GL, Vitamin D deficiency at 16 to 20 weeks' gestation is associated with impaired lung function and asthma at 6 years of age. *Ann Am Thorac Soc* 2014;11: 571-7.
26. Pike KC, Inskip HM, Robinson S, Lucas JS, Cooper C, Harvey NC, Godfrey KM, Roberts G, Southampton Women's Survey Study G, Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes. *Thorax* 2012;67: 950-6.
27. Wills AK, Shaheen SO, Granell R, Henderson AJ, Fraser WD, Lawlor DA, Maternal 25-hydroxyvitamin D and its association with childhood atopic outcomes and lung function. *Clin Exp Allergy* 2013;43: 1180-8.
28. Cremers E, Thijs C, Penders J, Jansen E, Mommers M, Maternal and child's vitamin D supplement use and vitamin D level in relation to childhood lung function: the KOALA Birth Cohort Study. *Thorax* 2011;66: 474-80.
29. Chawes BL, Bonnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H, Cord blood 25(OH)-vitamin D deficiency and childhood asthma, allergy and eczema: the COPSAC2000 birth cohort study. *PLoS One* 2014;9: e99856.

30. Just J, Bourgoïn-Heck M, Amat F, Clinical phenotypes in asthma during childhood. *Clin Exp Allergy* 2017;47: 848-55.
31. Rothers J, Wright AL, Stern DA, Halonen M, Camargo CA, Jr., Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J Allergy Clin Immunol* 2011;128: 1093-9 e1-5.
32. Merkus PJ, ten Have-Opbroek AA, Quanjer PH, Human lung growth: a review. *Pediatr Pulmonol* 1996;21: 383-97.
33. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH, Vitamin D deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med* 2011;183: 1336-43.
34. Saadoon A, Ambalavanan N, Zinn K, Ashraf AP, MacEwen M, Nicola T, Fanucchi MV, Harris WT, Effect of Prenatal versus Postnatal Vitamin D Deficiency on Pulmonary Structure and Function in Mice. *Am J Respir Cell Mol Biol* 2017;56: 383-92.
35. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW, Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. *Eur Respir J* 2014;43: 1051-8.
36. Nguyen M, Trubert CL, Rizk-Rabin M, Rehan VK, Besancon F, Cayre YE, Garabedian M, 1,25-Dihydroxyvitamin D3 and fetal lung maturation: immunogold detection of VDR expression in pneumocytes type II cells and effect on fructose 1,6 bisphosphatase. *J Steroid Biochem Mol Biol* 2004;89-90: 93-7.
37. Larque E, Morales E, Leis R, Blanco-Carnero JE, Maternal and Foetal Health Implications of Vitamin D Status during Pregnancy. *Ann Nutr Metab* 2018;72: 179-92.
38. Evans KN, Nguyen L, Chan J, Innes BA, Bulmer JN, Kilby MD, Hewison M, Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. *Biol Reprod* 2006;75: 816-22.
39. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S, Vitamin D, the immune system and asthma. *Expert Rev Clin Immunol* 2009;5: 693-702.

40. Vasiliou JE, Lui S, Walker SA, Chohan V, Xystrakis E, Bush A, Hawrylowicz CM, Saglani S, Lloyd CM, Vitamin D deficiency induces Th2 skewing and eosinophilia in neonatal allergic airways disease. *Allergy* 2014;69: 1380-9.
41. Matheu V, Back O, Mondoc E, Issazadeh-Navikas S, Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol* 2003;112: 585-92.
42. Ron M, Levitz M, Chuba J, Dancis J, Transfer of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 across the perfused human placenta. *Am J Obstet Gynecol* 1984;148: 370-4.
43. Brannon PM, Picciano MF, Vitamin D in pregnancy and lactation in humans. *Annu Rev Nutr* 2011;31: 89-115.
44. Bland JM, Altman DG, A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Comput Biol Med* 1990;20: 337-40.
45. Papapetrou PD, The interrelationship of serum 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in pregnancy at term: a meta-analysis. *Hormones (Athens)* 2010;9: 136-44.
46. Vinkhuyzen AAE, Eyles DW, Burne TH, Blanken LME, Kruithof CJ, Verhulst F, Jaddoe VW, Tiemeier H, McGrath JJ, Prevalence and predictors of vitamin D deficiency based on maternal mid-gestation and neonatal cord bloods: The Generation R Study. *J Steroid Biochem Mol Biol* 2016;164: 161-67.
47. Leffelaar ER, Vrijkotte TG, van Eijsden M, Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. *Br J Nutr* 2010;104: 108-17.
48. Dijkstra SH, van Beek A, Janssen JW, de Vleeschouwer LH, Huysman WA, van den Akker EL, High prevalence of vitamin D deficiency in newborn infants of high-risk mothers. *Arch Dis Child* 2007;92: 750-3.

