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Prenatal and child vitamin D levels and allergy and asthma in childhood

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BACKGROUND: Early-life vitamin D deficiency may impair immune system development contributing to allergy and asthma onset. Findings from prospective studies are inconsistent.
OBJECTIVE: To examine whether maternal and child vitamin D levels are associated with allergic and asthma-related symptoms throughout childhood in a Spanish birth cohort.
METHODS: 25-Hydroxyvitamin D_3 ($25(OH)D_3$) levels were measured in the serum of pregnant women ($N = 2525$) and children ($N = 803$). Information on allergic and asthma-related symptoms was obtained from repeated questionnaires from 1 to 9 years.
RESULTS: A total of 19% of mothers and 24% of children had deficient $25(OH)D_3$ levels (<20 ng/ml). Higher child $25(OH)D_3$ levels at 4 years were associated with lower odds of atopic eczema from 4 to 9 years (adjusted odds ratio = 0.90; 95% CI = 0.84–0.97 per 5 ng/ml). Higher maternal and child $25(OH)D_3$ levels were associated with a lower prevalence of late-onset wheezing at the limit of statistical significance (adjusted relative risk ratio (RRR_{adj}) = 0.86; 95% CI = 0.74–1.00 and $RRR_{adj} = 0.76$; 95% CI = 0.58–1.02 per 5 ng/ml, respectively). All the remaining associations were null.
CONCLUSION: Child $25(OH)D_3$ levels at pre-school age are associated with a reduced odds of atopic eczema in later childhood and both maternal and child levels may reduce the prevalence of late-onset wheezing.

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IMPACT:

- In this Spanish birth cohort, with a total of 19% of mothers and 24% of children with deficient levels of vitamin D, higher child vitamin D at 4 years of age was associated with reduced odds of atopic eczema up to 9 years. There was also some evidence that higher maternal and child vitamin D levels reduced the prevalence of late-onset wheezing.
- Although these findings need replication, they may imply optimal vitamin D levels at pre-school age to prevent atopic eczema.

INTRODUCTION

Vitamin D deficiency, generally defined as circulating vitamin D levels below 20 ng/ml,[1] is still high in countries with abundant sunshine such as Southern European countries with prevalence ranging from 18 to 75% in adults.[2] Of concern, pregnant women and infants are at increased risk of vitamin D deficiency.[3] Because vitamin D during early life plays a fundamental role in the development of the immune system,[4–6] vitamin D deficiency during these periods may contribute to the onset of allergy and asthma in childhood. However, evidence from prospective cohort studies is still inconsistent. Some studies have found a protective role of high maternal or child vitamin D levels on the development of allergies and asthma throughout childhood;[5,7–11] others have reported either no associations[12–15] or even an increased risk of allergies and asthma symptoms.[16–18] Controversial results across studies may be due to heterogeneity regarding the levels and ranges of vitamin D, weeks of gestation or child's age when vitamin D was measured, child's age at outcome assessment, definition of the outcomes, sample size or confounders considered. Also, most studies have collected information on allergic and asthma symptoms at only one time during childhood,[7,11,14,16,19] providing incomplete information on the onset and progression of symptoms

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throughout childhood. Moreover, previous studies have mainly evaluated the effects of vitamin D status during pregnancy[5,10,13,14,17,20] or in childhood;[8,9,15] only three studies have been able to assess the vitamin D effects in both periods in the same population.[7,11,21] Studies with vitamin D measured in both periods may help to identify which is the period when vitamin D has the major influence on the development of allergies and asthma.

In this study, we aimed to examine whether maternal and/or child vitamin D levels at pre-school age, influence the development of allergy and asthma in children from 1 until 9 years.

