ORIGINAL ARTICLE

Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes

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ABSTRACT

Background Studies exploring the relationship between prenatal vitamin D exposure and childhood asthma have yielded conflicting results. Higher vitamin D intake during pregnancy has been shown to lower the risk of childhood wheeze, yet a study of maternal late-pregnancy serum 25-hydroxyvitamin D suggested higher serum concentrations may be associated with increased childhood asthma.

Objective To assess the relationship between mothers' serum 25-hydroxyvitamin D status and asthma and wheeze phenotypes in their children at age 6 years. Also to explore the relationship between maternal 25-hydroxyvitamin D status and objective measures of childhood atopy and lung function.

Methods Serum 25-hydroxyvitamin D was measured at 34 weeks' gestation in the mothers of 860 children born at term. Wheeze was classified as either transient or persistent/late using questionnaire data collated from 6, 12, 24 and 36 months and 6 years. At 6 years spirometry was performed and atopic status was determined by skin prick testing, exhaled nitric oxide was measured in 451 children and bronchial hyperresponsiveness in 216 children.

Results There were no significant associations between maternal late-pregnancy 25-hydroxyvitamin D status and either asthma or wheeze at age 6 years. Maternal vitamin D status was not associated with transient or persistent/late wheeze; no significant association was found between persistent/late wheeze when subdivided according to atopic status. No associations were found with skin sensitisation or lung function.

Conclusions This study provides no evidence that exposure to higher concentrations of 25-hydroxyvitamin D in maternal serum during late pregnancy increases the risk of childhood asthma, wheeze or atopy.

Key messages

What is the key question?
- Does maternal 25-hydroxyvitamin D status influence the presence of asthma and wheeze phenotypes in their children at age 6 years.

What is the bottom line?
- We found no evidence that exposure to higher concentrations of maternal serum 25-hydroxyvitamin D during late pregnancy increases the risks of childhood asthma, wheeze or atopy.

Why read on?
- A number of publications have suggested that a lack of vitamin D is involved in the development of both atopy and asthma.

INTRODUCTION

Vitamin D has multiple effects beyond those upon calcium metabolism and skeletal integrity. A role in asthma and atopy has been suggested as many immune cells possess vitamin D receptors[1] and genetic association has been demonstrated between receptor variants and asthma.[2] However, the relationship between vitamin D and asthma has proven controversial. While Wjst and Dold proposed rising asthma prevalence to be a consequence of increased consumption of vitamin D-fortified foods,[3] Litonjua and Weiss argued, in contrast, that vitamin D deficiency may be responsible.[4] Epidemiological support can be found for either viewpoint. A Southampton study found increased infantile eczema and childhood asthma in the children of mothers with higher late-pregnancy serum 25-hydroxyvitamin D.[5] However, this small study was not specifically designed to look at atopic outcomes. Conversely, several cohort studies have found lower maternal vitamin D intake during pregnancy to be associated with increased childhood wheeze risk.[6–9]

Serum 25-hydroxyvitamin D reflects total body vitamin D.[10] Most vitamin D is derived from photosynthesis in the skin.[10] Serum measures may more accurately characterise total exposure than estimated intake, which does not account for sun exposure. Maternal 25-hydroxyvitamin D status measured in the Dutch KOALA study was not found to be associated with lung function at age 6 years; however, asthma prevalence, wheeze phenotypes and atopy were not assessed.[11]

This is the first study to prospectively assess the relationship between maternal 25-hydroxyvitamin D status during pregnancy and childhood asthma, wheeze and atopy, and the first to consider wheeze separately in atopic and non-atopic children. This is important as persistent wheeze with atopy differs in clinical presentation from non-atopic persistent wheeze and is likely to be of separate aetiology.[12] Data from a large population-based cohort were used to investigate whether higher maternal 25-hydroxyvitamin D status at 34 weeks' gestation is associated with increased

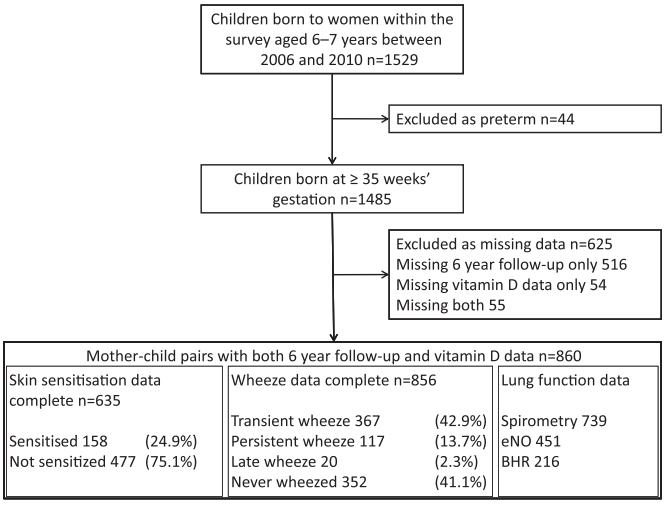

Figure 1 Participants in the study. BHR, bronchial hyperresponsiveness; eNO, exhaled nitric oxide.

childhood asthma or wheeze risk. Objective measures of lung function and skin sensitisation were used to test the secondary hypotheses that maternal 25-hydroxyvitamin D status is associated with evidence of altered immune or respiratory development.

METHODS

Participants

Participants were mother–child pairs from the Southampton Women's Survey.[13] Infants born <35 weeks' gestation were excluded. One thousand four hundred and eighty-five children were aged 6 years during the study period (2006–2010), maternal vitamin status and 6-year follow-up data were available for 860 pairs (figure 1). Parental consent was obtained and the Southampton and South West Hampshire Research Ethics Committee granted ethical approval (276/97, 307/97, 089/99, 06/Q1702/104).

Maternal serum 25-hydroxyvitamin D

Maternal blood was sampled at 34 weeks' gestation, centrifuged, separated and stored at −80°C. 25-Hydroxyvitamin D concentrations were measured by radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA, coefficient of variability <10%).

Maternal vitamin D intake

At 11 and 34 weeks' gestation average frequencies of consumption over the preceding 3 months were recorded using a validated 100-item food frequency questionnaire (FFQ).[14] Early and late pregnancy intakes were averaged.

Atopy

Skin prick testing was conducted at 1 and 3 years using cat, dog, house dust mite, grass pollens, egg and milk allergens (Hollister-Stier, Spokane, Washington, USA); at age 6, tree pollens (ALK Abelló, Hørsholm, Denmark) were also tested. For validity ≥3 mm positive and 0 mm negative control responses were required. Atopy was defined as any allergen response ≥3 mm.

Airway inflammation

Exhaled nitric oxide (eNO) was measured according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations using a NIOX chemiluminescence analyser (Aerocrine, Solna, Sweden).[15] A mean value was calculated from three readings if possible. eNO data were normalised then standardised as a z-score, high untransformed eNO values gave rise to high standardised scores.

Childhood asthma and wheeze

Research nurses administered the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire wheezing module.[16] Mothers were asked at each visit whether their child had experienced 'episodes of chestiness associated with wheezing or whistling in his/her chest since they were last seen' and at 6 years whether their child had 'ever been diagnosed with asthma by a doctor'. Current asthma was used to refer only to those who had experienced wheeze or received asthma medication within the last year. Wheeze phenotypes were:

- transient: wheeze at 6, 12, 24 or 36 months but no wheeze or asthma treatment at 6 years
- persistent: wheeze at 6, 12, 24 or 36 months and wheeze or asthma treatment at 6 years
- late onset: no wheeze at 6, 12, 24 or 36 months but wheeze or asthma treatment at 6 years.
- As the late-onset group contained few children, persistent and late-onset groups were combined then subclassified according to atopic status (figure 1).