Table 1. Maternal and child characteristics.

	n =4,951
Maternal characteristics	
Age, years	30.7 (4.9)
Body mass index at enrolment (kg/m ²) ¹	23.7 (18.7-35.7)
Educational level, higher (%)	51.0 (2,525)
History of asthma or atopy, yes (%)	39.0 (1,932)
Psychiatric symptoms (GSI) ¹	0.17 (0.0-1.26)
Parity, nullipara (%)	57.9 (2,869)
Smoking during pregnancy, yes (%)	30.3 (1,502)
Pet keeping, yes (%)	41.9 (2,072)
Damp patches or mold in house, yes (%)	16.2 (804)
Folate concentration in early pregnancy, nmol/L ¹	18.2 (4.1-51.2)
Mid-gestational blood sampling season (%)	
Spring	31.0 (1,537)
Summer	22.2 (1,097)
Autumn	23.9 (1,182)
Winter	22.9 (1,135)
25-Hydroxyvitamin D concentration in mid-gestation (nmol/L) ¹	53.0 (8.1-122.5)
Severely deficient (%)	20.1 (905)
Deficient (%)	26.1 (1,178)
Sufficient (%)	53.8 (2,424)
Child characteristics	
Female sex (%)	50.6 (2,504)
Gestational age at birth (weeks) ¹	40.1 (35.9-42.3)
Birth weight (grams)	3,449 (550)
Ethnic background (%)	
Dutch and Western	70.1 (3,471)
Turkish and Moroccan	11.3 (561)
African	10.8 (537)
Asian	7.7 (383)
Ever breastfeeding, yes (%)	88.3 (4,372)
Daycare attendance first year, yes (%)	61.6 (3,049)
At birth blood sampling season (%)	
Spring	24.1 (1,192)
Summer	26.7 (1,324)
Autumn	27.2 (1,349)
Winter	21.9 (1,086)
25-Hydroxyvitamin D concentration at birth (nmol/L) ¹	30.7 (5.3-82.7)
Severely deficient (%)	40.2 (1,320)
Deficient (%)	37.4 (1,229)
Sufficient (%)	22.4 (735)
25-Hydroxyvitamin D concentration at the age of 6 years (nmol/L) ¹	65.6 (19.1-134.8)
FEV ₁ (z-score)	0.17 (0.98)
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 years, yes (%)	5.7 (226)
Inhalant allergic sensitization at the age of 10 years, yes (%)	32.9 (1,131)
Physician-diagnosed inhalant allergy at the age of 10 years, yes (%)	13.3 (369)

Accepted Article

Values are means (SD), ¹medians (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 = 1,667), at the age of 6 years (n = 2,142), and Forced Expiratory Volume in 1 second (FEV₁) (n = 752), Forced Vital Capacity (FVC) (n = 752), FEV₁/FVC ratio (n = 752), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅) (n = 752), current asthma (n = 1,001), inhalant allergic sensitization (n = 1,512) and physician-diagnosed inhalant allergy (n = 2,173) was not imputed.

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

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI)	Z-score change (95% CI)	Z-score change (95% CI)	Z-score change (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Model 1: Main model							
25-hydroxyvitamin D per 10 nmol/L (n = 4,507)	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 = 1,178)	-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 = 2,424)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
25-hydroxyvitamin D per 10 nmol/L (n = 2,580)	-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 = 1,394)	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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold 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 years.

Table 3. Associations of 25-hydroxyvitamin D concentrations *at birth* with respiratory and allergy outcomes at age 10 years.