METHODS

Study population

The INMA Project is a network of population-based birth cohorts from seven different Spanish regions that aim to study the effect of environmental pollutants during early life (pregnancy and childhood) on growth, development and children's health. Pregnant women were recruited in the first trimester of pregnancy between 1997 and 2008 during the standard prenatal care visit.[20] In the present study we included 3246 pregnant women recruited from five of the INMA birth cohorts: Asturias, Gipuzkoa, Menorca, Sabadell, and Valencia. Inclusion criteria were: ≥16 years old, intention to deliver in the reference hospital, singleton pregnancy, and no assisted conception or communication issues. Pregnant women were followed during pregnancy until delivery. From birth, children were followed up regularly in varying intervals depending on the cohort until 9 years of age. During the follow-up periods, extensive information has been collected from children and their parents from a variety of sources including face-to-face interviews by trained INMA personnel or self-reported by the participants, physical health examinations, neuropsychological assessments, and passive collection of clinical records. In addition, multiple biomarkers of effect and exposure have been determined in biological samples (e.g., persistent and non-persistent chemicals, metals and trace elements, vitamins and nutrients, immune and inflammatory markers). Depending on the cohort, the visits were held in the school or the health care centre of reference.

Of the initial 3246 participants recruited in Asturias, Gipuzkoa, Menorca, Sabadell, and Valencia, a total of 2525 had information on circulating maternal vitamin D levels during pregnancy and child allergy and asthma outcomes at 1, 2, 4, 7 or 9 years and were included in the analysis (Supplementary Fig. S2). Among the 3246 mother–child pairs recruited, a total of 3108 live born children were followed at birth. Participation rate, considering those included at birth, varied across cohorts between 90 and 100% at 1 year, 87 and 99% at 2 years, 77 and 98% at 4 years, 64 and 97% at 7 years, and 60 and 88% at 9 years. In addition, circulating children vitamin D levels at 4 years were measured in a subsample of the population randomly selected from Asturias, Gipuzkoa, Sabadell, and Valencia. A total of 803 children had information on vitamin D levels at 4 years and allergy and asthma outcomes from 4 to 9 years and were also included in the analysis (Supplementary Fig. S2).

Vitamin D

Quantification of 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels in pregnant women at 13.3 weeks of gestation (standard deviation (SD) = 2.8) and at 4.40 years of the child (SD = 0.18) was performed by high-performance liquid chromatography (HP-LC) using BioRAD kit.[22] To adjust for the month at vitamin D measurement, we used deseasonalized $25(OH)D_3$ concentrations (see Supplementary information).

Allergy and asthma

Questionnaires administered to the mothers by trained nurses collected information on the occurrence (no/yes) of allergic rhinitis, atopic eczema, wheeze, and asthma from 1 until 9 years. The questionnaire was the Spanish/Catalan version of the validated International Study of Asthma Allergies and Childhood questionnaire (see full questions in the Supplementary information). We defined active wheezing in each follow-up if the child had wheezing and had taken asthma medication in the last year. Information on wheezing in the last year collected from 1 until 9 years was used to create four wheezing categories: (i) never wheezers; (ii) early wheezers (≤4 years only); (iii) late-onset wheezers (>4 years only), and persistent wheezers (≤4 and >4 years). We defined ever asthma as having ever been asthma diagnosed by a doctor considering all follow-ups until 9 years.

Covariates

Maternal and child characteristics were obtained through questionnaires administered to the mothers during pregnancy (maternal ethnicity, country of birth, age at delivery, pre-pregnancy body mass index (BMI), education, social class (coded according to the International Standard Classification for Occupations-88 system), smoking, parity, history of asthma/atopy (defined as maternal history of asthma, allergic rhinitis or eczema), adherence to Mediterranean diet score[23] and self-perception of physical activity), at 1 year of the child (duration of breastfeeding (any)) or at 4 years (adherence to Mediterranean diet score[23] and parental perception of physical activity)). Child's sex, birth weight, and gestational age were obtained from medical records. Child's weight and height at 4 years were measured by trained nurses.

Statistical analysis

We calculated Spearman's correlation coefficients between maternal and child $25(OH)D_3$ levels. The shape of the relationship between the exposures and the outcomes was tested by general additive models. All associations showed linearity (Supplementary Fig. S3); therefore, $25(OH)D_3$ levels were treated as continuous (per 5 ng/ml increase[11,15]). We performed multiple imputation of missing values for the covariates using chained equations. Distributions of variables were similar in observed and imputed data sets (Supplementary Table S1).

