

Prospective Association of 25-Hydroxyvitamin D₃ and D₂ with Childhood Lung Function, Asthma, Wheezing, and Flexural Dermatitis

Anna-Maija Tolppanen,^a Adrian Sayers,^b Raquel Granell,^b William D. Fraser,^c John Henderson,^b and Debbie A. Lawlor^a

Background: Higher serum total 25-hydroxyvitamin D (25(OH)D) concentrations have been associated with better lung function and lower risk of allergic disease. 25(OH)D₃ constitutes the majority of total 25(OH)D and has been suggested to be more potent than 25(OH)D₂. We studied the prospective associations of 25(OH)D₂ and 25(OH)D₃ with asthma, wheezing, flexural dermatitis, and lung function in children who participated in the Avon Longitudinal Study of Parents and Children—a population-based contemporary birth cohort of children born in 1991–1992 from South West England.

Methods: Serum 25(OH)D₂ and 25(OH)D₃ concentrations, measured at a mean age of 9.8 years, were related to incident cases of wheezing (study sample: $n = 3,323$, 141 cases; 4%), asthma ($n = 3,323$, 464 cases; 14%), and flexural dermatitis ($n = 3,748$, 300 cases; 8%), as well as lung function (forced expiratory volume in 1 second [FEV₁], forced vital capacity [FVC], and mid-forced expiratory flow assessed at a mean age of 15.5 years: $n = 2,259$).

Results: 25(OH)D₂ was inversely associated with flexural dermatitis (adjusted odds ratio per doubling of exposure = 0.83 [95% confidence interval = 0.72–0.94]) and wheezing (0.83 [0.68–1.00]), and 25(OH)D₃ was positively associated with flexural dermatitis (1.09 [1.00–1.18]) and wheezing (1.14 [1.03–1.28]). 25(OH)D₂ was weakly positively associated with FEV₁ and FVC. 25(OH)D₃ was not associated with lung function.

Conclusions: These results suggest that higher 25(OH)D₃ concentrations are associated with increased risk of wheezing and flexural dermatitis. Despite being one of the few prospective studies and being able to adjust for confounders, these findings need replication. Our results do not provide strong evidence that lower concentrations of vitamin D are detrimental to respiratory and allergic health in children.

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Associations between vitamin D status, indicated by serum 25-hydroxyvitamin D (25(OH)D) concentrations, and lung function^{1–8} or risk of atopic and allergic outcomes^{7–9} in adults have been inconsistent. These previous studies have often been cross-sectional,^{1–5,8} had a small sample size (<650 participants),^{1,3–7} and been conducted in clinical cohorts (eg, patients with asthma,^{3,4,7} interstitial lung disease,¹ or (ex-)smokers^{5,6}); thus, the prospective association of vitamin D in lung function in the general adult population is unclear. Furthermore, it is plausible that there are different etiologic characteristics related to the development of respiratory and immunologic function in childhood than to their maintenance and age-related decline in later adulthood.¹⁰ Thus, any association of vitamin D with markers of asthma and allergy and with lung function in childhood may differ from those in adulthood.

Large prospective cohort studies have found an association between high maternal intake of vitamin D or higher maternal 25(OH)D concentrations during pregnancy and lower risk of wheezing^{11–13} and asthma and allergies in childhood,^{14,15} although other cohort studies have concluded that higher vitamin D intake during pregnancy is associated with higher risk of asthma^{16,17} and that vitamin D supplementation during first year of life is related to increased risk of atopy and allergic rhinitis¹⁸ or found no association between maternal 25(OH)D concentrations and asthma or wheezing in childhood.¹⁹ Studies assessing child's own 25(OH)D concentrations and lung function^{8,20–24} or risk of allergic outcomes^{8,9,19,20,22,25–33} have been inconsistent. Similar to epidemiologic studies in adults, these studies have

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From the ^aMRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Bristol, UK; ^bSchool of Social and Community Medicine, University of Bristol, Bristol, UK; and ^cNorwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK.

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Correspondence: Anna-Maija Tolppanen, MRC Centre for Causal Analysis in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Oakfield House, 15–23 Oakfield Grove, Clifton, Bristol BS8 2BN, UK. E-mail: anna-maija.tolppanen@uef.fi.

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often been cross-sectional,^{8,9,20–22,24,26,27,31,33} small,^{17,20–24,26–30,32,33} or conducted among children with asthma.^{20–22,24,26} Six prospective studies have assessed the association between vitamin D status (indicated by 25(OH)D concentrations) and allergic or respiratory outcomes,^{17,19,23,25,29,30} and two randomized controlled trials have been conducted.^{28,32} Higher maternal 25(OH)D concentrations were associated with higher risk of asthma and eczema in one birth cohort study,¹⁷ whereas no associations were detected in another cohort.¹⁹ A U-shaped association between cord-blood 25(OH)D concentrations and aeroallergen sensitization²⁹ and an inverse association between cord-blood concentrations of 25(OH)D and wheezing have been reported.²⁵ Although vitamin D supplementation reduced the risk of asthma exacerbations²⁸ and recurrent asthma attacks³² in randomized controlled trials and better vitamin D status in childhood was prospectively associated with lower risk of asthma,³⁰ no association between 25(OH)D concentrations and lung function was observed in a general population of children.²³

Circulating 25(OH)D consists of 25(OH)D₃ (metabolite of vitamin D₃ synthesized in skin after ultraviolet B exposure and obtained from animal food sources) and 25(OH)D₂ (synthesized from vitamin D₂ obtained from plant sources). With respect to bone health, vitamin D₃ has been suggested to be more potent than D₂,³⁴ but no studies have examined whether associations of these two differ with respect to lung function or atopy. Exploring these differences is important because supplements can be manufactured from either form of vitamin D. If one form is more potent than the other, as has been argued,^{35,36} and has stronger evidence of effects on adverse respiratory and atopic outcomes, it would make sense to use that form in subsequent clinical trials.