Lung function

Spirometry was performed according to ATS/ERS guidelines,[17] using a portable spirometer with incentive software (KoKo V.4). Noseclips were not used to avoid discomfort. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were recorded with and without height standardisation[18] to explore whether any effect of maternal 25-hydroxyvitamin D status upon wheeze risk was mediated by an effect upon child's height.

Bronchial hyperresponsiveness (BHR) to methacholine (0.06–16 mg/ml) was measured using a dosimeter (Koko V.4) and a compressed air-driven nebuliser (Sidestream; Respironics, Chichester, UK). Challenges were conducted according to ATS/ERS guidelines[19] and terminated following the 16 mg/ml dose or a ≥20% fall in FEV_1. BHR was expressed as the inverse of the slope of the regression line through FEV_1 drop and logged methacholine concentration[20]; lower values indicate increased BHR.

Statistical methods

Poisson regression with robust variance was used to model RR for binary outcomes.[21] Children with transient or persistent/late wheeze were compared with those who had never wheezed. RRs for persistent/late wheeze with and without atopy were calculated using non-atopic children who had never wheezed as the comparator group. Relationships between maternal 25-hydroxyvitamin D status and continuous outcomes were explored using linear regression.

Potential confounders were identified a priori (table 1) and models developed for each outcome comprising all variables significantly associated with the outcome ($p<0.1$). Birthweight and gestation were initially excluded from the multivariable models as they may lie on the causal pathway. Season and year of blood sampling were not initially included to preserve variation in the exposure variable. The analyses were repeated including these variables if they were significantly associated with the outcomes. 25-Hydroxyvitamin D was analysed as a continuous variable. The relationship between this exposure and each outcome was checked for linearity by fitting a quadratic term.

Table 1 Comparison of Southampton Women's Survey mother–child pairs with complete data with those lacking either maternal 25-hydroxyvitamin D or 6-year follow-up data but born in the same time period

	Mother–child pairs in analysis (n = 860)	Mother–child pairs with missing data (n = 625)	p Value
Maternal characteristics			
Age at child's birth, mean (SD)	30.37 (3.81)	29.63 (3.76)	<0.001
Primiparous, n (%)			
No	462 (53.72)	369 (59.04)	0.042
Yes	398 (46.28)	256 (40.96)	
Qualifications, n (%)			
None	14 (1.63)	35 (5.61)	0.001
GCSE D–G	84 (9.78)	67 (10.74)	
GCSE A*–C	248 (28.87)	179 (28.69)	
A Level	249 (28.99)	186 (29.81)	
HND	64 (7.45)	40 (6.41)	
Degree	200 (23.28)	117 (18.75)	
Parents' social class, n (%)			
1	88 (10.35)	57 (12.26)	0.031
2	425 (50.00)	201 (43.23)	
3N	234 (27.53)	123 (26.45)	
3M	67 (7.88)	54 (11.61)	
4	34 (4.00)	23 (4.95)	
5	2 (0.24)	7 (1.51)	
Smoked in pregnancy, n (%)			
No	723 (85.46)	469 (78.17)	<0.001
Yes	123 (14.54)	131 (21.83)	
Maternal asthma, n (%)			
No	673 (78.9)	468 (75.97)	0.184
Yes	180 (21.1)	148 (24.03)	
Maternal childhood eczema, n (%)			
No	703 (82.51)	501 (81.33)	0.561
Yes	149 (17.49)	115 (18.67)	
Maternal rhinitis, n (%)			
No	494 (57.91)	372 (60.39)	0.341
Yes	359 (42.09)	244 (39.61)	
Maternal atopy, n (%)			
No	406 (53.28)	255 (56.67)	0.253
Yes	356 (46.72)	195 (43.33)	
Pre-pregnancy body mass index (kg/m^2), median (IQR)	24.32 (22.01–27.53)	24.05 (21.93–27.35)	0.996
Height (cm), mean (SD)	163.50 (6.65)	162.81 (6.01)	0.041
Serum 25-hydroxyvitamin D (nmol/litre), median (IQR)	59.00 (40.52–84.89)	53.00 (38.47–79.19)	0.027
Paternal characteristics			
Paternal asthma, n (%)			
No	697 (82.39)	486 (80.46)	0.351
Yes	149 (17.61)	118 (19.54)	
Paternal childhood eczema, n (%)			
No	739 (88.29)	531 (88.06)	0.893
Yes	98 (11.71)	72 (11.94)	
Paternal rhinitis, n (%)			
No	558 (66.59)	400 (66.12)	0.852
Yes	280 (33.41)	205 (33.88)	
Child's characteristics			
Gender, n (%)			
Boy	445 (51.74)	333 (53.45)	0.516
Girl	415 (48.26)	290 (46.55)	
Birth weight (kg), mean (SD)	3483.63 (494.52)	3467.09 (498.12)	0.529
Gestational age, (weeks), median (IQR)	40.14 (39.14–41.00)	40.14 (39.14–41.00)	0.914
Months of breastfeeding, n (%)			
None	132 (15.70)	131 (23.69)	<0.001
<1	168 (19.98)	116 (20.98)	
1–3	157 (18.67)	122 (22.06)	
4–6	156 (18.55)	72 (13.02)	
7–11	139 (16.53)	81 (14.65)	
12 or more	89 (10.58)	31 (5.61)	
Age of introducing solid food (weeks), median (IQR)	17.38 (15.04–17.67)	17.38 (15.04–17.38)	0.282

Continued

Table 1 Continued

	Mother–child pairs in analysis (n = 860)	Mother–child pairs with missing data (n = 625)	p Value
Mother smoked during child's infancy, n (%)			
No	707 (82.88)	439 (76.88)	0.005
Yes	146 (17.12)	132 (23.12)	
Cats/dogs in home during child's infancy, n (%)			
No	432 (50.47)	295 (53.25)	0.307
Yes	424 (49.53)	259 (46.75)	
Age at testing (years), median (IQR)	6.46 (6.34–6.61)	6.44 (6.35–6.60)	0.797

As the analyses were designed a priori to test a limited number of hypotheses, use of a Bonferroni correction was considered overconservative.[22] We focused on results with p values ≤0.025 and considered consistency of the findings in our interpretation.

RESULTS

Participants

Participant mothers were similar in terms of asthma, atopy and allergic disorders to those mothers for whom maternal 25-hydroxyvitamin D status or follow-up data were incomplete. Participant mothers were older, taller, less likely to smoke in pregnancy, more likely to be primiparous and of higher educational attainment and social class than those with incomplete data. Participant children were less likely to be exposed to environmental tobacco smoke and more likely to have been breastfed than those with incomplete data (table 1). Similarly, children contributing skin prick, spirometry, eNO or BHR data were broadly similar to those who did not.

The median (IQR) maternal 25-hydroxyvitamin D concentration was 59.0 nmol/litre (40.5–84.9 nmol/litre). The highest serum 25-hydroxyvitamin D value was 203 nmol/litre; 29% of women had values >80 nmol/litre. Serum 25-hydroxyvitamin D concentrations were slightly lower in women lost to follow up (53.0 (38.5–79.2) nmol/litre), probably reflecting socioeconomic and associated lifestyle factors. A total of 87 children of 860 (10.1%) had current doctor-diagnosed asthma, while 504 of 856 (58.9%) had experienced wheeze at or before age 6 years. A total of 137 of 856 children (16.0%) were assigned to the persistent/late wheeze phenotype; of these, 48.9% were atopic and 51.1% non-atopic (table 2). Technically acceptable measures of FEV_1, BHR and eNO were available from 739, 216 and 451 children, respectively (figure 1). There was no evidence for a nonlinear relationship between maternal 25-hydroxyvitamin D and any wheeze phenotype, atopy or measure of lung function (data not shown).