Mothers included were more likely to be older, higher educated, Spanish and to have a normal weight compared to those excluded (Supplementary Table S2A). Children with vitamin D levels at 4 years were more likely to have older mothers who did not smoke during pregnancy, and have a higher education level and social class (Supplementary Table S3A). In order to tackle the selection bias that potentially arises when restricting the analysis for the population with available information on the exposure and outcomes, we calculated the inverse probability weighting for both populations (mothers and children). We used information available for all participants at recruitment to predict the probability of participation in the current study and used the inverse of those probabilities as weights in the analyses so that the results would be representative of the initial sample ($n = 3246$) (Supplementary Tables S2B and S3B). We therefore assumed that the characteristics of the included participants (78% in the case of mothers and 29% in the case of children) predicted those of non-included participants.

We used multivariable logistic mixed-effects models to assess the associations between $25(OH)D_3$ levels and allergic rhinitis, atopic eczema, and active wheezing from 1 to 9 years for maternal $25(OH)D_3$ and from 4 to 9 years for child $25(OH)D_3$. We included an interaction term between vitamin D and age at outcome assessment to assess whether the vitamin D effect differed over time. We used multivariable logistic and multinomial logistic regression models to assess the associations between maternal and child $25(OH)D_3$ levels and ever asthma and wheezing patterns, respectively. Estimates are expressed as odds ratio (OR) in case of logistic mixed-effect models and logistic regression models and relative risk ratio (RRR) in case of multinomial logistic regression models. We used Direct Acyclic Graphs to identify the minimum set of confounders to adjust the models (Supplementary Figs. S4 and S5). Models were adjusted for region of residence, maternal ethnicity, country of birth, age at delivery, pre-pregnancy BMI, education, social class, smoking, and parity. Given that the Menorca cohort had no available information on diet and physical activity during pregnancy, we tested these two covariates as potential confounders in a subsequent analysis excluding the Menorca cohort. Child models were adjusted for region of residence, maternal ethnicity, country of birth, age at delivery, pre-pregnancy BMI, education, social class, smoking, parity, sex of the child, breastfeeding duration, child's BMI, adherence to Mediterranean diet, and physical activity at 4 years of age, and child's age at each follow-up.

To assess the robustness of our results we first repeated all models using the complete-case dataset. Second, to rule out reverse causality in the associations of child $25(OH)D_3$ levels with allergy and asthma outcomes at 4 years, we repeated the models including only those children who developed symptoms after 4 years of age (i.e., at 7 and/or at 9 years). Third, we tested potential effect modification by region of residence and sex[10] by including an interaction term in the models and performing a stratified analysis.

Analyses were conducted using STATA 16.1 statistical software (Stata Corporation, College Station, Texas) and R version 4.0.2 (R Foundation, Vienna, Austria). Statistical significance was set at p value <0.05 for multivariate analyses and p value <0.1 for the interaction test.

RESULTS

Maternal and child median plasma 25(OH)D_3 levels were 29.2 ng/ml (range: 21.9–37.0 ng/ml) and 27.5 ng/ml (range: 20.3–36.9 ng/ml), respectively (Table 1). A total of 19% of mothers and 24% of children had deficient levels of 25(OH)D_3 (<20 ng/ml). Maternal and child 25(OH)D3 levels were weakly correlated (Spearman $p = 0.15$; $p < 0.001$).

In the population with maternal 25(OH)D3 during pregnancy ($N = 2525$), the prevalence of allergic rhinitis increased from 3% at 4 years to 23% at 9 years of age while the prevalence of atopic eczema remained stable throughout childhood (between 19 and 23%) (Table 2). The prevalence of active wheezing decreased from 1 year (31%) to 9 years (6%). A total of 45% of children were classified as never wheezers, 40% as early wheezers, 3% as late wheezers, and 12% as persistent wheezers. The prevalence of ever asthma was 9%. Prevalence and tendency of allergy and asthma symptoms throughout childhood were similar in the population with child 25(OH)D3 levels at 4 years ($N = 803$) (Table 2).