We investigated the prospective associations between serum 25(OH)D₂ and 25(OH)D₃ concentrations and asthma, wheezing, flexural dermatitis, and lung function in childhood, and we compared whether the associations of 25(OH)D₂ and 25(OH)D₃ with these outcomes were different. Because vitamin D together with parathyroid hormone regulates calcium and phosphate homeostasis,³⁷ we investigated whether parathyroid hormone, calcium, and phosphate were related to outcomes and whether the associations of 25(OH)D₂ and 25(OH)D₃ were independent of parathyroid hormone, calcium, or phosphate levels.

METHODS

Participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort from South West England. The cohort consisted of 14,062 live births from 14,541 enrolled pregnant women who were expected to give birth between 1 April 1991 and 31 December 1992.³⁸ From 7 years of age, all children have been invited for regular clinical assessments. Informed consent was obtained from the parents and ethical approval from the ALSPAC Law and Ethics

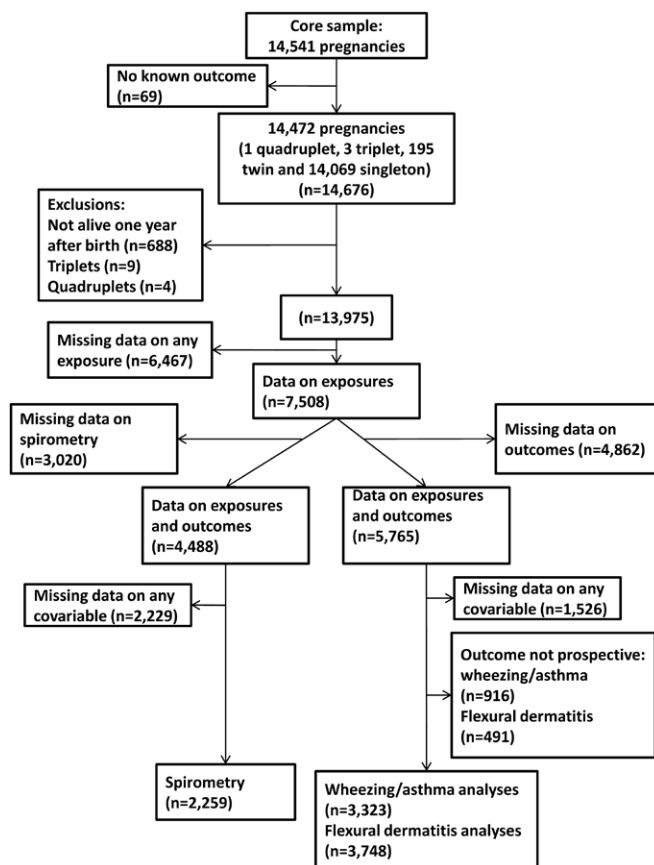


FIGURE. Flow diagram of participants.

Research Committee and local research ethics committee at baseline and all follow-up assessments.

Single and twin births were included in this study. Complete data on exposures, confounders, and wheezing or asthma were available on 3323 children (24% of live births in the cohort); on exposures, confounders, and flexural dermatitis on 3748 children (27%); and on exposures, confounders, and lung function at 15 years of age on 2259 children (16%) (Fig.).

Outcomes

Data on asthma and wheezing during the previous year were obtained from the child's main caregiver (most commonly mother) by questionnaires when children were on average 6.8, 7.6, 8.6, 10.7, 12.8, and 13.5 years old ("In the past 12 months, has he/she had asthma?" and "In the past 12 months, has he/she had wheezing or whistling on his/her chest when he/she breathed?"). Children 15–16 years of age responded to the question, "Have you had two or more episodes of wheeze during the last year?" For analyses with these outcomes, we excluded any participant with a history of asthma or wheezing at any assessment before, or around the same time as, the phlebotomy (prevalent asthma/wheezing, $n = 916$). Flexural dermatitis was measured according to the The International Study of Asthma and Allergies in Childhood (ISAAC) protocol³⁹ at clinics conducted when the participants were 7–8, 9–10,

10–11, 11–12, 12–13, and 13–14 years of age. Participants with prevalent flexural dermatitis, defined as those identified at any clinic before, at the time of or 1 year after the 25(OH)D measurement were excluded ($n = 491$). For all three binary outcomes (asthma, wheezing, and flexural dermatitis), the first indication of the child having a new outcome always followed the measurement of 25(OH)D concentrations by at least 1 year.

Lung function was measured at the 15-year clinic at a mean age of 15.5 (standard deviation [SD] = 0.29) years by spirometry (Vitalograph 2120, Maids Moreton, UK) according to the American Thoracic Society/European Respiratory Society criteria.⁴⁰ The equipment was calibrated at the start of each day with a 3-liter calibration syringe to ensure volume accuracy to be within $\pm 3.5\%$, and linearity of the pneumotachometer was checked weekly at different flow rates using the same accuracy thresholds. Measurements were made with the child seated and wearing a nose clip. The best measurements from three reproducible flow-volume curves were used for analyses. Measurements were repeated before and 10 minutes after inhalation of 400 μg salbutamol by metered aerosol and spacer (Volumatic). Flow-volume curves were reviewed by one respiratory physician (J.H.) to ensure adherence to standards. Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and forced maximal mid-expiratory flow (FEF_{25-75}) were converted to sex-, age-, and height-adjusted SD units.⁴¹ The correlation between pre- and postsalbutamol measurements was strong ($r_{\text{FVC}} = 0.95$; $r_{\text{FEV}_1} = 0.94$; $r_{\text{FEF}_{25-75}} = 0.87$), so the results are reported for postsalbutamol lung function measurements only. Bronchodilator reversibility was defined as a 12% or greater increase in FEV_1 postsalbutamol.⁴²