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	OR (95% CI) n = 3,950	OR (95% CI) n = 3,439	OR (95% CI) n = 2,778
Model 1: Main model							
25-hydroxyvitamin D	0.00 (-0.02,	0.01 (-0.01,	-0.01 (-0.03,	-0.00 (-0.02,	1.03 (0.94,	0.97 (0.92, 1.02)	1.04 (0.97,
Clinical cut-offs							
Severely deficient (n =	-0.06 (-0.17,	-0.12 (-0.23, -	0.10 (-0.01,	0.03 (-0.07,	0.72 (0.43,	1.13 (0.84, 1.51)	0.90 (0.59,
Deficient (n = 1,229)	-0.03 (-0.13,	-0.03 (-0.12,	-0.01 (-0.11,	-0.04 (-0.13,	0.87 (0.55,	1.23 (0.96, 1.59)	1.01 (0.70,
Sufficient (n = 735)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D							
25-hydroxyvitamin D	-0.01 (-0.03,	-0.00 (-0.03,	0.00 (-0.03,	-0.00 (-0.03,	1.05 (0.92,	0.96 (0.89, 1.03)	1.04 (0.93,
Clinical cut-offs							
Severely deficient (n =	-0.05 (-0.19,	-0.07 (-0.21,	0.02 (-0.13,	-0.03 (-0.16,	0.59 (0.29,	1.20 (0.82, 1.77)	1.07 (0.59,
Deficient (n = 697)	-0.02 (-0.15,	0.05 (-0.07,	-0.13 (-0.26, -	-0.09 (-0.21,	0.64 (0.35,	1.34 (0.96, 1.87)	0.95 (0.58,
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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold 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 years.

Supplementary material

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

Sara M. Mensink-Bout, MD^{1,2}, Evelien R. van Meel, MD^{1,2}, Johan C. de Jongste, MD PhD²,
Trudy Voortman, PhD³, Irwin K. Reiss, MD, PhD⁴, Nicolette W. De Jong, PhD⁵, Vincent W.V.
Jaddoe, MD, PhD^{1,6}, Liesbeth Duijts, MD PhD^{2,4}

¹The Generation R Study Group, ²Department of Pediatrics, Division of Respiratory Medicine and Allergology, ³Department of Epidemiology, ⁴Department of Pediatrics, Division of Neonatology, ⁵Department of Internal Medicine, Division of Allergology, and ⁶Department of Pediatrics; Erasmus MC, University Medical Center, Rotterdam, The Netherlands.

Table E-1. Comparison of maternal and child's characteristics between children included and not included in the analyses

	Participants	Non-participants	p-value for difference
	n = 4,951	n = 1,086	
Maternal characteristics			
Age, years	30.7 (4.9)	28.4 (5.3)	<0.001
Body mass index at enrolment (kg/m ²) ¹	23.7 (18.8-35.6)	24.4 (18.4-37.2)	<0.001
Educational level, higher (%)	50.5 (2,380)	29.2 (282)	<0.001
History of asthma or atopy, yes (%)	37.3 (1,629)	38.8 (71)	N.S.
Psychiatric symptoms (GSI) ¹	0.15 (0.0-1.31)	0.13 (0.0-1.86)	N.S.
Parity, nullipara (%)	58.2 (2,869)	49.9 (538)	<0.001
Smoking during pregnancy, yes (%)	24.3 (1,083)	23.3 (45)	N.S.
Pet keeping, yes (%)	34.1 (1,462)	26.5 (48)	0.036
Damp patches or mold in house, yes (%)	10.6 (451)	9.5 (71)	N.S.
Folate concentration in early pregnancy, nmol/L ¹	17.5 (6.0-37.4)	14.2 (5.0-34.9)	<0.001
Mid-gestational blood sampling season (%)			
Spring	29.3 (1,321)	29.2 (289)	N.S.
Summer	22.2 (999)	24.2 (239)	
Autumn	25.3 (1,139)	23.5 (232)	
Winter	23.3 (1,049)	23.2 (229)	
Child characteristics			
Female sex (%)	50.6 (2,504)	48.0 (521)	N.S.
Gestational age at birth (weeks) ¹	40.1 (35.9-42.3)	40.0 (36.0-42.3)	N.S.
Birth weight (grams)	3,447 (547)	3,408 (553)	N.S.
Ethnic background (%)			
Dutch and Western	70.7 (3,447)	53.7 (548)	<0.001
Turkish and Moroccan	11.5 (561)	24.2 (247)	
African	10.3 (503)	14.2 (145)	
Asian	7.4 (363)	7.8 (80)	
Ever breastfeeding, yes (%)	92.4 (3,715)	93.2 (165)	N.S.
Daycare attendance first year, yes (%)	62.6 (1,940)	56.8 (67)	N.S.
At birth blood sampling season (%)			
Spring	24.1 (1,192)	24.3 (264)	N.S.
Summer	26.7 (1,324)	26.7 (290)	
Autumn	27.2 (1,349)	28.7 (312)	
Winter	21.9 (1,086)	20.3 (220)	

Values are means (SD), medians¹ (2.5-97.5th percentile) or valid percentages (absolute numbers) based on observed data. P-value for difference was calculated using Student's t-test for continuous normally distributed variables, Mann-Whitney U test for continuous not normally distributed variables, and chi-square test for categorical variables. N.S. (non-significant).