We observed few associations between maternal 25(OH)D_3 levels and allergy and asthma outcomes in the offspring (Table 3). Exposure to higher 25(OH)D_3 levels during pregnancy were associated with a lower prevalence of late-onset wheezing (adjusted relative risk ratio (RRR_{adj}) = 0.86; 95% CI = 0.74–1.00, per 5 ng/ml increase of 25(OH)D_3), but the association was at the limit of statistical significance. Children with higher 25(OH)D_3 levels at 4 years had lower odds of atopic eczema from 4 to 9 years (adjusted odds ratio (OR_{adj}) = 0.90; 95% CI = 0.84–0.97). Increasing child 25(OH)D_3 levels at 4 years were also associated with a lower prevalence of late-onset wheezing ($RRR_{adj} = 0.76$; 95% CI = 0.58–1.02), but this association was at the limit of statistical significance. The associations between maternal and child 25(OH)D_3 levels and allergic rhinitis, atopic eczema, and active wheezing were largely consistent across ages (p values for interaction >0.10). Neither maternal nor child 25(OH)D_3 levels were associated with childhood allergic rhinitis, active wheezing, or ever asthma (Table 3). The addition of adherence to the Mediterranean diet and physical activity as confounders in the maternal models did not change the effect estimates (Supplementary Table S4).

Analyses using complete-case data sets and only including those children who developed symptoms after 4 years, yielded similar results (Supplementary Tables S5 and S6). There was no indication of modification of the 25(OH)D_3 effect on allergy and asthma outcomes by region of residence (Supplementary Tables S7 and S8). We did observe that the protective role of higher child 25(OH)D_3 levels in relation to late-onset wheezing was only observed among females ($RRR_{adj} = 0.55$; 95% CI = 0.35–0.87 for females and $RRR_{adj} = 1.01$; 95% CI = 0.64–1.54 for males) (Supplementary Table S8).

DISCUSSION

In this Spanish mother–child cohort, higher child circulating 25(OH)D_3 levels at pre-school age were associated with a lower odds of atopic eczema from 4 to 9 years. We also observed that higher maternal and child 25(OH)D_3 levels were associated with a lower prevalence of late-onset wheezing, although these associations were at the limit of statistical significance.

Comparison with other studies

The two previous studies that assessed maternal and child vitamin D levels in the same population and their association with atopic eczema development,[7,11] observed that higher maternal vitamin D levels were more protective against the development of atopic eczema than child levels.[7,11] In both studies, children were not followed after the age when child vitamin D was measured and hence the effects in later childhood could not be explored.

Table 1. Maternal and child characteristics of the included populations.