Exposures and Blood-Based Covariables

Serum 25(OH)D₂, 25(OH)D₃, phosphate, calcium, and parathyroid hormone were assayed on nonfasting blood samples collected at a mean age of 9.9 years for the majority of participants. If no samples were available from the 9.9-year assessment, samples from a mean age of 11.8 years or, second, the 7.6-year assessments were used. The number of children with samples not available from the 9.9-year clinic for flexural dermatitis, asthma/wheezing, and spirometry analyses were 1,148 (31%), 1,000 (30%), and 534 (24%), respectively. The mean age at sample collection in the whole study sample was 9.8 years (SD = 1.18 years). 25(OH)D₂, 25(OH)D₃, and deuterated internal standard were measured with high-pressure liquid chromatography tandem mass spectrometer in the multiple reaction mode. Interassay coefficients of variation for the assay were $<10\%$ across a working range of 1–250 ng/ml for both 25(OH)D₂ and 25(OH)D₃.

Total serum calcium, phosphate, and albumin concentrations were measured by standard laboratory methods on Roche Modular analyzers (Roche Diagnostics Ltd, West Sussex, UK). Serum calcium was adjusted for albumin using a normogram of calcium and albumin distributions of the samples analyzed in the clinical chemistry laboratory where the

measurements were performed. Albumin-adjusted calcium was used in all statistical analyses. Intact parathyroid hormone (1–84) was measured by electrochemiluminescent immunoassay on an Elecsys 2010 immunoanalyzer (Roche, Lewes, UK). Interassay coefficient of variation was $<6\%$ from 2 to 50 pmol/l. The assay sensitivity (replicates of the zero standard) was 1 pmol/l.

Potential Confounders

We considered sex, age, ethnicity, socioeconomic position, exposure to ultraviolet B, body mass index (BMI), and maternal history of allergy and asthma to be important confounders because of their known or suspected associations with 25(OH)D₃ or 25(OH)D₂ concentrations and lung function, asthma, wheeze, or dermatitis. We adjusted for pubertal stage because we felt that this might be associated with lung function and 25(OH)D₃ or 25(OH)D₂. Data on education level and occupational social class of parents; ethnicity; and maternal history of asthma, eczema, and rhinoconjunctivitis were obtained by parent-completed questionnaires. BMI was calculated from weight and height measurements obtained at the same clinic as the blood samples. Weight and height were measured in light clothing and without shoes. Weight was measured to the nearest 0.1 kg using Tanita scales. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer.

Parents' education was recorded as a grouped variable according to increasing levels of achievement: "None or CSE" (certificate of secondary education, ie, subject-specific qualifications of a lower level than ordinary levels, generally obtained at 16 years of age, which was the minimal school-leaving age from 1974 in UK)/"vocational training"/"ordinary level" (subject-specific qualifications generally obtained at 16 years of age)/"advanced level" (subject-specific qualifications generally obtained at 18 years of age and required for university entry)/"university degree." Occupational social class was based on the Registrar General's classification of occupations and grouped into five categories (from highest to lowest): "i" (professional); "ii" (managerial and technical); "iii nonmanual" (skilled nonmanual); "iii manual" (skilled manual); and "iv/v" (semiskilled or unskilled).

Time spent outdoors during summer months on school days, school weekends, and holidays was reported as "None," "1 hour/day," "1–2 hours /day," and "3 or more hours/day" in parent-completed questionnaires at a mean age of 8.5 years. Responses were coded as follows: "None" = 0, "1" = 1, "1–2" = 1.5, and "3" = 5. Average hours spent outdoors per summer day (1 June to 31 August) were calculated using term dates from Bristol City Council's Education Committee term dates for 2001–2002 (summer term 1 June to 23 July, holidays 24 July to 31 August). Information on protection from ultraviolet B exposure (use of sunblock, covering clothing or hat, and avoidance of midday sun) were obtained from the same questionnaires. A summary variable for ultraviolet B protection score was derived by scoring the responses to questions on

use of sunblock, covering clothing or hat, and avoidance of midday sun as “Always” = 3, “Usually” = 2, “Sometimes” = 1, “Never” = 0, and summing these scores. This gives a single variable that ranges from zero to 12, with zero indicating the least meticulous protection from ultraviolet B radiation. Puberty stage was assessed by self-reported Tanner staging of pubic hair and breast development at the time of spirometry assessment.

Statistical Analyses

Statistical analyses were conducted with Stata 11.0 (Stata Corp LP, College Station, TX). 25(OH)D₃ was modeled according to date of phlebotomy using linear regression with trigonometric sine and cosine functions to adjust for seasonal variability. 25(OH)D₃ was log_e-transformed to reduce heteroscedasticity. The residual was used as 25(OH)D₃ exposure variable in regression analyses. In supplementary analyses, we also report associations of unadjusted (for season) 25(OH)D₃ and total 25(OH)D.

To include all participants on whom a 25(OH)D₂ was assayed, those with a value below the detectable limit of the assay (0.5 ng/ml) were given a value of 0.5 ng/ml and indicated using a binary covariable in all regression models. To account for age differences at the time of assessment, we generated age- and sex-standardized z-scores for serum 25(OH)D₃, 25(OH)D₂, adjusted calcium, phosphate, and parathyroid hormone using the internal cohort data with age in 1-month categories.