Binary outcomes were compared by χ^2 test, categorical outcomes by a χ^2 test for trend, and continuous variables using t tests, after transformation if appropriate, or a rank sum test.

Table 2 Distribution of child participants between outcome groups

Wheeze outcome	N (%)
Current doctor-diagnosed asthma (aged 6 years)	87/860 (10.1)
Current wheeze in the last 12 months (aged 6 years)	117/860 (13.6)
Ever wheezed at or before 6 years	504/856 (58.9)
Never wheezed	352/856 (41.8)
Transient wheeze (before 3 years not after)	367/856 (42.0)
Persistent/late wheeze (beyond or after 3 years)	137/856 (16.0)
Atopic persistent/late wheeze	46/632 (7.3)
Non-atopic persistent/late wheeze	48/632 (7.6)
Atopic outcome	
Skin sensitisation	158/635 (24.9)

Asthma and wheeze

There was no association between maternal 25-hydroxyvitamin D status at 34 weeks' gestation and current asthma. In addition, there was no association between 25-hydroxyvitamin D status and wheeze at or before 6 years, or current wheeze in the year preceding the 6-year follow-up (table 3). There were no associations with the transient or persistent/late wheeze phenotypes and subdividing the persistent/late phenotype by atopic status did not reveal any associations (table 3).

Atopy and eNO

Maternal 25-hydroxyvitamin D status at 34 weeks' gestation was not associated with skin sensitisation at 1, 3 or 6 years or with eNO at age 6 years (table 4).

Lung function

Maternal 25-hydroxyvitamin D at 34 weeks' gestation was not associated with absolute or standardised values of FEV_1 or FVC at 6 years. Maternal 25-hydroxyvitamin D status was not associated with BHR (table 5).

Alternative multivariable models

Birthweight was not associated with any outcome and was therefore not considered a confounder. Gestation was associated with all wheeze and skin sensitisation outcomes, except current asthma and persistent/late wheeze with atopy. The absence of an association between maternal 25-hydroxyvitamin D and these variables remained when gestation was included in the multivariable models. Birthweight was associated with absolute measures of FEV_1 and FVC; including birthweight in the multivariable models did not reveal any associations between maternal 25-hydroxyvitamin D and these measures (online tables E1–E3). The absence of any association between maternal 25-hydroxyvitamin D and childhood wheeze, atopy or lung function variables was unchanged by adjusting for season and year of blood sampling (online tables E4–E6).

Maternal vitamin D intake

The median (IQR) average total daily maternal vitamin D intake was 4.2 μg/day (3.0–6.7 μg/day). During early pregnancy 39% of women took vitamin D containing supplements, while during late pregnancy, 22% took these supplements. Median (IQR) supplementary intake in these women was 4.1 μg/day (1.5–8.3 μg/day) in early pregnancy and 5.8 μg (2.5–12.2 μg/day) vitamin D/day in late pregnancy. Only 11.5% of women achieved an average intake of 10 μg/day, which is currently recommended by the Department of Health.[23] Correlation coefficients for early, late and average intake with status at 34 weeks' gestation were 0.25, 0.33 and 0.33, respectively. Total and food-derived maternal intake were not associated with asthma or any wheeze outcome (online tables E7 and E10). No associations were found between intake and skin sensitisation (online tables E8 and E11) or any measure of lung function

Table 3 Relationship between maternal late-pregnancy 25-hydroxyvitamin D status and offspring asthma and wheeze at age 6 years

	Univariable model			Final model		
	RR (95% CI)	p Value	n	RR (95% CI)	p Value	n
Current doctor-diagnosed asthma at 6 years	0.97 (0.91 to 1.04)	0.36	860	0.98 (0.92 to 1.04)	0.56	836
Current wheeze at 6 years	0.98 (0.92 to 1.03)	0.40	860	0.99 (0.94 to 1.05)	0.76	833
Any wheeze at or before 6 years	1.00 (0.98 to 1.01)	0.61	856	1.00 (0.98 to 1.02)	0.95	823
Transient wheeze	1.00 (0.98 to 1.02)	0.85	719	1.00 (0.98 to 1.02)	0.89	707
Persistent/late wheeze	0.98 (0.93 to 1.03)	0.37	489	0.98 (0.94 to 1.03)	0.49	475
Persistent/late wheeze with atopy	0.90 (0.81 to 0.99)	0.03	257	0.91 (0.84 to 0.99)	0.04	251
Persistent/late wheeze without atopy	0.99 (0.91 to 1.06)	0.73	259	1.01 (0.94 to 1.09)	0.73	253

Data presented as change in RR per 10 nmol/litre change in maternal serum 25-hydroxyvitamin D status at 34 weeks' pregnancy.
Adjusted models: asthma—maternal education, maternal asthma, paternal asthma and paternal rhinitis; wheeze at age 6—maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy; wheeze at or before 6 years—maternal body mass index (BMI), child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis; transient wheeze—maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis; persistent/late wheeze—maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis; persistent/late wheeze with atopy—child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy; persistent/late wheeze without atopy—maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy.

(online tables E9 and E12). A positive association was found between food-derived vitamin D and eNO (online table E5) but the lack of a similar association with total intake (food and supplements) (online table E2) suggests that this is not a clinically robust association. There was no evidence for an association between total maternal vitamin D intake and any outcome when, for consistency with previous birth cohort analyses, the results were energy adjusted and analysed as a categorical variable according to quartile of vitamin D intake (tables E13–E15).

DISCUSSION

This study found no evidence that higher maternal late-pregnancy serum 25-hydroxyvitamin D is associated with increased risk of childhood asthma or atopy. There were no significant associations between maternal 25-hydroxyvitamin D status at 34 weeks' gestation and asthma, transient or persistent/late wheeze, skin sensitisation at 1, 3 or 6 years or eNO, FEV_1, FVC or BHR at 6 years.

It has been suggested that serum 25-hydroxyvitamin D levels in pregnancy should be above 80 nmol/litre; this was achieved by 29% of the women in this study. Supplementation and 25-hydroxyvitamin D levels were higher in the current study than in our previous cohort in which a positive association was found between maternal 25-hydroxyvitamin D status and childhood eczema and asthma.[5] While higher rates of supplementation might reduce the present study's ability to detect any effect of dietary insufficiency, failure to confirm a harmful effect of high 25-hydroxyvitamin D status cannot be attributed to lower exposure; it is more likely that the earlier study was underpowered to assess specific clinical outcomes.

