

ORIGINAL ARTICLE

Associations of maternal and fetal 25-hydroxyvitamin D levels with childhood eczema: The Generation R Study

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Abstract

Background: Exposure to low levels of vitamin D in fetal life might affect the developing immune system, and subsequently the risk of childhood eczema. We examined whether 25-hydroxyvitamin D levels in mid-gestation and at birth were associated with the risk of eczema until the age of 4 years.

Methods: In a population-based prospective cohort study of 3019 mothers and their children, maternal blood samples in mid-gestation and umbilical cord blood samples at birth were used to determine 25-hydroxyvitamin D levels (severely deficient <25.0 nmol/l, deficient 25.0–49.9 nmol/l, sufficient 50.0–74.9 nmol/l, optimal ≥75.0 nmol/l). Eczema was prospectively assessed by annual questionnaires until the age of 4 years. Eczema patterns included never, early (age ≤1 year only), late (age >1 year only), and persistent eczema (age ≤ and >1 year). Data were assessed using the generalized estimating equations and multinomial regression models.

Results: Compared with the optimal 25-hydroxyvitamin D group, sufficient, deficient, and severely deficient groups of 25-hydroxyvitamin D level in mid-gestation were not associated with the risk of overall eczema (odds ratios [95% confidence interval]: 1.09 [0.82, 1.43], 1.04 [0.87, 1.25], and 0.94 [0.81, 1.10], p-values for trend >0.05), nor with eczema per year or eczema patterns in children up to the age of 4 years. Similarly, we observed no associations of 25-hydroxyvitamin D groups at birth with any eczema outcome.

Conclusion: Our results suggest that levels of 25-hydroxyvitamin D in mid-gestation and at birth are not associated with the risk of overall eczema, eczema per year, or eczema patterns among children until the age of 4 years.

Eczema is a common relapsing, inflammatory, pruritic skin condition with an estimated prevalence of up to 22.5% among children at the age of 7 years (1). It has been suggested that its origin is partly in pregnancy and early infancy (2). Changes in levels of vitamins, supplements, or diet during pregnancy, such as vitamin E, folic acid, and n-3

or n-6 polyunsaturated fatty acids, have been associated with the risk of childhood eczema (3, 4). Also, lower exposure to vitamin D in fetal life might affect the developing immune system, leading to unbalanced inflammatory processes and skin barrier impairment, and subsequently increased risk of eczema (5). Animal studies have suggested that vitamin D

has a role in regulating the innate and adaptive immune system (6). *In vitro* studies have shown that the treatment of various human cell lines with 1,25-dihydroxy vitamin D, the active form of 25-hydroxyvitamin D, promotes the development and maturation of Th2 cells (6) and inhibits inflammatory activity (7) such as that of IgE-activated mast cells (8). These processes might have a role in initiation and amplification of inflammatory process underlying the development of eczema. Also, systemic administration of vitamin D receptor agonists improved allergen-triggered eczema of mice and showed induction of skin barrier gene and antimicrobial peptide gene expression (9). Previous studies in humans reported inconsistent results for the associations of maternal 25-hydroxyvitamin D levels during pregnancy and at birth with the risk of childhood eczema (5, 10–17). Differences in results might be explained by different methods of vitamin D measurements or adjustment for different confounders. We hypothesized that lower 25-hydroxy vitamin D levels in early life lead to an increased risk of eczema at a young age.

Therefore, we examined among 3019 mothers and their children participating in a population-based prospective cohort study whether 25-hydroxyvitamin D levels in mid-gestation and at birth were associated with eczema from birth until the age of 4 years.

Materials and methods

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands (18).

The Medical Ethical Committee of the Erasmus Medical Center (MEC 217.595/2002/202) approved the study protocol. This study was performed in participants of Dutch origin, only to prevent confounding or effect modification by the large heterogeneity of other small ethnic groups with different skin types and diet. Written informed consent was obtained from 3840 parents of participating children until the age of 4 years (Figure S1). Twins ($n = 119$) and children of whom we had no data on vitamin D levels in mid-gestation and at birth ($n = 597$) and on eczema at any time point ($n = 105$) were excluded leaving a total of 3019 children for the current analyses.