Table E-2. Comparison of maternal and child's characteristics between children included in the study with and without information on 25-hydroxyvitamin D concentrations at age 6 years.

	Participants child 25- hydroxyvitamin D model n = 2,809	Non-participants child 25- hydroxyvitamin D model n = 2,142	p-value for difference
Maternal characteristics			
Age, years	31.0 (4.8)	30.4 (4.9)	<0.001
Body mass index at enrolment (kg/m ²) ¹	23.7 (18.9-35.6)	23.8 (18.6-36.1)	N.S.
Educational level, higher (%)	52.2 (1,467)	49.4 (1,058)	N.S.
History of asthma or atopy, yes (%)	38.3 (1,077)	39.9 (854)	N.S.
Psychiatric symptoms (GSI) ¹	0.17 (0.0-1.25)	0.18 (0.0-1.23)	0.026
Parity, nullipara (%)	58.5 (1,643)	57.2 (1,226)	N.S.
Smoking during pregnancy, yes (%)	29.8 (837)	31.0 (665)	N.S.
Pet keeping, yes (%)	41.9 (1,176)	41.8 (896)	N.S.
Damp patches or mold in house, yes (%)	15.8 (444)	16.8 (360)	N.S.
Folate concentration in early pregnancy, nmol/L ¹	18.3 (4.1-50.7)	18.0 (4.1-51.7)	N.S.
Mid-gestational blood sampling season (%)			
Spring	30.4 (854)	31.9 (683)	0.017
Summer	20.9 (586)	23.9 (512)	
Autumn	24.7 (694)	22.8 (488)	
Winter	24.0 (675)	21.5 (460)	
25-Hydroxyvitamin D in mid-gestation (nmol/L) ¹	52.7 (8.4-121.8)	53.6 (7.9-124.6)	N.S.
Child characteristics			
Female sex (%)	49.6 (1,392)	51.9 (1,112)	N.S.
Gestational age at birth (weeks) ¹	40.1 (36.0-42.3)	40.1 (35.8-42.4)	N.S.
Birth weight (grams)	3,451 (549)	3,438 (565)	N.S.
Ethnic background (%)			
Dutch and Western	71.4 (2,006)	68.4 (1,465)	N.S.
Turkish and Moroccan	11.1 (311)	11.7 (250)	
African	10.6 (298)	11.1 (238)	
Asian	6.9 (194)	8.8 (188)	
Ever breastfeeding, yes (%)	89.0 (2,499)	87.5 (1,874)	N.S.
Daycare attendance first year, yes (%)	61.9 (1,740)	61.1 (1,309)	N.S.
At birth blood sampling season (%)			
Spring	25.1 (704)	22.8 (488)	N.S.
Summer	26.7 (749)	26.8 (575)	
Autumn	26.7 (751)	27.9 (598)	
Winter	21.5 (605)	22.5 (481)	
25-Hydroxyvitamin D at birth (nmol/L) ¹	31.1 (5.4-82.5)	30.2 (5.2-82.9)	N.S.

Values are means (SD), medians¹ (2.5-97.5th percentile) or valid percentages (absolute numbers) based on imputed data. Data on 25-hydroxyvitamin D in mid-gestation (n=444) and at birth (n=1,667) was not imputed. P-value for difference was calculated using Student's t-test for continuous normally distributed variables, Mann-

Whitney U test for continuous not normally distributed variables, and chi-square test for categorical variables. N.S. (non-significant).

Table E-3. Associations of 25-hydroxyvitamin D concentrations *in mid-gestation* with respiratory and allergy outcomes at age 10 years according to 25-hydroxyvitamin D in groups based on tertiles.