Vitamin D supplementation during pregnancy is known to benefit calcium metabolism and bone health[24] and may protect against cardiovascular, autoimmune and malignant disease via 'fetal imprinting'.[25] The National Institute for Health and Clinical Excellence[26] suggested that pregnant women may wish to consider vitamin D supplementation. Recently a randomised controlled trial demonstrated that daily supplementation with 4000 IU vitamin D can increase maternal serum 25-hydroxyvitamin D concentration without adverse events.[27] However, few adequately powered studies have considered the effects of increased maternal 25-hydroxyvitamin D upon relevant clinical outcomes.[28]

Relationship between maternal late-pregnancy serum 25-hydroxyvitamin D status and childhood asthma and wheeze

This study found no evidence that higher late-pregnancy maternal serum 25-hydroxyvitamin D is associated with increased asthma. Although an inverse relationship between energy-adjusted maternal vitamin D intake and asthma at age 5 was found in a Finnish cohort,[6] this was significant only for food-derived not total (food and supplement) intake. Associations with food-derived intake only may be vulnerable to confounding by other nutrients present in vitamin D-rich foods and socioeconomic factors or they may arise as a result of multiple comparisons. The majority of studies reporting inverse associations between early vitamin D exposure and adverse respiratory outcomes reported associations with wheeze but not asthma. Many of these studies relied on estimated maternal intake[6–9] and relatively short follow-up.[7 8]

Vitamin D intake studies are vulnerable to confounding by socioeconomic and lifestyle factors and by the effects of other nutrients found in vitamin D-containing foods. Such confounding has been suggested to explain the absence of an association between 25-hydroxyvitamin D status and lung function in adults with chronic obstructive pulmonary disease, despite an association with vitamin D intake; absence of an association between vitamin D receptor genotype and lung function strengthened this argument.[29] Studies with short

Table 4 Relationship between maternal late-pregnancy 25-hydroxyvitamin D status and offspring atopy and airway inflammation

	Univariable model			Final model		
	RR or β (95% CI)	p Value	n	RR or β (95% CI)	p Value	n
Atopy age 1 year	0.94 (0.88 to 1.01)	0.08	773	0.96 (0.90 to 1.03)	0.24	685
Atopy age 3 years	0.99 (0.94 to 1.05)	0.81	676	0.99 (0.94 to 1.04)	0.58	661
Atopy age 6 years	0.97 (0.93 to 1.02)	0.26	635	0.99 (0.95 to 1.04)	0.71	545
Exhaled nitric oxide	−0.014 (−0.044 to 0.016)	0.36	451	−0.0204 (−0.050 to 0.009)	0.18	434

Data presented as change in RR per 10 nmol/litre change in maternal serum 25-hydroxyvitamin D status at 34 weeks' pregnancy.
Adjusted models: atopy age 1 year—child's gender, parents' social class, maternal atopy; atopy age 3 years—child's gender, exposure to smoke in infancy, maternal eczema; atopy age 6 years—child's age at testing, child's gender, parents' social class, maternal asthma, paternal rhinitis, maternal atopy; exhaled nitric oxide—child's age at testing, maternal asthma, paternal rhinitis, maternal height.

Table 5 Relationship between maternal late-pregnancy 25-hydroxyvitamin D status and offspring lung function at 6 years

	Univariable model				Final model			
	β	(95% CI)	p Value	n	β	95% CI	p Value	n
FEV_1 absolute	−0.0007	−0.0054 to 0.0039	0.76	739	−0.0001	−0.0046 to 0.0043	0.95	731
FEV_1 z-score	0.012	−0.0078 to 0.033	0.23	739	0.011	−0.0091 to 0.031	0.28	739
FVC absolute	−0.001	−0.0068 to 0.0045	0.69	739	−0.0001	−0.0054 to 0.0052	0.96	730
FVC z-score	0.013	−0.010 to 0.036	0.27	739	0.012	−0.011 to 0.035	0.31	739
BHR slope	−0.084	−0.194 to −0.025	0.13	216	−0.102	−0.211 to −0.008	0.07	208

Data presented as change in RR per 10 nmol/litre change in maternal serum 25-hydroxyvitamin D status at 34 weeks' pregnancy.
Adjusted models: FEV_1 absolute—child's age at testing, child's gender, parity, maternal BMI, maternal height; FEV_1 z-score—child's gender; FVC absolute—child's age at testing, child's gender, age at introduction of solid foods, maternal BMI, maternal height; FVC z-score—child's gender; BHR—maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid foods.
BHR, bronchial hyperresponsiveness; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

follow-up are obliged to use childhood wheeze as an outcome in the absence of a reliable asthma diagnosis in young children. Follow-up to 5 years was conducted in a New Zealand cohort and an inverse association found between cord blood 25-hydroxyvitamin D and wheeze, but again no association was found with asthma.[30] An inverse association was also found between cord blood 25-hydroxyvitamin D status and respiratory infections in early infancy, leading these authors to conclude that the beneficial effects of vitamin D upon innate immunity may indirectly reduce wheeze risk in early childhood. The present study did not address the issue of childhood infection.

Relationship between maternal late pregnancy serum 25-hydroxyvitamin D status and immune and respiratory development

While it remains possible that vitamin D exerts an affect upon immune predisposition to wheeze with infection, rather than altered atopic immunity, an alternative explanation for the lack of a demonstrable association between early vitamin D exposure and asthma could be the existence of different asthma phenotypes. Subdividing the persistent/late wheeze phenotype by atopic status did not reveal any association with maternal vitamin D status, although it remains possible that the phenotypes used in this study were excessively heterogeneous and that early vitamin D exposure may have differential effects upon late-onset compared with persistent wheeze, for example.

Animal studies suggest vitamin D may promote a proallergic T helper 2 phenotype.[31] Similarly, epidemiological evidence suggests that high early life exposure to vitamin D supplementation[32] or high maternal 25-hydroxyvitamin D status might predispose children to allergic disorders.[5] No previous prospective epidemiological study, however, has investigated the relationship between maternal 25-hydroxyvitamin D status and objective measures of atopy. In this respect, the null findings in this study are reassuring: higher serum 25-hydroxyvitamin D concentrations in late pregnancy do not appear to increase skin sensitisation at 1, 3 or 6 years or eosinophilic airways inflammation at 6 years.

Early vitamin D exposure has been shown to alter the volume dependence of lung mechanics in an animal model, suggestive of altered tissue structure.[33] Altered development affecting lung structure and airway calibre would also be consistent with the results of maternal intake studies. However, this study confirms, in a larger cohort with more extensive characterisation of lung function, the findings of the KOALA study[11]; there was no evidence of a clinically significant alteration of lung function according to maternal late-pregnancy 25-hydroxyvitamin D status.

As the repeatability of the serum 25-hydroxyvitamin D levels is relatively low, there is a significant chance that a single serum measurement will lead to misclassification of exposure. As this misclassification is random, this may bias studies, such as this, based upon single serum samples towards the null or no effect. Furthermore, epidemiological studies are limited in their ability to discriminate causal from closely linked factors. This study cannot exclude the existence of a relationship between vitamin D exposure and wheeze or atopy, which is hidden by an opposing relationship between incompletely controlled for seasonal and other factors upon these outcomes. Another feature of the study design which may have limited the likelihood of identifying an association between maternal 25-hydroxyvitamin D status and childhood wheeze outcomes is the use of frequent prospective questionnaires; this may have set too low a threshold to reflect significant pathology.

While the present study did not have complete follow-up and those followed up differed from those who were not in terms of several socioeconomic variables, this should not alter the conclusions unless the nature of any relationship between maternal 25-hydroxyvitamin D status and wheeze or atopic outcomes differed according to socioeconomic status or if the relationship were non-linear. We have no evidence to support either assertion. The null results in this study may have arisen as a consequence of measuring 25-hydroxyvitamin D status in late pregnancy only. However, while much respiratory development, particularly that of the airways, occurs early in pregnancy,[34] significant maturation of the immune system is believed to occur in late pregnancy.[35] Furthermore, the null result was supported by analyses based upon intake data which covered both the first and second trimesters.

In summary, neither higher late-pregnancy maternal 25-hydroxyvitamin D status nor higher vitamin D intake during pregnancy was significantly associated with asthma or any wheeze phenotype. Moreover there was no evidence that early exposure to higher concentrations of 25-hydroxyvitamin D had a deleterious effect upon lung function or atopic sensitisation. Together, these findings suggest the risk posed by vitamin D supplementation in terms of asthma and atopic diseases may not be a concern.