	Population with maternal 25(OH)D_3 during pregnancy $n = 2525$	Population with child 25(OH)D_3 levels at 4 years $n = 803$
Maternal characteristics		
Ethnicity; Caucasian	96.8	97.5
Country of birth; Spain	92.9	93.5
Age at delivery (years)	31.7 (4.3)	32.4 (4.1)
Pre-pregnancy BMI		
Underweight (<18.5 kg/m^2)	4.2	4.1
Normal (18.5–24.9 kg/m^2)	69.5	68.0
Overweight (25–29.9 kg/m^2)	18.5	18.9
Obese (≥30 kg/m^2)	7.8	9.0
Education		
Primary or less	27.9	20.5
Secondary	39.9	43.2
High	32.2	36.3
Social class		
Semi-skilled/unskilled	21.0	23.9
Skilled manual/non-manual	30.2	27.2
Professionals and managers	48.8	49.9
Smoking		
Never smoke	68.3	73.4
Quit early in pregnancy	15.1	12.0
During pregnancy	16.6	14.6
Parity, nulliparous	56.3	55.6
History of asthma/atopy; yes	25.4	25.4
Adherence to Mediterranean diet at 12 weeks		
Low	40.8	39.4
Medium	29.9	30.8
High	29.3	29.8
Physical activity, self-perception previous year		
Sedentary	6.8	7.8
Little active	25.1	26.0
Moderately active	41.1	39.8
Quite-very active	27.0	26.4
25(OH)D_3 at 13 weeks of pregnancy (ng/ml)	29.2 (21.9–37.0)	28.4 (20.9–36.5)
Deficient (<20 ng/ml)	19.2	22.5
Insufficient (20–30 ng/ml)	33.1	32.0
Sufficient (>30 ng/ml)	47.7	45.5
Child characteristics		
Sex; male	51.7	50.4
Birth weight (g)	3254.6 (468.8)	3280.6 (462.2)
Preterm (<37 weeks)	4.3	4.5
Breastfeeding duration		
Never	15.1	14.0
1–16 weeks	26.5	23.7
17–24 weeks	16.1	18.3
>24 weeks	42.3	44.0
BMI at 4 years		
Normal	80.4	87.3
Overweight or obese	14.6	12.7
Adherence to Mediterranean diet at 4 years		
Low	30.1	24.9
Medium	51.0	53.5
High	18.9	21.6

Table 1. continued

	Population with maternal 25(OH)D_3 during pregnancy $n = 2525$	Population with child 25(OH)D_3 levels at 4 years $n = 803$
Physical activity at 4 years		
Sedentary/little active	6.5	4.3
Moderately active	33.5	33.6
Quite active	44.9	47.9
Very active	15.1	14.2
25(OH)D_3 at 4 years (ng/ml)	27.4 (20.2–36.9)	27.5 (20.3–36.9)
Deficient (<20 ng/ml)	24.4	24.0
Insufficient (20–30 ng/ml)	33.1	33.3
Sufficient (>30 ng/ml)	42.5	42.7
Age at outcome assessment		
1 year	1.4 (0.6)	1.6 (0.7)
2 years	2.5 (0.4)	2.7 (0.4)
4 years	4.4 (0.2)	4.4 (0.2)
7 years	7.4 (0.8)	7.6 (0.7)
9 years	9.4 (0.6)	9.1 (0.6)

Values are percentages for categorical variables and means (standard deviation) or median (percentile 25–percentile 75) for continuous variables. Non-imputed data.
BMI body mass index, *25(OH)D_3* 25-hydroxyvitamin D_3.

Considering those studies that only assessed child vitamin D levels, two Australian prospective studies observed that lower levels of vitamin D in the first 6 years of life were associated with increased risk of atopy at 10[9] and at 14 years,[8] similar to our study. On the contrary, in the large UK ALSPAC cohort, higher levels of vitamin D at 10 years increased the risk of atopy at 15 years.[16] Finally, in a Norwegian cohort, vitamin D levels at 1 year were not related to the presence or severity of atopic eczema at 2 years.[15] Mixed findings across studies may be due to the different child's ages when vitamin D and outcomes were assessed. Differences in child vitamin D levels might also explain the conflicting results: levels in ALSPAC and in the Norwegian cohort were maybe too low (median 23–25 ng/ml) and had narrow ranges (e.g., ALSPAC interquartile range: 0.4 ng/ml) to observe a protective effect compared with the Australian and INMA cohorts (median: 29 ng/ml). Finally, the selection of the study population could also play a role (i.e., an Australian cohort only included children with a high genetic risk of allergy and asthma). Results from randomised control trials are also controversial: a study in Boston (mean 9 years of age) found that oral vitamin D supplementation improved atopic dermatitis severity in children,[24] whereas two other studies in Toronto[25] and Rome[26] did not find any improvement in atopic dermatitis severity after child vitamin D supplementation at 6 and 7 years of age, respectively. Regarding the studies that only assessed maternal vitamin D levels (and not child levels), they have also found inconsistent results.[5,10,13,18,27] Indeed, findings from randomised control trials in pregnant women[24–26] are also inconclusive. Despite the huge number of studies conducted to date, the effects of early-life vitamin D exposure on atopic eczema are still unclear.[28]