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	OR (95% CI) n = 3,950	OR (95% CI) n = 3,439	OR (95% CI) n = 2,778
Model 1: Main model							
1 st Tertile, 0.0 – 37.5 nmol/L (n = 1,503)	-0.03 (-0.12, 0.06)	-0.11 (-0.19, -0.02)	0.10 (0.01, 0.20)	0.08 (-0.00, 0.17)	0.86 (0.56, 1.33)	0.92 (0.73, 1.17)	0.74 (0.51, 1.07)
2 nd Tertile, 37.6 – 69.3 nmol/L (n = 1,503)	-0.01 (-0.08, 0.07)	-0.05 (-0.12, 0.02)	0.07 (-0.01, 0.14)	0.05 (-0.02, 0.12)	0.87 (0.60, 1.26)	0.86 (0.71, 1.04)	0.89 (0.66, 1.20)
3 rd Tertile, 69.4 – 161.9 nmol/L (n = 1,501)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
1 st Tertile, 0.0 – 37.5 nmol/L (n = 844)	0.02 (-0.10, 0.14)	-0.08 (-0.19, 0.03)	0.14 (0.02, 0.25)	0.11 (-0.00, 0.22)	0.77 (0.43, 1.36)	0.89 (0.66, 1.20)	0.86 (0.52, 1.41)
2 nd Tertile, 37.6 – 69.3 nmol/L (n = 890)	0.04 (-0.06, 0.14)	-0.02 (-0.11, 0.07)	0.08 (-0.02, 0.18)	0.08 (-0.01, 0.17)	0.67 (0.41, 1.08)	0.85 (0.66, 1.09)	0.86 (0.58, 1.29)
3 rd Tertile, 69.4 – 161.9 nmol/L (n = 846)	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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold 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 years.

Table E-4. Associations of 25-hydroxyvitamin D concentrations *at birth* with respiratory and allergy outcomes at age 10 years according to 25-hydroxyvitamin D in groups based on tertiles.

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	Z-score change (95% CI) n = 4,199	OR (95% CI) n = 3,950	OR (95% CI) n = 3,439	OR (95% CI) n = 2,778
Model 1: Main model							
1 st Tertile, 0.1 – 21.3 nmol/L (n = 1,092)	-0.07 (-0.17, 0.04)	-0.11 (-0.21, -0.01)	0.07 (-0.04, 0.18)	0.01 (-0.09, 0.11)	0.73 (0.43, 1.23)	1.00 (0.75, 1.32)	0.79 (0.53, 1.19)
2 nd Tertile, 21.3 – 41.5 nmol/L (n = 1,100)	-0.00 (-0.09, 0.09)	-0.00 (-0.09, 0.08)	-0.01 (-0.10, 0.08)	-0.00 (0.09, 0.08)	0.96 (0.63, 1.46)	1.03 (0.82, 1.30)	0.88 (0.63, 1.23)
3 rd Tertile, 41.6 – 144.9 nmol/L (n = 1,092)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
1 st Tertile, 0.1 – 21.3 nmol/L (n = 614)	-0.03 (-0.17, 0.11)	-0.07 (-0.21, 0.06)	0.05 (-0.09, 0.19)	0.00 (-0.13, 0.13)	0.63 (0.31, 1.28)	1.03 (0.71, 1.49)	0.86 (0.49, 1.53)
2 nd Tertile, 21.3 – 41.5 nmol/L (n = 621)	0.06 (-0.06, 0.17)	0.07 (-0.04, 0.18)	-0.04 (-0.16, 0.08)	-0.01 (-0.11, 0.10)	0.74 (0.42, 1.31)	1.05 (0.77, 1.41)	0.84 (0.53, 1.32)
3 rd Tertile, 41.6 – 144.9 nmol/L (n = 624)	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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold 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 years.

Table E-5. Associations of 25-hydroxyvitamin D concentrations *in mid-gestation* with respiratory and allergy outcomes in children with a Dutch ethnic background at age 10 years.