The association of potential confounders with exposures and spirometry results was assessed with linear regression and the association with asthma, wheezing, and flexural dermatitis with logistic regression. The association between exposures and outcomes was analyzed using a nonparametric bootstrap with replacement in conjunction with ordinary least squares linear regression (continuous outcomes) or logistic regression (categorical outcomes), based on 1000 replications. The bootstrapping procedure enabled us to statistically compare associations of 25(OH)D₂ with outcomes to those of 25(OH)D₃ with outcomes. The difference between the association of 25(OH)D₂ and 25(OH)D₃ was calculated from the bootstrap replicate distribution. Beta estimates and standard errors were empirically calculated from the mean and SD, respectively, of the bootstrap distribution. *P* values were calculated using bootstrap means and standard errors and compared with a z-distribution. To numerically compare the associations of two forms of 25(OH)D, we scaled them the same by multiplying the beta coefficients from the regression models by log_e(2). These can then be interpreted as mean differences or odds ratios per doubling in exposure. Analyses were performed for both sexes combined, and results of sex-specific analyses are reported only if there was statistical evidence of Sex × Exposure interaction.

RESULTS

The medians (interquartile ranges) of 25(OH)D₃, 25(OH)D₂, phosphate, albumin-adjusted calcium, and parathyroid

hormone were 24.9 (24.7–25.1) ng/ml, 1.3 (0.5–2.7) ng/ml, 1.53 (1.43–1.64) mmol/l, 2.37 (2.31–2.44) mmol/l, and 4.4 (3.3–5.8) pmol/l, respectively. Altogether, 141 (4%) of children had incident cases of wheezing, 464 (14%) had incident cases of asthma, and 300 (8%) incident cases of flexural dermatitis after exposure assessment. Of 2259 children who had data on exposures, confounders, and spirometry outcomes, 181 (8%) were responsive to bronchodilator, ie, had 12% or greater increase in FEV₁ after salbutamol administration.

Table 1 shows the distribution of exposures, confounders, and outcomes in those participants who were excluded from flexural dermatitis analyses and spirometry analyses owing to missing data in comparison to included participants. Differences between excluded and included children in asthma and wheezing analyses were similar to differences between children who were excluded and included in flexural dermatitis analyses (data not shown). Children with missing data had lower ultraviolet B protection score and spent less time outdoors, were more likely to be of non-white ethnicity, and have lower parental socioeconomic position or maternal history of allergy. Children who were included in the flexural dermatitis analyses were younger; had lower BMI; and were less likely to have incident cases of wheezing, asthma, or flexural dermatitis than the excluded children. There were no differences in the distribution of exposure variables. Children who were included in the spirometry analyses were older, had slightly higher FVC and phosphate concentration, were more likely to be girls or have incident cases of wheezing or flexural dermatitis, and less likely to have incident cases of asthma than excluded children. There were no differences in other exposure or spirometry-based outcome variables between included and excluded children.

Associations of the potential confounders with age- and sex-adjusted exposures are shown in eTable 1 (<http://links.lww.com/EDE/A648>). Higher BMI and non-white ethnicity were associated with lower serum 25(OH)D₃ and 25(OH)D₂ concentrations and with higher parathyroid hormone concentrations. Higher socioeconomic position was associated with higher concentrations of 25(OH)D₃ and lower concentrations of 25(OH)D₂ and calcium. More careful protection from ultraviolet B was associated with higher concentrations of 25(OH)D₃ and lower concentrations of parathyroid hormone. Children who spent more time outdoors had higher serum concentrations of 25(OH)D₃ and 25(OH)D₂. Children with higher 25(OH)D₃ concentrations had less advanced pubertal stage at 15 years of age.

eTable 2 (<http://links.lww.com/EDE/A648>) shows the associations of exposures and confounders with asthma, wheezing, and flexural dermatitis. Higher BMI was associated with higher risk of asthma. Children from lower socioeconomic background and those with maternal history of asthma or allergy had higher odds of asthma, wheezing, and flexural dermatitis. Children who were less meticulous about protection from ultraviolet B radiation had higher risk of asthma.

TABLE 1. Distribution of Confounders, Outcomes, and Exposures in Children Who Were Excluded from Flexural Dermatitis or Spirometry Analyses in Comparison to Children Included in These Analyses