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Contributors JSAL, KMG, HMI, CC, SMR and GCR designed the research; KCP and JSAL conducted the research; HI and KCP analysed the data; KCP, JSAL and GCR wrote the paper; GCR had primary responsibility for the final content. All authors read and approved the final manuscript.

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Competing interests All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that none of the authors have support from any company for the submitted work; KCP, HMI, SMR, JSAL, CC, NCH, KMG and GR have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners or children have no financial relationships that may be relevant to the submitted work; and KCP, HMI, SMR, JSAL, CC, NCH, KMG and GR have no non-financial interests that may be relevant to the submitted work.

Ethics approval Ethics approval was provided by Southampton and South West Hampshire Local Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The Southampton Women's Survey is a complex study with ongoing data collection of many of the waves of the study. We encourage data sharing through collaboration, wherever possible, to maximise the use of the study data. In the first instance, enquiries should be made to the MRC Lifecourse Epidemiology Unit Director, Professor Cyrus Cooper on cc@mrc.soton.ac.uk.

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METHODS

Participants

Participants were mother-child pairs from the Southampton Women's Survey.[E1] Women aged 20-34 years were recruited during 1998-2002; those who became pregnant were followed through pregnancy and their children visited at 6, 12, 24 and 36 months and 6 years. To exclude effects of prematurity, whilst maximising power, infants born < 35 weeks' gestation were excluded. Children aged six years during 2006-2010 were invited for follow-up; 1485 mother-child pairs were eligible, maternal vitamin status and 6-year follow-up data were available for 860 pairs (Figure 1). Questionnaire data were collected during a home-visit, during this visit spirometry was also attempted. Due to resource limitations clinic-based bronchial provocation testing and exhaled nitric oxide measurements were limited to unselected subsets of participants. Parental consent was obtained and ethical approval was granted by the Southampton and South West Hampshire Local Research Ethics Committee (LREC Number 276/97, 307/97, 089/99 and 06/Q1702/104).

Maternal serum 25-hydroxyvitamin D

Maternal venous blood was sampled at 34 weeks' gestation. To ensure stability samples were centrifuged, separated and stored at -80^{o}C within 24 hours of sampling. 25-hydroxyvitamin D concentrations were measured by a Vitamin D External Quality Assessment Scheme compliant laboratory, with quality control samples in each batch. The radioimmunoassay used (DiaSorin, Minnesota, USA) had a coefficient of variability < 10%.

Maternal vitamin D intake

At 11 and 34 weeks' gestation average frequencies of consumption over the preceding 3 months were recorded using a 100-item food frequency questionnaire (FFQ). The FFQ has

been validated against 4-day food diaries and maternal micronutrient concentrations.[E2] Nutrient intakes were calculated by multiplying frequency of consumption by nutrient content for each food or supplement. Early and late pregnancy intakes were averaged to provide an estimate of pregnancy intake. Energy adjustment can be used to correct for over or underestimation of intake in the FFQ, however, intake data were not automatically adjusted for total energy intake as supplementary intake vitamin D from supplements do not increase in line with energy intake.

Atopy

Skin prick testing was conducted at 1 and 3 years using cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), grass pollens, egg and milk allergens (Hollister-Stier, Spokane, WA); at age 6, tree pollens (ALK Abelló Hørsholm, Denmark) were also tested. For validity a ≥ 3 mm positive and a 0 mm negative control response were required, atopy was defined as any allergen response ≥ 3 mm.

Airway inflammation

Exhaled nitric oxide (eNO) was measured online by trained research nurses according to ERS/ATS recommendations.[E3] Measurements were recorded during controlled expiration at 50 ml/sec using a NIOX® chemiluminescence analyser (Aerocrine, Sweden). A mean value was calculated from three readings where possible. eNO data were normalised using an inverse square root transformation then standardised as a z-score. The sign of the values was reversed so that high untransformed eNO values gave rise to high standardised scores.

Childhood asthma and wheeze

Respiratory assessment was conducted by research nurses using questions from the ISAAC core questionnaire wheezing module.[E4] Specifically, mothers were asked at each visit whether their child had experienced 'episodes of chestiness associated with wheezing or whistling in his/her chest since they were last seen' and at 6 years whether their child had 'ever been diagnosed with asthma by a doctor'. The asthma outcome was refined to current asthma by including only 6-year-old children diagnosed with asthma who had experienced asthma symptoms or received asthma medication within the last year. The wheeze phenotypes were based upon those of the Tuscon Children's Respiratory Study;[E5] questionnaire data from 6, 12, 24 and 36 months and six years were combined to define:

Transient wheeze: wheeze at ages 6, 12, 24 or 36 months but no wheeze and no asthma treatment at 6 years.

Persistent wheeze: wheeze at ages 6, 12, 24 or 36 months and wheeze or asthma treatment at 6 years.

Late-onset wheeze: no wheeze at ages 6, 12, 24 or 36 months but wheeze or asthma treatment at 6 years.

The persistent and late-onset groups were combined because the late-onset group contained few children (Figure1). The persistent/late wheeze group was sub-classified according to atopic status determined by skin prick testing.

Lung function

Spirometry was performed according to ATS/ERS guidelines,[E6] although without noseclips to avoid discomfort. Flow-volume loops were measured for all children by experienced research nurses using a portable Koko spirometer with incentive software (KoKo version 4; PDS Instrumentation; Louisville, USA). Absolute values of FEV_1 and FVC were recorded

with and without standardisation for height[E7] to explore whether any effect of maternal 25-hydroxyvitamin D status upon wheeze risk was mediated by an effect upon child's height.

Bronchial hyperresponsiveness (BHR) was measured by bronchial provocation challenge. Methacholine was administered using a dosimeter (Koko; PDS Instrumentation; Louisville, USA) and a compressed air-driven nebuliser (Sidestream®; Respironics, UK). Challenges were conducted according to ATS/ERS guidelines using incremental methacholine concentrations (0.06 mg/ml to 16 mg/ml).[E8] Challenges were terminated following the 16 mg/ml dose or a 20% fall in FEV_1. BHR was expressed as the inverse of the slope of the regression line through FEV_1 drop and logged methacholine concentration:
Log.slope=100/[regression slope of FEV_1 drop and $\log_{10}$(cumulative methacholine dose) + 10]
The constant removes negative values and the inverse transformation produces a normally distributed variable.[E9] Lower inverse log.slope values indicate increased BHR.

Statistical methods

Poisson regression with robust variance was used to model relative risk for binary outcomes. This is more appropriate than logistic regression for common outcomes where odds ratios cannot be interpreted as relative risks and so are hard to interpret.[E10] Transient and persistent/late wheeze phenotypes were mutually exclusive; children suffering one of these types of wheeze were not at risk of the other so relative risks were calculated by comparing children with transient or persistent/late wheeze to those who had never wheezed. Relative risks for persistent/late wheeze with atopy and persistent/late wheeze without atopy were calculated using non-atopic children who had never wheezed as the comparator group. Relationships between maternal 25-hydroxyvitamin D status and continuous outcome variables were explored using linear regression.

Potential confounders were identified *a priori* and tested for association with each respiratory outcome. Models were developed comprising all the confounding variables listed in Table 1 which were significantly associated with each outcome ($p < 0.1$). Birthweight and gestation were initially excluded from the multivariable models as they may lie on the causal pathway. Similarly, season and year of blood sampling were initially excluded from the multivariable models to preserve variation in the exposure variable which may drive an effect upon outcome. The analyses were repeated including these variables if they were significantly associated with the outcomes. 25-hydroxyvitamin D was analysed as a continuous variable to maximise power, however, the relationship between this exposure and each outcome was checked for linearity by fitting a quadratic term.