25-hydroxyvitamin D

We collected maternal venous blood samples in mid-gestation (median 20.5 [range 18.1–24.9] weeks of gestation) and umbilical cord blood samples at birth (median 40.1 [range 27.6–43.4] weeks of gestation). Measurements of 25-hydroxyvitamin D levels from maternal and cord blood were conducted at the Eyles Laboratory at the Queensland Brain Institute, University of Queensland, Australia (19). Details are provided in Data S1. Levels of 25-hydroxyvitamin D were categorized as severely deficient <25.0 nmol/l (<10.0 mg/l), deficient 25.0–49.9 nmol/l (10.0–19.9 mg/l), sufficient 50.0–

74.9 nmol/l (20.0–29.9 mg/l), and optimal ≥ 75.0 nmol/l (≥ 30.0 mg/l) (20).

Childhood eczema

Information on physician-diagnosed eczema was obtained by parental questionnaires at the ages of 6 months and 1, 2, 3, and 4 years based on questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) ('Was your child diagnosed with eczema in the last 6 months/last year?') (Not as far as I know; yes, did not go to the doctor; yes, went to the general practitioner; yes, went to the hospital) (21). Answers were categorized into 'no' (not as far as I know; yes, did not go to the doctor) or 'yes' (yes, went to the general practitioner; yes, went to the hospital). The response rates at 1, 2, 3, and 4 year follow-up were 73%, 71%, 76%, 72%, and 73%, respectively. Based on annual data on eczema, we defined the following eczema patterns: never eczema (no eczema from birth until the age of 4 years), early eczema (age ≤ 1 year only), late eczema (age >1 year only), and persistent eczema (age $\leq$ and >1 year) (22).

Covariates

We obtained information on maternal age, pre-pregnancy body mass index (BMI), educational level (primary; secondary; higher), history of eczema, allergy and asthma (no; yes), parity (nulliparous; multiparous), smoking during pregnancy (no; yes), and pet keeping (no; yes) from questionnaires during pregnancy. Maternal psychological distress in the second trimester of pregnancy was assessed using the global severity index of the brief symptom inventory (23). Maternal folate levels were measured in early pregnancy (median 13.2 [range 6.2–17.9] weeks of gestation), as previously described (24). Season of blood sampling was recorded at time of the blood sampling for 25-hydroxyvitamin D levels. We obtained information on child's sex, gestational age, and birth weight from midwife and hospital records at birth. Information on breastfeeding (never; partial until the age of 4 months; exclusive until the age of 4 months) was collected by questionnaires at the ages of 2, 6 and 12 months. Information on vitamin D supplementation (no; yes) was collected at the age of 2 months by a questionnaire. In the Netherlands, it is recommended that all children until the age of 4 years use 10 mg per day as vitamin D supplement (25).

Statistical analysis

We used generalized estimating equations (GEE) to examine the associations of 25-hydroxyvitamin D levels (categorized and continuous) in mid-gestation and at birth with the longitudinal odds of eczema at the ages of 6 months, and 1, 2, 3, and 4 years independently and overall. The GEE models take correlations between repeated measurements of eczema within the same child into account. We used multinomial regression models for the associations of 25-hydroxyvitamin D levels in mid-gestation and at birth with eczema patterns. Models were adjusted for maternal age, pre-pregnancy BMI, educational level, history of eczema, allergy and asthma,

parity, smoking during pregnancy, pet keeping, psychological distress, folate level in first trimester, and mid-gestational season of blood sampling, child's sex, gestational age at birth, birth weight, breastfeeding, season of blood sampling at birth and mutually for mid-gestational, and cord blood 25-hydroxyvitamin D levels. Prevalence of missing data of covariates was $\leq 20.0\%$, except for maternal psychological distress (31.4%) and folate (22.2%). To reduce potential bias from missing data and to increase precision, we performed a multiple imputation analysis of covariates, 25-hydroxyvitamin D levels in mid-gestation and at birth, and eczema outcomes (per year and eczema patterns) generating 10 independent datasets using the Markov chain Monte Carlo method and calculated pooled estimates (26). Multiple imputations of all missing variables were required to successfully perform GEE modeling. We present the results based on imputed analyses only. Measures of association are presented as odds ratios (ORs) with their 95% confidence intervals (CIs). Statistical analyses were performed using SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Subject characteristics