	Flexural Dermatitis Analyses			Spirometry Analyses		
	No.	Excluded	Included (n = 3,478)	No.	Excluded	Included (n = 2,259)
Age at exposure assessment (years)	3,397	9.84 (0.02)	9.71 (0.02)	4,886	9.72 (0.02)	9.91 (0.02)
BMI at exposure assessment (kg/m ²)	3,397	17.67 (0.06)	17.23 (0.05)	4,886	17.46 (0.05)	17.39 (0.07)
Ultraviolet B protection score	4,046	7.62 (0.04)	7.39 (0.04)	5,535	7.55 (0.03)	7.40 (0.05)
Average hours/day spent outdoors during summer	4,036	4.10 (0.02)	4.01 (0.02)	5,525	4.10 (0.02)	4.00 (0.02)
Standardized FVC	1,619	NA	NA	1,598	−0.03 (0.03)	0.03 (0.02)
Standardized FEV ₁	1,560	NA	NA	1,441	−0.01 (0.02)	0.02 (0.02)
Standardized FEF _{25–75}	1,619	NA	NA	1,598	0.01 (0.01)	0.00 (0.01)
Serum 25(OH)D ₃ concentration (ng/ml) ^a	3,363	24.9 (0.01)	24.9 (0.01)	4,847	24.9 (0.01)	24.9 (0.01)
Serum 25(OH)D ₂ concentration (ng/ml) ^a	3,358	1.4 (0.5–2.8)	1.3 (0.5–2.7)	4,847	1.4 (0.5–2.8)	1.2 (0.5–2.6)
Serum calcium concentration mmol/l	3,386	2.38 (0.01)	2.38 (0.01)	4,857	2.37 (0.01)	2.38 (0.01)
Serum phosphate concentration (mmol/l)	3,348	1.54 (0.01)	1.54 (0.01)	4,837	1.53 (0.01)	1.55 (0.01)
Serum parathyroid hormone concentration (pmol/l) ^a	3,748	4.4 (3.3–5.8)	4.3 (3.3–5.7)	4,860	4.3 (3.3–5.7)	4.5 (3.4–5.9)
Sex	10,398			11,887		
Boys	5,415	52.1	50.8	6,215	52.3	48.9
Girls	4,983	47.9	49.2	5,672	47.7	51.1
Ethnicity	9,985			11,474		
White	9,431	93.6	96.6	10,777	93.9	96.8
Non-white	644	6.5	3.4	697	6.1	3.2
Head of household social class	7,817			9,306		
i (highest)	860	11.0	18.1	1,117	12.0	18.7
ii	3,088	39.5	46.6	3,742	40.2	48.3
iii (nonmanual)	2,065	26.4	23.5	2,431	26.1	22.8
iii (manual)	1,236	15.8	8.7	1,408	15.1	6.9
iv/v (lowest)	568	7.3	3.1	608	6.5	3.4
Paternal education	8,254			9,743		
None/CSE (lowest)	2,544	30.8	15.7	2,816	28.9	14.1
Vocational	712	8.6	8.1	857	8.8	7.0
O level	1,726	20.9	22.0	2,066	21.2	21.5
A level	2,006	24.3	29.8	2,412	24.8	31.4
Degree (highest)	1,266	15.3	24.4	1,592	16.3	26.1
Maternal education	8,735			10,224		
None/CSE (lowest)	2,159	24.7	9.7	2,643	22.9	8.0
Vocational	967	11.1	7.0	1,086	10.6	6.3
O level	2,989	34.2	36.0	3,531	34.5	35.1
A level	1,736	19.9	28.5	2,126	20.8	30.0
Degree (highest)	884	10.1	19.3	1,139	11.1	20.7
Maternal history of asthma	8,598			10,087		
No	7,577	88.1	89.1	8,906	88.3	89.0
Yes	1,021	11.9	10.9	1,181	11.7	11.0
Maternal history of allergy	8,546			10,035		
No	3,563	41.7	48.8	4,238	42.2	46.0
Yes	4,983	58.3	54.2	5,797	57.8	54.0
Incident asthma	5,084			6,573		
No	3,136	61.7	74.4	4,328	65.9	70.7
Yes	1,948	38.3	25.6	2,245	34.2	29.3
Incident wheezing	3,953			5,442		
No	3,471	87.8	90.5	4,874	89.6	88.0
Yes	482	12.2	9.5	568	10.4	12.0

(Continued)

TABLE 1. (Continued)

	Flexural Dermatitis Analyses			Spirometry Analyses		
	No.	Excluded	Included (n = 3,478)	No.	Excluded	Included (n = 2,259)
Incident flexural dermatitis	3,731			5,246		
No	3,193	85.6	92.0	4,738	90.3	85.2
Yes	538	14.4	8.0	508	9.7	14.8
≥12% increase in FEV ₁	1,577	NA	NA	1,464		
No		NA	NA	1,334	91.1	91.2
Yes		NA	NA	130	8.9	8.0
Puberty stage at spirometry assessment	1,961	NA	NA	2,325		
1–3		NA	NA	215	9.3	8.4
4		NA	NA	1,244	53.5	52.4
5		NA	NA	866	37.3	39.2

Data are given as mean (standard error) for continuous variables except as median (interquartile range) for serum 25(OH)D₂ and parathyroid hormone concentrations and as percentages for categorical variables.

^a25(OH)D₂, 25(OH)D₃, and parathyroid hormone concentrations were log-transformed.

TABLE 2. Prospective Association of 25(OH)D₂, 25(OH)D₃, Phosphate, Calcium, and Parathyroid Hormone Concentrations (Assessed at Mean Age 9.8 Years) with Asthma (464/3,323 [14.0%]), Wheezing (141/3,323 [4.2%]), and Flexural Dermatitis (300/3,748 [8.0%]), Assessed a Minimum of 1 Year After the Exposure

Exposure	Outcome	Odds Ratio Per Doubling of Exposure (95% CI)		
		Model 1	Model 2	Model 3
25(OH)D ₂	Wheezing	0.81 (0.69 to 0.98)	0.83 (0.69 to 1.02)	0.83 (0.68 to 1.00)
	Asthma	0.98 (0.89 to 1.08)	0.89 (0.77 to 1.01)	0.89 (0.78 to 1.02)
	Flexural dermatitis	0.85 (0.75 to 0.97)	0.83 (0.73 to 0.94)	0.83 (0.72 to 0.94)
25(OH)D ₃	Wheezing	1.14 (1.03 to 1.26)	1.15 (1.03 to 1.27)	1.14 (1.03 to 1.28)
	Asthma	0.98 (0.92 to 1.05)	1.03 (0.94 to 1.12)	1.02 (0.93 to 1.12)
	Flexural dermatitis	1.07 (0.99 to 1.16)	1.08 (0.99 to 1.17)	1.09 (1.00 to 1.18)
Albumin-adjusted calcium	Wheezing	0.99 (0.88 to 1.10)	0.98 (0.87 to 1.10)	0.96 (0.85 to 1.08)
	Asthma	0.97 (0.90 to 1.04)	1.01 (0.92 to 1.10)	1.00 (0.91 to 1.10)
	Flexural dermatitis	0.98 (0.90 to 1.05)	0.97 (0.90 to 1.06)	0.97 (0.89 to 1.06)
Phosphate	Wheezing	1.07 (0.95 to 1.21)	1.07 (0.95 to 1.20)	1.09 (0.97 to 1.23)
	Asthma	1.02 (0.95 to 1.09)	1.02 (0.93 to 1.13)	1.04 (0.94 to 1.14)
	Flexural dermatitis	1.05 (0.97 to 1.15)	1.06 (0.98 to 1.15)	1.06 (0.97 to 1.16)
Parathyroid hormone	Wheezing	0.99 (0.90 to 1.10)	0.99 (0.88 to 1.10)	0.99 (0.88 to 1.11)
	Asthma	0.97 (0.92 to 1.04)	0.92 (0.85 to 1.01)	0.92 (0.84 to 1.00)
	Flexural dermatitis	1.07 (1.00 to 1.15)	1.08 (0.99 to 1.17)	1.08 (1.00 to 1.18)