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	OR (95% CI) n = 2,559	OR (95% CI) n = 1,998	OR (95% CI) n = 1,815
Model 1: Main model							
25-hydroxyvitamin D per 10 nmol/L (n = 2,686)	0.00 (-0.01, 0.02)	0.01 (-0.00, 0.03)	-0.01 (-0.03, 0.01)	-0.01 (-0.02, 0.01)	1.06 (0.99, 1.14)	1.06 (1.02, 1.10)	1.09 (1.03, 1.16)
Clinical cut-offs							
Severely deficient (n = 177)	-0.07 (-0.23, 0.09)	-0.08 (-0.23, 0.07)	-0.03 (-0.20, 0.13)	-0.01 (-0.16, 0.14)	1.22 (0.58, 2.57)	0.67 (0.41, 1.08)	0.48 (0.21, 1.12)
Deficient (n = 655)	-0.00 (-0.09, 0.09)	-0.02 (-0.10, 0.07)	0.01 (-0.08, 0.11)	0.03 (-0.06, 0.12)	0.99 (0.63, 1.57)	0.83 (0.65, 1.07)	0.77 (0.52, 1.16)
Sufficient (n = 1,854)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: Child 25-hydroxyvitamin D model							
25-hydroxyvitamin D per 10 nmol/L (n = 1,567)	-0.00 (-0.02, 0.02)	0.00 (-0.01, 0.02)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)	1.10 (1.00, 1.21)	1.05 (1.00, 1.11)	1.06 (0.97, 1.14)
Clinical cut-offs							
Severely deficient (n = 109)	0.00 (-0.20, 0.21)	0.00 (-0.18, 0.19)	-0.04 (-0.25, 0.16)	-0.01 (-0.20, 0.19)	1.32 (0.51, 3.39)	0.73 (0.40, 1.34)	0.86 (0.33, 2.29)
Deficient (n = 399)	0.05 (-0.07, 0.16)	0.03 (-0.07, 0.14)	0.01 (-0.11, 0.13)	0.04 (-0.07, 0.15)	0.65 (0.34, 1.24)	0.86 (0.62, 1.18)	0.76 (0.44, 1.30)
Sufficient (n = 1,059)	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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold in the house, folate concentration and season of blood sampling, and child's sex, gestational age at birth, birthweight, 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 years.

Table E-6. Associations of 25-hydroxyvitamin D concentrations *at birth* with respiratory and allergy outcomes in children with a Dutch ethnic background at age 10 years.

	FEV ₁	FVC	FEV ₁ /FVC	FEF ₇₅	Current asthma	Inhalant allergic sensitization	Inhalant allergy
	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	Z-score change (95% CI) n = 2,488	OR (95% CI) n = 2,559	OR (95% CI) n = 1,998	OR (95% CI) n = 1,815
Model 1: Main model							
25-hydroxyvitamin D per 10 nmol/L (n = 2,035)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.03)	1.02 (0.91, 1.14)	1.01 (0.95, 1.08)	1.07 (0.98, 1.17)
Clinical cut-offs							
Severely deficient (n = 502)	-0.05 (-0.18, 0.08)	-0.10 (-0.22, 0.02)	0.05 (-0.08, 0.19)	-0.01 (-0.13, 0.12)	0.89 (0.47, 1.70)	0.91 (0.63, 1.31)	0.70 (0.41, 1.20)
Deficient (n = 894)	-0.02 (-0.12, 0.09)	-0.03 (-0.12, 0.07)	0.00 (-0.11, 0.11)	-0.04 (-0.13, 0.07)	0.86 (0.51, 1.46)	1.05 (0.78, 1.40)	0.97 (0.65, 1.46)
Sufficient (n = 639)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Model 2: child 25-hydroxyvitamin D model							
25-hydroxyvitamin D per 10 nmol/L (n = 1,166)	0.00 (-0.03, 0.03)	-0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)	1.05 (0.90, 1.23)	0.97 (0.89, 1.06)	1.09 (0.97, 1.23)
Clinical cut-offs							
Severely deficient (n = 305)	-0.05 (-0.22, 0.12)	-0.05 (-0.21, 0.10)	-0.03 (-0.21, 0.14)	-0.05 (-0.22, 0.11)	0.62 (0.26, 1.44)	1.11 (0.69, 1.79)	0.79 (0.39, 1.62)
Deficient (n = 505)	-0.02 (-0.15, 0.12)	0.04 (-0.08, 0.17)	-0.12 (-0.26, 0.02)	-0.09 (-0.22, 0.04)	0.71 (0.36, 1.40)	1.06 (0.72, 1.55)	0.74 (0.42, 1.31)
Sufficient (n = 356)	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 second (FEV₁), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF₇₅). 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 mold

in the house and folate concentration, and child's sex, gestational age at birth, birthweight, 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 years.

Figure E-1. Flow chart of participants included for analysis