Our results suggest a protective role of maternal and child 25(OH)D_3 levels on the development of late-onset wheezing. These findings have to be interpreted with caution because the sample size of children who developed late-onset wheezing was limited (53 in the maternal population and 21 in the child population) and maternal and child 25(OH)D_3 levels were not associated either with active wheezing or ever asthma. Only one previous study assessed wheezing patterns and observed that low vitamin D levels at birth increased the risk of early wheezing (<3 years) but not of late wheezing (5 years);[5] of note, children were not followed after 5 years, so it is unknown whether the effects on early wheezing persisted later in childhood. Previous prospective studies and randomised control trials on vitamin D and wheezing and asthma have yielded inconsistent results. Of the two studies that assessed vitamin D both in pregnancy and childhood, one found higher maternal vitamin D levels to reduce the risk of asthma at 4 years[7] and neither of them found child vitamin D levels to be associated with reduced risk of wheezing or asthma.[7,11] Two meta-analyses of prospective studies on maternal vitamin D including 16 and 34 studies, respectively, also did not find any consistent association with wheezing or asthma.[12,29] The combined results of two large randomised control trials on prenatal vitamin D supplementation, the Vitamin D Antenatal Asthma Reduction Trial (VDAART) and Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC2010), resulted in a reduced risk of asthma/recurrent wheeze in the offspring at 3 years of age.[30] However, the VDAART followed children until 6 years of age, and the observed association at 3 years disappeared.[31] Regarding child vitamin D, few prospective studies have evaluated this association and results are also inconclusive.[9,16,32] Further studies assessing child vitamin D levels and following children later in childhood are needed to confirm our results.

We found no association between maternal and/or child vitamin D levels and allergic rhinitis. Our findings are in line with a previous meta-analysis[12] and also with results of the VIVA birth cohort in the US, where no association was observed between either vitamin D levels at birth or at 8 years with ever allergic rhinitis at school age.[21]

Interpretation of the results

Vitamin D is involved in many aspects of atopic eczema onset: it modulates the development of the immune system by inhibiting β-lymphocyte function and reducing the secretion of immunoglobulin E[4] and plays a role in keratinocytes differentiation by modulating its growth and suppressing inflammatory responses while promoting immune tolerance.[5] Our finding that higher vitamin D levels at pre-school age are more protective against atopic dermatitis than maternal levels has not been previously reported. We may hypothesise that vitamin D levels measured in children accurately reflect the biologically active levels and hence those involved in the mechanisms preventing eczema. In fact, the amount of vitamin D that can cross the placental barrier and be biologically available for the foetus is still unclear. We also hypothesise that vitamin D levels at 4 years probably reflect child levels of previous years, as shown in an Australian cohort, where vitamin D levels at 1 year were similar to those at 4 years.[9] Since atopic dermatitis has its onset in the first 5 years of life, vitamin D from birth to pre-school age might be critical for the proper development of the immune system and epidermal barrier function.

Vitamin D can protect against wheezing and asthma development through several mechanisms. Low vitamin D levels have been shown to be associated with an increased risk of lower respiratory tract infections[12,13] and allergic sensitisation.[9] Both conditions promote inflammation of the airways and may trigger the appearance of wheezing and asthma later in life. Different susceptibilities between populations can explain the discrepancies observed across studies on wheezing/asthma. Genetic polymorphisms in proteins involved in vitamin D metabolism, receptor binding affinity, or transportation can modify the predisposition to vitamin D deficiency or allergy onset.[33,34] Also, it is suggested that the immune system can develop differently according to geographical region.[35]

Strengths and limitations

The main strengths of our study are its population-based prospective design with vitamin D measurements during

Table 2. Prevalence of allergy and asthma outcomes.