Model 1 is unadjusted (the exposures are standardized for age and sex and 25(OH)D₃ is adjusted for season and ethnicity). Model 2 is adjusted for ethnicity, maternal history of asthma and allergy, head of household social class, mother's and partner's education, time spent outdoors during summer, protection from ultraviolet B exposure, and BMI. Model 3 is adjusted for Model 2 plus serum concentrations of other hormones or metabolites that are related to vitamin D homeostasis (eg, association of 25(OH)D₂ is adjusted for 25(OH)D₃, phosphate, albumin-adjusted calcium, and parathyroid hormone).

eTable 3 (<http://links.lww.com/EDE/A648>) shows the associations of confounders with lung function measurements. Non-white ethnicity, lower socioeconomic position, lower BMI, and less advanced puberty stage were associated with lower lung function. Children from lower socioeconomic background and those who had parental report of a diagnosis of asthma were more likely to have clinically meaningful increase in postsalbutamol FEV₁.

Table 2 shows the associations of 25(OH)D₂, 25(OH)D₃, phosphate, calcium, and parathyroid hormone with incident cases of asthma, wheezing, and flexural dermatitis assessed at least 1 year after phlebotomy. Higher 25(OH)D₂ concentrations were associated with lower risk of wheezing and flexural dermatitis, whereas higher 25(OH)D₃ concentrations were associated with higher risk of wheezing and flexural dermatitis in age- and sex-adjusted model (Model

TABLE 3. Prospective Association of 25(OH)D₂, 25(OH)D₃, Phosphate, Calcium, and Parathyroid Hormone Concentrations (Assessed at Mean Age 9.8 Years) with Lung Function After 400 µg Salbutamol Dose (Assessed at Age 15 Years, N = 2,259)

Exposure	Outcome	SD Change in Outcome Per Doubling of Exposure (95% CI)		
		Model 1	Model 2	Model 3
25(OH)D ₂	FVC	0.02 (−0.02 to 0.07)	0.04 (−0.01 to 0.09)	0.04 (0.00 to 0.09)
	FEV ₁	0.03 (−0.01 to 0.09)	0.06 (0.01 to 0.10)	0.06 (0.01 to 0.10)
	FEF _{25–75}	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.01)
25(OH)D ₃	FVC	0.01 (−0.03 to 0.04)	0.00 (−0.03 to 0.04)	0.00 (−0.03 to 0.03)
	FEV ₁	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.03)	0.00 (−0.03 to 0.03)
	FEF _{25–75}	−0.01 (−0.01 to 0.00)	−0.01 (−0.01 to 0.00)	−0.01 (−0.01 to 0.00)
Albumin-adjusted calcium	FVC	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.04)
	FEV ₁	0.00 (−0.03 to 0.04)	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.04)
	FEF _{25–75}	0.00 (0.00 to 0.01)	0.00 (−0.01 to 0.01)	0.00 (0.00 to 0.01)
Phosphate	FVC	−0.01 (−0.04 to 0.02)	−0.01 (−0.04 to 0.02)	−0.01 (−0.04 to 0.02)
	FEV ₁	−0.01 (−0.04 to 0.01)	−0.01 (−0.04 to 0.01)	−0.01 (−0.04 to 0.02)
	FEF _{25–75}	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.00)
Parathyroid hormone	FVC	−0.02 (−0.05 to 0.01)	−0.01 (−0.04 to 0.01)	−0.01 (−0.04 to 0.02)
	FEV ₁	−0.03 (−0.06 to 0.00)	−0.02 (−0.05 to 0.01)	−0.02 (−0.05 to 0.01)
	FEF _{25–75}	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.01)	0.00 (−0.01 to 0.01)

Model 1 is unadjusted (the exposures are standardized for age and sex and 25(OH)D₃ is adjusted for season and ethnicity). Model 2 is adjusted for ethnicity, maternal history of asthma and allergy, head of household social class, mother's and partner's education, time spent outdoors during summer, protection from ultraviolet B exposure, BMI, asthma, and pubertal stage at 15 years. Model 3 is adjusted for Model 2 plus serum concentrations of other hormones/metabolites which are related to vitamin D homeostasis (eg, association of 25(OH)D₂ is adjusted for 25(OH)D₃, phosphate, albumin-adjusted calcium, and parathyroid hormone).

1, additional adjustment for seasonality and ethnicity for 25(OH)D₃). These associations remained after adjusting for confounders and other analytes (Models 2 and 3). Higher 25(OH)D₂ concentrations were weakly associated with lower risk of asthma in the confounder-adjusted model (Model 2). The associations of 25(OH)D₃ and 25(OH)D₂ with wheezing and flexural dermatitis were statistically different ($P_{\text{difference}} = 0.004$ and 0.001 , respectively). Higher serum parathyroid hormone concentrations were associated with higher risk of flexural dermatitis in an age- and sex-adjusted model (Model 1) and with higher risk of flexural dermatitis and lower risk of asthma after adjusting for additional confounders (Model 2) and other analytes (Model 3). Parathyroid hormone concentrations were not associated with wheezing, and calcium and phosphate concentrations were not associated with wheezing, asthma, or flexural dermatitis.