Maternal and child characteristics are presented in Table 1. Observed data showed a median 25-hydroxyvitamin D level in mid-gestation of 65.5 nmol/l (range 1.5–193.2) and at birth 39.3 nmol/l (range: 0.1–144.9) ($p < 0.001$). The prevalence of eczema ranged from 7.2% to 18.2% and, of early, late, persistent eczema 12.1%, 12.9%, and 13.1%, respectively. Because we imputed the missing values in almost all variables that were used in this analysis, we presented their values in comparison with the observed values (Table S1). Compared with those included in the study, all maternal and child characteristics of those lost to follow-up differed, except for maternal pre-pregnancy body mass index, history of eczema, allergy and asthma, and psychological distress, child's sex and blood sampling season of 25-hydroxyvitamin D levels (Table S2).

25-hydroxy vitamin D and eczema

Compared with the optimal 25-hydroxy vitamin D group, sufficient, deficient, and severely deficient groups of 25-hydroxy vitamin D level in mid-gestation were not associated with the risk of overall eczema in children up to the age of 4 years (OR (95% CI): 1.09 (0.82, 1.43), 1.04 (0.87, 1.25), and 0.94 (0.81, 1.10), p -values for trend > 0.05) (Table 2). Also, per year analysis showed no association between 25-hydroxy vitamin D groups in mid-gestation and childhood eczema (Table 2). We observed no association of the sufficient, deficient, and severely deficient groups of 25-hydroxy vitamin D level at birth with overall eczema, compared with the optimal 25-hydroxy vitamin D group (OR [95% CI]: 0.93 [0.70, 1.23], 0.92 [0.71, 1.20], and 0.89 [0.69, 1.17], p -values for trend > 0.05). Similarly, per year analysis showed no association between 25-hydroxy vitamin D groups at birth and

Table 1 Observed characteristics of mothers and their children

	Observed
Maternal characteristics	
Age (years)	30.5 (7.6)
Pre-pregnancy body mass index (kg/m ²)	22.9 (4.0)
Educational level, higher (%)	64.0 (1909)
History of eczema, allergy and asthma, yes (%)	34.7 (951)
Parity, nulliparous (%)	59.8 (1804)
Smoking during pregnancy, yes (%)	23.7.0 (656)
Pet keeping, yes (%)	41.6 (1122)
Psychological distress	0.2 (0.2)
Folate level in 1st trimester (nmol/L)	19.9 (13.7)
Mid-gestational blood sampling season (%)	
Spring	28.4 (787)
Summer	23.3 (646)
Autumn	23.4 (649)
Winter	24.9 (691)
25-hydroxyvitamin D level in mid-gestation (nmol/L)	65.5 (43.7)
Child characteristics	
Sex, male (%)	50.9 (1514)
Gestational age at birth (weeks)	40.2 (1.7)
Birth weight (g)	3550 (655)
Breastfeeding, exclusive until the age of 4 months (%)	27.2 (696)
Vitamin D supplementation at the age of 2 months, yes (%)	71.0 (1850)
At birth blood sampling season (%)	
Spring	24.1 (727)
Summer	26.4 (797)
Autumn	27.9 (842)
Winter	21.6 (653)
25-hydroxyvitamin D level at birth (nmol/L)	39.3 (28.8)
Eczema, yes (%)	
Age of 6 months	15.1 (319)
Age 1 year	18.2 (518)
Age 2 years	12.8 (340)
Age 3 years	8.1 (201)
Age 4 years	7.2 (177)
Eczema patterns (%)	
Never	61.9 (1066)
Early	12.1 (210)
Late	12.9 (222)
Persistent	13.1 (225)

Values for categorical variables are expressed as percentages (absolute numbers), and values for continuous variables are expressed as medians (interquartile range).

childhood eczema (Table 2). The 25-hydroxy vitamin D levels in mid-gestation and at birth were not associated with eczema patterns (Table 3).