Outcomes	Population with maternal 25(OH)D_3 during pregnancy, $N = 2525$			Population with child 25(OH)D_3 levels at 4 years, $N = 803$		
	N children with information for each outcome (with+without symptoms)	Children with symptoms/ children without symptoms	Prevalence (%)	*N* children with information for each outcome (with+without symptoms)	Children with symptoms/ children without symptoms	Prevalence (%)
Allergic rhinitis						
4 years	2100	67/2033	3.2	794	21/773	2.6
7 years	1910	366/1544	19.2	726	138/588	19.0
9 years	826	190/636	23.0	351	69/282	19.7
Atopic eczema						
1 year	2429	539/1890	22.2	–	–	–
2 years	1296	301/995	23.2	–	–	–
4 years	2100	407/1693	19.4	794	135/659	17.0
7 years	1910	406/1504	21.3	726	138/588	19.0
9 years	1085	254/1085	23.4	351	96/255	27.4
Active wheezing[a]						
1 year	1453	444/1009	30.6	–	–	–
4 years	1772	218/1554	12.3	784	91/693	11.6
7 years	1902	155/1747	8.1	722	55/667	7.6
9 years	1080	60/1020	5.6	351	27/324	7.7
Wheezing patterns[b]						
Never	763	–	44.6	283	–	42.0
Early (1–4 years)	688	–	40.3	298	–	44.3
Late-onset (5–7/9 years)	53	–	3.1	21	–	3.1
Persistent (1–7/9 years)	205	–	12.0	71	–	10.6
Ever asthma	1715	158/1557	9.2	678	50/628	7.4

[a]Active wheezing was defined as having had any wheezing episode during the 12 months prior to the follow-up in addition to had taken any asthma or wheezing medication during the same period.
[b]Wheezing in the past 12 months was used to create four wheezing categories: never wheezers, early wheezers (≤4 years only), late-onset wheezers (>4 years only), and persistent wheezers (≤4 and >4 years).

pregnancy and at pre-school age, and the outcomes assessed at multiple timepoints throughout childhood. Also, we applied multiple imputation and inverse probability weighting to account for selection bias in order to increase the validity of our results. However, we may have missed important variables that explained the attrition and hence, completely remove the bias. However, the study has some limitations. First, relying on only one vitamin D assessment during pregnancy may not be representative of the entire period although it has been suggested that a single 25(OH)D measurement can be used to identify women who are at risk of low 25(OH)D levels at other stages of pregnancy.[36] Moreover, the immune system starts to develop in the first trimester of pregnancy, consequently, vitamin D may exert immunomodulatory effects in utero as early as the first trimester.[37] Also, child vitamin D was measured at 4 years, probably after most of the allergic outcomes, especially atopic eczema, tend to have already developed. Indeed, of the children who developed atopic eczema at any time during childhood (47%), 77% developed it before 4 years and 23% developed it after that, at 7 or at 9 years (data not shown). However, in the sensitivity analysis only including those children who developed atopic eczema after 4 years, we still observed a reduced odds of atopic eczema associated with child vitamin D levels. In addition, although 25(OH)D levels are considered the most reliable indicator of vitamin D status[38] and most of the 25(OH)D is in 25(OH)D_3 form, circulating levels of 25(OH)D_2 were not assessed which could contribute to exposure misclassification. In addition, despite vitamin D levels were assessed using HP-LC and not mass spectrometry, considered the gold standard technique to assess vitamin D levels, there is evidence of a good agreement between both techniques in terms of accuracy and precision.[39,40] Second, although allergy and asthma-related symptoms were collected using well-validated questionnaires answered by the mothers with the help of trained nurses, we cannot rule out some outcome misclassification due to under- or over-reporting of symptoms, which may have led to attenuation or overestimation of the results. Furthermore, although questions mostly referred to the last 12 months, at some points were administered 2 or 3 years apart, which may have introduced bias. In addition, the classification of children in the different wheezing patterns can be biased due to the missingness in some of the follow-ups. Also, we did not have information on allergic sensitisation, which support the diagnosis of allergic rhinitis. Finally, we did not correct for multiple comparisons that might have led to false-positive findings (type I error). However, statistical correction for multiple comparisons assumes that the tested hypotheses are independent, which is not the case in these analyses since the exposures, but especially the allergic and asthma outcomes are related to each other.[41] Also, it increases the chances of false negative findings (type II error), which in the context of public health research might have worse consequences.