There was statistical evidence for sex difference in associations between 25(OH)D₂ and flexural dermatitis ($P_{\text{interaction}} = 0.07$, P for other Sex $\times$ Exposure interactions ≥ 0.10). Higher concentrations of 25(OH)D₂ were more clearly associated with lower risk of flexural dermatitis in girls (adjusted odds ratio = 0.76 [95% confidence interval = 0.61–0.93]) than in boys (0.88 [0.72–1.05]).

Table 3 shows the association of 25(OH)D₂, 25(OH)D₃, phosphate, calcium, and parathyroid hormone with FVC, FEV₁, and FEF_{25–75}. Higher serum 25(OH)D₂ concentrations were associated with higher FVC and FEV₁, but not with FEF_{25–75} in the confounder-adjusted and fully adjusted models (Models 2 and 3). Higher serum 25(OH)D₃ concentrations

were weakly associated with lower FEF_{25–75} in all models. 25(OH)D₃ concentrations were not associated with lung function, and the associations of 25(OH)D₃ and 25(OH)D₂ with FEV₁ were statistically different ($P_{\text{difference}} = 0.04$, $P_{\text{difference}}$ for other outcomes ≥ 0.1). Serum calcium, phosphate, and parathyroid hormone concentrations were not associated with lung function. There was no evidence for Sex $\times$ Exposure interaction for any of the spirometry outcomes ($P_{\text{interaction}} \geq 0.16$).

Table 4 shows the association of 25(OH)D₂, 25(OH)D₃, phosphate, calcium, and parathyroid hormone concentrations with bronchodilator responsiveness of FEV₁. 25(OH)D₂, 25(OH)D₃, calcium, and phosphate concentrations were not associated with bronchodilator responsiveness. Those with higher parathyroid hormone concentrations were less likely to have improvement of $>12\%$ in FEV₁ after salbutamol in the age- and sex-adjusted (Model 1), confounder-adjusted models (Model 2), and after adjusting for other serum analytes (Model 3).

The associations of unadjusted (for season) 25(OH)D₃ (eTables 4 and 5, <http://links.lww.com/EDE/A648>) and total 25(OH)D (eTables 6 and 7, <http://links.lww.com/EDE/A648>) were broadly similar to those of season-adjusted 25(OH)D₃, although the associations with flexural dermatitis were closer to null than the associations of season-adjusted 25(OH)D₃.

DISCUSSION

This is the first large study investigating the prospective association between circulating concentrations of 25(OH)

TABLE 4. Prospective Association of 25(OH)D₂, 25(OH)D₃, Phosphate, Calcium, and Parathyroid Hormone Concentrations (Assessed at Mean Age 9.8 Years) with $\geq 12\%$ Increase in FEV₁ After 400 μ g Salbutamol Dose (Assessed at Age 15 Years, N = 2,259, n = 181; 8.0%)

Exposure	Odds Ratio Per Doubling of Exposure (95% CI)		
	Model 1	Model 2	Model 3
25(OH)D ₂	1.06 (0.89 to 1.25)	1.08 (0.90 to 1.29)	1.10 (0.90 to 1.32)
25(OH)D ₃	1.02 (0.93 to 1.12)	1.01 (0.90 to 1.12)	1.00 (0.90 to 1.12)
Albumin-adjusted calcium	0.96 (0.85 to 1.06)	0.98 (0.88 to 1.08)	0.94 (0.83 to 1.05)
Phosphate	1.06 (0.96 to 1.17)	1.05 (0.95 to 1.17)	1.09 (0.98 to 1.22)
Parathyroid hormone	0.90 (0.81 to 0.99)	0.89 (0.79 to 0.99)	0.88 (0.78 to 0.98)

Model 1 is unadjusted (the exposures are standardized for age and sex and 25(OH)D₃ is adjusted for season and ethnicity). Model 2 is adjusted for ethnicity, maternal history of asthma and allergy, head of household social class, mother's and partner's education, time spent outdoors during summer, protection from ultraviolet B exposure, BMI, asthma, and pubertal stage at 15 years. Model 3 is adjusted for Model 2 plus serum concentrations of other hormones/metabolites which are related to vitamin D homeostasis (eg, association of 25(OH)D₂ is adjusted for 25(OH)D₃, phosphate, albumin-adjusted calcium, and parathyroid hormone).

D₂ and 25(OH)D₃ with asthma, wheezing, flexural dermatitis, and lung function in children or adolescents and comparing the association of 25(OH)D₂ and 25(OH)D₃ with these outcomes. Previous prospective studies that have investigated these outcomes in children have been considerably smaller than our study, and they have not assessed associations of 25(OH)D₂.^{19,23,25,30,31} We found inverse associations of 25(OH)D₂ with wheezing and flexural dermatitis and weak positive associations with FVC and FEV₁, which are measures of lung volume and airway obstruction, bronchoconstriction, or bronchodilatation, respectively. By contrast, 25(OH)D₃ was positively associated with wheezing and flexural dermatitis and also weakly positively associated with FEF_{25–75}, an index of airway obstruction.

In humans, 25(OH)D₃ contributes 80–90% of the total 25(OH)D concentration and is thought to be more potent than 25(OH)D₂.^{35,36} Thus, from previous, largely cross-sectional studies that have shown higher concentrations of total 25(OH)D to be associated with reduced risk of asthma^{20,23,25,30,31,33} and better lung function,^{1–5,8,20–22,24} we might have expected that higher 25(OH)D₃ concentrations would be associated with lower risk of wheezing, asthma, and flexural dermatitis and with better lung function. However, cross-sectional studies may be importantly biased by reverse causality, ie, those with asthma or poorer lung function might be less able to spend time outdoors and as a result have lower 25(OH)D₃ and total 25(OH)D because of lower ultraviolet B exposure. In addition, these studies in adults, many of which have been completed in clinical populations, may not be generalizable to healthy children or adolescents.