As the analyses were designed *a priori* to test a limited number of hypotheses and not all the tests were independent, use of a Bonferroni correction was considered over-conservative.[E11] As a compromise we focused on results with P-values ≤ 0.025 and considered consistency of the findings in our interpretation.

Table E1 Relationship between maternal 25-hydroxyvitamin D status at 34 weeks' pregnancy and offspring asthma and wheeze at age 6 years (adjusted for gestation and birthweight)

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Current doctor-diagnosed asthma at 6 years	0.97	(0.91, 1.04)	0.36	860	0.98	(0.92, 1.04)	0.56	836
Current wheeze at 6 years	0.98	(0.92, 1.03)	0.40	860	0.99	(0.94, 1.05)	0.74	833
Any wheeze at or before 6 years	1.00	(0.98, 1.01)	0.61	856	1.00	(0.98, 1.02)	0.96	832
Transient wheeze	1.00	(0.98, 1.02)	0.85	719	1.00	(0.98, 1.02)	0.88	707
Persistent/late wheeze	0.98	(0.93, 1.03)	0.37	489	0.98	(0.94, 1.03)	0.44	475
Persistent/late wheeze with atopy	0.90	(0.81, 0.99)	0.03	257	0.91	(0.84, 0.99)	0.04	251
Persistent/late wheeze without atopy	0.99	(0.91, 1.06)	0.73	259	1.01	(0.94, 1.09)	0.72	253

Data presented as change in relative risk per 10 nmol/l 25-hydroxyvitamin D.

Adjusted models: asthma - maternal education, maternal asthma, paternal asthma and paternal rhinitis; wheeze at age 6 - maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy, gestation; wheeze at or before 6 years - maternal BMI, child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis, gestation; transient wheeze - maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis, gestation; Persistent/late - maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis, gestation; Persistent/late wheeze with atopy-child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy; Persistent/late wheeze without atopy-maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy, gestation.

Table E2 Relationship between maternal 25-hydroxyvitamin D status at 34 weeks' pregnancy and offspring atopy and airway inflammation (adjusted for gestation and birthweight)

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Atopy age 1 year	0.94	(0.88, 1.01	0.08	773	0.96	(0.90, 1.03	0.24	685
Atopy age 3 years	0.99	(0.94, 1.05)	0.81	676	0.99	(0.94, 1.04)	0.6	661
Atopy age 6 years	0.97	(0.93, 1.02)	0.26	635	0.99	(0.95, 1.04)	0.74	545
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
Exhaled nitric oxide at 6 years	0.0139	(-0.0441, 0.0163)	0.37	451	-0.0205	(-0.0503, 0.0094)	0.18	434

Data presented as change in relative risk per 10 nmol/l 25-hydroxyvitamin D for binary outcomes and as change in transformed unit of eNO per 10 nmol/l 25-hydroxyvitamin D for exhaled nitric oxide outcome.

Adjusted models: atopy age 1 year - child's gender, gestation, parents' social class, maternal atopy; atopy age 3 years - child's gender, exposure to smoke in infancy, gestation, maternal eczema, atopy age 6 years - child's age at testing, child's gender, gestation, parents social class, maternal asthma, paternal rhinitis, maternal atopy; exhaled nitric oxide - child's age at testing, maternal asthma, paternal rhinitis, maternal height.

Table E3 Relationship between maternal 25-hydroxyvitamin D status at 34 weeks' pregnancy and offspring lung function (adjusted for gestation and birthweight) aged 6 years

	Univariable Model				Final Model			
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
FEV_1 absolute	-0.0007	(-0.0054, 0.0039)	0.76	739	-0.0002	(-0.0046, 0.0042)	0.93	725
FEV_1 z-score	0.0124	(-0.0078, 0.0326)	0.23	739	0.0109	(-0.0091, 0.0309)	0.28	739
FVC absolute	-0.0011	(-0.0068, 0.0045)	0.69	739	-0.0002	(-0.0055, 0.0051)	0.94	724
FVC z-score	0.0131	(-0.0103, 0.0365)	0.27	739	0.0122	(-0.0112, 0.0356)	0.31	739
BHR slope	-0.0840	(-0.1935, 0.0244)	0.13	216	-0.1020	(-0.2115, 0.0075)	0.07	208

Data presented as change in relative risk per 10 nmol/l 25-hydroxyvitamin D.

Adjusted models: FEV_1 absolute - child's age at testing, child's gender, birthweight, parity, maternal BMI, maternal height; FEV_1 z-score - child's gender; FVC absolute - child's age at testing, child's gender, age at introduction of solid foods, birthweight, maternal BMI, maternal height, FVC z-score - child's gender; BHR - maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid foods

Table E4 Relationship between maternal 25-hydroxyvitamin D at 34 weeks' pregnancy and offspring asthma and wheeze at age 6 years (adjusted for season and year of blood sampling)

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Current doctor-diagnosed asthma at 6 years	0.97	(0.91, 1.04)	0.36	860	0.99	(0.92, 1.06)	0.76	832
Current wheeze at 6 years	0.98	(0.92, 1.03)	0.40	860	1.00	(0.93, 1.07)	0.94	829
Any wheeze at or before 6 years	1.00	(0.98, 1.01)	0.61	856	1.00	(0.98, 1.02)	0.89	828
Transient wheeze	1.00	(0.98, 1.02)	0.85	719	1.00	(0.97, 1.03)	0.95	704
Persistent/late wheeze	0.98	(0.93, 1.03)	0.37	489	0.99	(0.94, 1.05)	0.77	472
Persistent/late wheeze with atopy	0.90	(0.81, 0.99)	0.03	257	0.98	(0.88, 1.09)	0.67	250
Persistent/late wheeze without atopy	0.99	(0.91, 1.06)	0.73	259	0.98	(0.88, 1.09)	0.69	253

Data presented as change in relative risk 10 nmol/l 25-hydroxyvitamin D.

Adjusted models: asthma - maternal education, maternal asthma, paternal asthma and paternal rhinitis, year and season of blood sampling; wheeze at age 6 - maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy, year and season of blood sampling; wheeze at or before 6 years - maternal BMI, child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis, year and season of blood sampling; transient wheeze - maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis, year and season of blood sampling; Persistent/late - maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis, year and season of blood sampling; Persistent/late wheeze with atopy-child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy, year and season of blood sampling; Persistent/late wheeze without atopy-maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy, year and season of blood sampling.

Table E5 Relationship between maternal 25-hydroxyvitamin D status at 34 weeks' pregnancy and offspring atopy and airway inflammation (adjusted for season and year of blood sampling)

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Atopy age 1 year	0.94	(0.88, 1.01)	0.08	773	0.98	(0.90, 1.08)	0.73	681
Atopy age 3 years	0.99	(0.94, 1.05)	0.81	676	1.01	(0.95, 1.08)	0.79	659
Atopy age 6 years	0.97	(0.93, 1.02)	0.26	635	1.01	(0.96, 1.07)	0.75	542
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
Exhaled nitric oxide age 6 years	0.0139	(-0.0441, 0.0103)	0.37	451	-0.0028	(-0.0561, 0.0146)	0.25	434

Data presented as change in relative risk per 10 nmol/l 25-hydroxyvitamin D for binary outcomes and as change in transformed unit of eNO per 10 nmol/l 25-hydroxyvitamin D for exhaled nitric oxide outcome.