Table 3. Association[a] of maternal and child 25(OH)D_3 levels (per 5 ng/ml increase) with allergic rhinitis, atopic eczema, active wheezing, ever asthma and wheezing patterns during childhood.

Outcome[a]	Maternal 25(OH)D_3 during pregnancy[b]		Child 25(OH)D_3 at 4 years[c]	
	N[d]	OR [95% CI]	*N*[d]	OR [95% CI]
Allergic rhinitis	2184	0.98 [0.89–1.08]	802	0.99 [0.90–1.10]
Atopic eczema	2516	0.99 [0.95–1.03]	802	0.90 [0.84–0.97]**
Active wheezing	2381	0.97 [0.91–1.02]	801	0.92 [0.82–1.04]
Ever asthma	2191	0.98 [0.90–1.06]	678	0.98 [0.83–1.15]
Wheezing patterns	1709	**RRR [95% CI]**	673	**RRR [95% CI]**
Never	763	Ref.	283	Ref.
Early	688	0.98 [0.93–1.03]	298	0.94 [0.86–1.02]
Late-onset	53	0.86 [0.74–1.00]*	21	0.76 [0.58–1.02]
Persistent	205	1.02 [0.95–1.10]	71	0.98 [0.88–1.10]

25(OH)D_3 25-hydroxyvitamin D_3, *CI* confidence interval, *OR* odds ratio, *RRR* relative risk ratio.
[a]Multivariable logistic mixed-effects models for allergic rhinitis, atopic eczema, and active wheezing from 1 to 9 years for maternal 25(OH)D_3 models and from 4 to 9 years for child 25(OH)D_3 models; logistic regression models for ever asthma; and multinomial logistic regression models for wheezing patterns.
[b]Maternal models were adjusted for region of residence, maternal ethnicity, country of birth, age at delivery, pre-pregnancy body mass index, education, social class, smoking, and parity.
[c]Child models were adjusted for region of residence, maternal ethnicity, country of birth, age at delivery, pre-pregnancy body mass index, education, social class, smoking, parity, child sex, breastfeeding duration, child body mass index, adherence to Mediterranean diet and physical activity at 4 years, and child age at each follow-up.
[d]Number of participants with maternal or child 25(OH)D_3 levels and at least one outcome available at one timepoint.
*p value < 0.1.
**p value < 0.05.

CONCLUSION

Our results suggest that higher child 25(OH)D_3 levels at pre-school age are associated with a reduced odds of atopic eczema at later ages and that both maternal and child levels may reduce the prevalence of late-onset wheezing. Given the large inconsistencies of previous studies, our study adds some evidence of the potential protective effect of vitamin D levels at pre-school age on atopic eczema development. However, prospective cohort studies assessing both vitamin D levels in pregnancy and in childhood and following children later in childhood are needed to confirm our findings. If confirmed, this will imply optimal vitamin D levels at pre-school age to prevent atopic eczema development.

DATA AVAILABILITY

The data that support the findings of this study are available from INfancia y Medio Ambiente (INMA) study but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of INMA study.

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AUTHOR CONTRIBUTIONS

J. Sangüesa and M.C. designed the study. J. Sunyer was the coordinator of the whole INMA Project. M.V., M.T., A.T., A.C.R.-D. and J.V. are the PIs of the different cohorts included. A.E., A.I., J.J. and L.L.-G. recruited participants or helped to obtain biological samples. R.G.-E., M.G., A.A. and J.G. gave advice about exposure or outcome assessment and how to do the analysis. R.G.-E. also provided statistical support and advice. Finally, J. Sangüesa and M.C. wrote the manuscript and the others revised and approved it.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Our study was approved by the regional ethical committees of each cohort and all participants signed written informed consent.

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