Vitamin D is an active mediator of both innate and adaptive immune systems and has a key role in balance between different types of T-helper cells, ie, Th1 and Th2 (eg, reviewed by Litonjua⁴³ and Adams and Hewison⁴⁴). It has been suggested that the associations with lung function and diseases are mediated via modulation of macrophage function.⁴⁵ Vitamin D may also play a dual role by both

enhancing and suppressing Th2-mediated responses. These proposed contradictory effects have been hypothesized to be explained by the timing and chronicity of vitamin D administration relative to allergen sensitization.⁴³ Most studies assessing 25(OH)D in childhood, with similar outcomes to those we examined, have found either low concentrations to be associated with adverse outcomes^{9,20,21,25,26,28,30} or null association,^{19,23} although one small cross-sectional study found an association between higher 25(OH)D concentrations and increased risk of asthma at 8 years of age,³³ and a U-shaped association between cord-blood 25(OH)D concentrations and aeroallergen sensitization in childhood was observed in another small study.²⁹ Eight of these previous studies have been prospective.^{17,19,23,25,28–30,32} A small randomized controlled trial, which investigated the efficacy of vitamin D₃ supplementation on asthma exacerbations, found that supplementation reduced the risk of asthma exacerbations.²⁸ Another trial found an association between vitamin D supplementation and reduction in recurrent asthma attacks,³² and better vitamin D status was prospectively associated with lower risk of asthma among 689 children assessed at 6 and 14 years of age.³⁰ Higher cord-blood concentrations of 25(OH)D were associated with lower risk of wheezing but not of asthma in a study of 922 children.²⁵ However, no association between maternal vitamin D status and asthma or wheezing at childhood was found in a birth cohort of 1724 children,¹⁹ and another study, with 219 participants, found a U-shaped association between cord-blood 25(OH)D concentrations and aeroallergen sensitization in childhood.²⁹ Higher 25(OH)D concentrations during pregnancy were associated with higher risk of asthma in 178 mother-child dyads.¹⁷ To our knowledge, only one previous study has investigated the association between child's own 25(OH)D concentrations and lung function in a general population of children.²³ That study was considerably smaller (n = 436), but, consistent with our findings with 25(OH)D₃, it reported no association between 25(OH)D concentrations and lung function.

For some investigated outcomes, the associations for 25(OH)D₂ were different from those for 25(OH)D₃, which may reflect distinct biologic activities. However, most of the mechanistic work has focused on vitamin D₃, and so currently there are no biologic explanations for different associations between the two forms. Similarly, there are no biologic explanations for the association between parathyroid hormone and flexural dermatitis. The sources for the two 25(OH)D forms are different: vitamin D₂ is obtained solely from diet and supplements, whereas most vitamin D₃ is formed in the skin after ultraviolet B exposure, and dietary intake normally has a lesser role. Thus, confounders affecting ultraviolet B and outdoor exposure would affect only 25(OH)D₃ concentrations and confound associations between 25(OH)D₃ and outcomes, but have no effect on the associations of 25(OH)D₂ and outcomes. Thus, different confounding structures and residual confounding are also a possible explanation for inconsistencies in our results.⁴⁶

Strengths of this study include the large sample size, prospective design, ability to adjust for multiple potential confounders, standardized measurement methodology for lung function, and repeat assessment of questionnaire-reported wheezing and asthma that allowed us to exclude participants with evidence of prevalent symptoms or disease and to include only new cases reported at least 1 year after the exposure measurement. This together with prospective design decreases the risk of detecting associations owing to reverse causality. The proportion of asthma and flexural dermatitis cases was similar to those observed in UK-wide surveys,^{47,48} whereas the prevalence of wheezing was much lower (12%) in our sample than among UK children in general (28%).⁴⁸ This may reflect difficulties in separating wheezing and asthma by children's parents.

Our study has some limitations. As is common in birth cohort studies, there was loss to follow-up, with those who continued to attend the follow-up clinics being more likely to be from higher socioeconomic background³⁸; also, the proportion of incident cases of wheezing, asthma, and flexural dermatitis cases was higher among children who were excluded owing to missing data. However, the distribution of standardized spirometry outcomes was similar in complete cases and those with complete outcome data. The distribution of 25(OH)D₃ in our study was similar to that of other Northern hemisphere populations,⁴⁹ and so our results are likely to be generalizable to these populations, although not necessarily those exposure to higher levels of ultraviolet B in the Southern hemisphere.

The data on asthma and wheezing were extracted from parent-completed questionnaires and not from medical records. In comparison with medical records, parental reports may underestimate the prevalence of asthma, although there is evidence of generally high levels of agreement between parental reports and medical record (82%).⁵⁰ We would expect any underreporting to be nonsystematic (ie, we would not expect it to be related to serum concentrations of exposures), which

would be expected to bias the associations of exposures with asthma toward the null. Because the proportion of incident cases of wheezing in our study was much lower than in nationwide survey,⁴⁸ it is likely that the associations with wheezing are also underestimated.

To conclude, we found higher 25(OH)D₃, the main constituent of total 25(OH)D, and higher total 25(OH)D to each be associated with greater risk of wheezing and flexural dermatitis, but 25(OH)D₃ and total 25(OH)D not related to diagnosed asthma or lung function. By contrast, higher concentrations of 25(OH)D₂ were associated with reduced risk of wheezing, flexural dermatitis, and better lung function. The magnitude of the association with lung function was weak and unlikely to be of clinical importance to a particular child, but it could have longer term impact in the context of long-term lung development. Long-term studies with trajectories of lung function based on repeat assessments within individuals would be required to detect such effects. Further prospective studies are needed to confirm our findings. Confirmation would be reassuring in the sense that it would reduce concerns about possible adverse respiratory health effects of policies to reduce ultraviolet B exposure in childhood (with clear health benefits in terms of skin cancer), but which may have also reduced 25(OH)D₃ concentrations.

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