Adjusted models: atopy age 1 year - child's gender, parents' social class, maternal atopy, season and year of blood sampling; atopy age 3 years - child's gender, exposure to smoke in infancy, maternal eczema, season and year of blood sampling; atopy age 6 years - child's age at testing, child's gender, parents social class, maternal asthma, paternal rhinitis, maternal atopy, season and year of blood sampling; exhaled nitric oxide - child's age at testing, maternal asthma, paternal rhinitis, maternal height, season and year of blood sampling.

Table E6 Relationship between maternal 25-hydroxyvitamin D status at 34 weeks' pregnancy and offspring lung function aged 6 years (adjusted for season and year of blood sampling)

	Univariable Model				Final Model			
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
FEV_1 absolute	-0.0007	(-0.0054,0.0039)	0.76	739	0.0002	(-0.0050, 0.0054)	0.94	728
FEV_1 z-score	0.0112	(-0.0078,0.033)	0.23	739	0.0026	(-0.0209, 0.0261)	0.83	736
FVC absolute	-0.0011	(-0.0068,0.0045)	0.69	739	0.0001	(-0.0061, 0.0064)	0.97	727
FVC z-score	0.0130	(-0.010,0.036)	0.27	739	0.0022	(-0.0253, 0.0297)	0.88	736
BHR slope	-0.084	(-0.194, -0.025)	0.13	216	-0.1898	(-0.3232, -0.0563)	0.006	208

Data presented as change in relative risk per 10 nmol/l 25-hydroxyvitamin D.

Adjusted models: FEV_1 absolute - child's age at testing, child's gender, parity, maternal BMI, maternal height, season and year of blood sampling; FEV_1 z-score - child's gender, season and year of blood sampling; FVC absolute - child's age at testing, child's gender, age at introduction of solid foods, maternal BMI, maternal height, season and year of blood sampling; FVC z-score - child's gender, season and year of blood sampling; BHR - maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid foods, season and year of blood sampling

Table E7 Relationship between maternal average total daily vitamin D intake during pregnancy and offspring asthma and wheeze at age 6 years

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Current doctor-diagnosed asthma at 6 years	1.01	(0.95, 1.07)	0.77	689	1.03	(0.97, 1.09)	0.36	677
Current wheeze at 6 years	1.00	(0.95, 1.06)	0.90	689	1.01	(0.96, 1.07)	0.62	675
Any wheeze at or before 6 years	1.00	(0.98, 1.01)	0.63	685	1.00	(0.98, 1.02)	0.97	674
Transient wheeze	0.99	(0.97, 1.02)	0.51	581	0.99	(0.97, 1.02)	0.66	575
Persistent/late wheeze	1.00	(0.96, 1.05)	0.91	388	1.01	(0.97, 1.06)	0.52	385
Persistent/late wheeze with atopy	1.03	(0.96, 1.11)	0.38	207	1.04	(0.97, 1.11)	0.30	205
Persistent/late wheeze without atopy	0.99	(0.91, 1.08)	0.87	210	0.98	(0.90, 1.07)	0.67	208

Data presented as change in relative risk per mcg/day total intake of vitamin D.

Adjusted models: asthma - maternal education, maternal asthma, paternal asthma and paternal rhinitis; wheeze at age 6 - maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy; wheeze at or before 6 years - maternal BMI, child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis; transient wheeze - maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis; Persistent/late - maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis; Persistent/late wheeze with atopy-child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy; Persistent/late wheeze without atopy-maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy.

Table E8 Relationship between maternal average total daily vitamin D intake during pregnancy and offspring atopy and airway inflammation

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Atopy age 1 year	1.00	(0.95, 1.06)	0.98	623	1.00	(0.95, 1.06)	0.98	556
Atopy age 3 years	1.01	(0.96, 1.06)	0.78	547	1.00	(0.95, 1.05)	0.98	538
Atopy age 6 years	1.03	(0.99, 1.07)	0.09	509	1.03	(0.99, 1.07)	0.20	446
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
Exhaled nitric oxide 6 years	0.0040	(-0.0252, 0.0332)	0.79	355	0.0042	(-0.0245, 0.0328)	0.77	346

Data presented as change in relative risk per mcg/day total intake of vitamin D for binary outcomes and as change in transformed unit of eNO per mcg/dy of vitamin D intake for exhaled nitric oxide outcome.

Adjusted models: atopy age 1 year - child's gender, parents' social class, maternal atopy; atopy age 3 years - child's gender, exposure to smoke in infancy, maternal eczema, atopy age 6 years - child's age at testing, child's gender, parents social class, maternal asthma, paternal rhinitis, maternal atopy; exhaled nitric oxide - child's age at testing, maternal asthma, paternal rhinitis, maternal height.

Table E9 Relationship between average total daily maternal vitamin D intake during pregnancy and offspring lung function at age 6 years

	Univariable Model				Final Model			
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
FEV_1 absolute	-0.0016	(-0.0061, 0.0028)	0.48	590	-0.0019	(-0.0061, 0.0024)	0.38	584
FEV_1 z-score	-0.0112	(-0.0304, 0.0080)	0.25	590	-0.0091	(-0.0282, 0.0099)	0.35	590
FVC absolute	-0.0021	(-0.0075, 0.0034)	0.46	590	-0.0022	(-0.0072, 0.0029)	0.40	584
FVC z-score	-0.0130	(-0.0355, 0.0096)	0.26	590	-0.0118	(-0.0343, 0.0107)	0.30	590
BHR slope	-0.0283	(-0.1509, 0.0943)	0.65	169	-0.0189	(-0.1409, 0.1031)	0.76	165

Data presented as change in lung function measure per mcg/kg total intake of vitamin D.

Adjusted models: FEV_1 absolute - child's age at testing, child's gender, parity, maternal BMI, maternal height; FEV_1 z-score - child's gender; FVC absolute - child's age at testing, child's gender, age at introduction of solid foods, maternal BMI, maternal height, FVC z-score - child's gender; BHR - maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid foods

Table E10 Relationship between average maternal daily vitamin D intake from food during pregnancy and offspring asthma and wheeze at age 6 years

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Current doctor-diagnosed asthma at 6 years	1.06	(0.93, 1.21)	0.36	689	1.06	(0.95, 1.19)	0.30	677
Current wheeze at 6 years	1.03	(0.92, 1.14)	0.64	689	1.01	(0.92, 1.11)	0.84	675
Any wheeze at or before 6 years	1.01	(0.98, 1.05)	0.40	685	1.02	(0.99, 1.05)	0.26	674
Transient wheeze	1.01	(0.97, 1.06)	0.54	581	1.02	(0.97, 1.06)	0.49	575
Persistent/late wheeze	1.04	(0.95, 1.14)	0.37	388	1.02	(0.94, 1.10)	0.61	385
Persistent/late wheeze with atopy	1.13	(0.99, 1.30)	0.08	207	1.06	(0.91, 1.23)	0.45	205
Persistent/late wheeze without atopy	0.93	(0.77, 1.11)	0.42	210	0.83	(0.69, 1.00)	0.05	208

Data presented as change in relative risk per mcg/day food intake of vitamin D. Excludes vitamin D intake from supplements.
Adjusted models: asthma - maternal education, maternal asthma, paternal asthma and paternal rhinitis; wheeze at age 6 - maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy; wheeze at or before 6 years - maternal BMI, child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis; transient wheeze - maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis; Persistent/late - maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis; Persistent/late wheeze with atopy-child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy; Persistent/late wheeze without atopy-maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy.

Table E11 Relationship between average maternal daily intake of vitamin D from food during pregnancy and offspring atopy and airway inflammation

	Univariable Model				Final model			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Atopy age 1 year	1.07	(0.96, 1.18)	0.22	623	1.03	(0.93,1.15)	0.53	556
Atopy age 3 years	1.05	(0.95, 1.16)	0.31	547	1.04	(0.94, 1.15)	0.45	538
Atopy age 6 years	1.07	(1.00, 1.14)	0.04	509	1.03	(0.94, 1.12)	0.51	446
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
Exhaled nitric oxide 6 years	0.0752	(0.0162, 0.1343)	0.01	355	0.0838	(0.0261, 0.1416)	0.004	346

Data presented as change in relative risk per mcg/day total intake of vitamin D for binary outcomes and as change in transformed unit of eNO per mcg/dy of vitamin D intake for exhaled nitric oxide outcome. Excludes vitamin D intake from supplements.

Adjusted models: atopy age 1 year - child's gender, parents' social class, maternal atopy; atopy age 3 years - child's gender, exposure to smoke in infancy, maternal eczema, atopy age 6 years - child's age at testing, child's gender, parents social class, maternal asthma, paternal rhinitis, maternal atopy; exhaled nitric oxide - child's age at testing, maternal asthma, paternal rhinitis, maternal height.

Table E12 Relationship between average maternal daily intake of vitamin D from food during pregnancy intake and offspring lung function aged 6 years

	Univariable Model				Final Model			
	beta	(95% CI)	P-value	n	beta	(95% CI)	P-value	n
FEV_1 absolute	0.0000	(-0.0095, 0.0096)	0.99	590	-0.0009	(-0.0099, 0.0082)	0.85	584
FEV_1 z-score	-0.0049	(-0.0461, 0.0364)	0.82	590	0.0023	(-0.0385, 0.0431)	0.91	590
FVC absolute	0.0037	(-0.0080, 0.0154)	0.53	590	0.0018	(-0.0091, 0.0128)	0.74	584
FVC z-score	0.0150	(-0.0333, 0.0633)	0.54	590	0.0191	(-0.0293, 0.0674)	0.44	590
BHR slope	-0.0067	(-0.2754, 0.2599)	0.96	169	0.0199	(-0.2478, 0.2876)	0.88	165

Data presented as change in unit of lung function measure per mcg/day food intake of vitamin D. Excludes vitamin D intake from supplements. Adjusted models: FEV_1 absolute - child's age at testing, child's gender, parity, maternal BMI, maternal height; FEV_1 z-score - child's gender; FVC absolute - child's age at testing, child's gender, age at introduction of solid foods, maternal BMI, maternal height, FVC z-score - child's gender; BHR - maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid foods.

Table E13 Relationship between total energy-adjusted daily maternal vitamin D intake during pregnancy and offspring asthma and wheeze at age 6 years

	Quartile 1	Quartile 2		Quartile 3		Quartile 4		
	RR	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	P for trend
Current doctor-diagnosed asthma at 6 years	1	1.11	(0.59, 2.09)	0.80	(0.38, 1.66)	1.23	(0.65, 2.33)	0.74
Current wheeze at 6 years	1	0.87	(0.51, 1.47)	0.92	(0.52, 1.61)	0.90	(0.53, 1.52)	0.75
Any wheeze at or before 6 years	1	1.08	(0.91, 1.28)	1.05	(0.88, 1.25)	1.03	(0.86, 1.23)	0.87
Transient wheeze	1	1.12	(0.90, 1.40)	1.08	(0.86, 1.35)	1.03	(0.81, 1.30)	0.93
Persistent/late wheeze	1	0.91	(0.60, 1.39)	0.95	(0.61, 1.49)	1.03	(0.66,1.60)	0.89
Persistent/late wheeze with atopy	1	0.33	(0.12, 0.92)	0.39	(0.15, 0.98)	0.84	(0.40, 1.78)	0.97
Persistent/late wheeze without atopy	1	0.82	(0.38, 1.77)	0.61	(0.28, 1.33)	0.65	(0.28, 1.49)	0.21

Adjusted models: asthma - maternal education, maternal asthma, paternal asthma and paternal rhinitis; wheeze at age 6 - maternal education, maternal asthma, paternal rhinitis, pets in the home during the child's infancy; wheeze at or before 6 years - maternal BMI, child's gender, maternal education, maternal asthma, paternal asthma, maternal rhinitis; transient wheeze - maternal BMI, child's gender, mother's parity, maternal asthma, maternal rhinitis; Persistent/late - maternal education, smoking during pregnancy, maternal asthma, paternal asthma, maternal rhinitis; Persistent/late wheeze with atopy-child's gender, maternal asthma, paternal asthma, paternal rhinitis, pets in the home during infancy; Persistent/late wheeze without atopy-maternal age, smoking during pregnancy, maternal asthma, paternal asthma, pets in the home during infancy.

Table E14 Relationship between daily energy-adjusted maternal vitamin D intake during pregnancy and offspring atopy and airway inflammation

	Quartile 1	Quartile 2		Quartile 3		Quartile 4		
	RR	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	P-trend
Atopy age 1 year	1	1.13	(0.54, 2.38)	1.22	(0.61, 2.46)	1.48	(0.75, 2.89)	0.24
Atopy age 3 years	1	1.21	(0.74, 1.97)	0.88	(0.53, 1.47)	0.93	(0.55, 1.56)	0.51
Atopy age 6 years	1	0.86	(0.53, 1.40)	0.69	(0.43, 1.11)	1.15	(0.76, 1.74)	0.59
		beta	(95% CI)	beta	(95% CI)	beta	(95% CI)	
Exhaled nitric oxide age 6 years	0	-0.127	(-0.422, 0.168)	-0.089	(-0.393, 0.215)	-0.065	(-0.370, 0.239)	0.76

Adjusted models: atopy age 1 year - child's gender, parents' social class, maternal atopy; atopy age 3 years - child's gender, exposure to smoke in infancy, maternal eczema, atopy age 6 years - child's age at testing, child's gender, parents social class, maternal asthma, paternal rhinitis, maternal atopy; exhaled nitric oxide - child's age at testing, maternal asthma, paternal rhinitis, maternal height.

Table E15 Relationship between daily energy-adjusted maternal vitamin D intake during pregnancy and offspring lung function at age 6 years

	Quartile 1	Quartile 2		Quartile 3		Quartile 4		P-trend
	beta	beta	(95% CI)	beta	(95% CI)	beta	(95% CI)	
FEV_1 absolute	0	-0.009	(-0.055, 0.036)	0.022	(-0.023, 0.067)	-0.006	(-0.051, 0.039)	0.87
FEV_1 z-score	0	0.005	(-0.199, 0.209)	0.084	(-0.119, 0.286)	-0.027	(-0.226, 0.173)	0.97
FVC absolute	0	0.019	(-0.035, 0.074)	0.047	(-0.007, 0.102)	0.012	(-0.041, 0.065)	0.50
FVC z-score	0	0.155	(-0.086, 0.397)	0.180	(-0.060, 0.420)	0.039	(-0.197, 0.276)	0.75
BHR slope	0	0.365	(-0.726, 1.457)	0.291	(-0.887, 1.468)	-0.327	(-1.473, 0.818)	0.60

Adjusted models: FEV_1 absolute - child's age at testing, child's gender, parity, maternal BMI, maternal height; FEV_1 z-score - child's gender; FVC absolute - child's age at testing, child's gender, age at introduction of solid foods, maternal BMI, maternal height, FVC z-score - child's gender; BHR - maternal age, smoking in pregnancy, paternal eczema, age at introduction of solid food.

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