Discussion

The results of this population-based prospective cohort study suggest that the levels of 25-hydroxy vitamin D in

Table 2 Associations of 25-hydroxyvitamin D levels with childhood eczema per year and overall (n = 3019)

	Odds ratio (95% CI) for eczema					
	6 months n = 688	1 year n = 614	2 years n = 528	3 years n = 371	4 years n = 384	Overall n = 1462
25-hydroxyvitamin D in mid-gestation						
Severely deficient, n = 155	1.05 (0.54, 2.05) 35/155	0.91 (0.55, 1.49) 31/155	1.05 (0.60, 1.83) 33/155	0.97 (0.50, 1.87) 19/155	1.33 (0.76, 2.32) 25/155	1.09 (0.82, 1.43) 80/155
Deficient, n = 747	1.15 (0.72, 1.85) 179/747	0.98 (0.74, 1.31) 153/747	0.95 (0.70, 1.29) 131/747	0.99 (0.62, 1.58) 90/747	0.93 (0.61, 1.43) 94/747	1.04 (0.87, 1.25) 384/747
Sufficient, n = 919	1.03 (0.75, 1.43) 207/919	0.92 (0.72, 1.17) 180/919	0.81 (0.61, 1.07) 145/919	0.90 (0.62, 1.30) 103/919	0.93 (0.67, 1.30) 112/919	0.94 (0.81, 1.10) 424/919
Optimal, n = 1199	Reference 267/1199	Reference 250/1199	Reference 220/1199	Reference 159/1199	Reference 153/1199	Reference 574/1199
p-value for trend	0.30	0.94	0.77	0.98	0.81	0.87
25-hydroxyvitamin D at birth						
Severely deficient, n = 684	0.69 (0.35, 1.36) 142/684	0.99 (0.64, 1.55) 136/684	0.96 (0.57, 1.62) 116/684	1.16 (0.58, 2.32) 92/684	1.23 (0.59, 2.56) 99/684	0.93 (0.70, 1.23) 335/684
Deficient, n = 1401	0.79 (0.47, 1.33) 309/1401	1.01 (0.66, 1.57) 284/1401	1.01 (0.65, 1.59) 242/1401	1.03 (0.57, 1.88) 170/1401	0.98 (0.52, 1.87) 166/1401	0.92 (0.71, 1.20) 661/1401
Sufficient, n = 741	0.83 (0.50, 1.39) 181/741	0.90 (0.57, 1.41) 153/741	0.95 (0.57, 1.57) 135/741	0.85 (0.45, 1.61) 84/741	0.98 (0.56, 1.71) 94/741	0.89 (0.69, 1.17) 371/741
Optimal, n = 193	Reference 56/193	Reference 41/193	Reference 35/193	Reference 25/193	Reference 25/193	Reference 95/193
p-value for trend	0.15	0.88	0.61	0.71	0.46	0.86

Values are odds ratios (95% confidence interval) using longitudinal generalized estimating equation models. 'n = represents the number of cases per group.' Groups of 25-hydroxy vitamin D were defined as severely deficient <25.0 nmol/l, deficient 25.0–49.9 nmol/l, sufficient 50.0–74.9 nmol/l, and optimal ≥75.0 nmol/l. Models were adjusted for maternal age, pre-pregnancy body mass index, education, history of eczema, allergy and asthma, parity, smoking, pet keeping, psychological distress, folate level, mid-gestational season of blood sampling, child's sex, gestational age at birth, birth weight, breastfeeding, vitamin D supplementation at the age of 2 months, at birth season of blood sampling, and mutually for mid-gestational and cord blood 25-hydroxyvitamin D.

mid-gestation and at birth were not associated with overall eczema, eczema per year, or eczema patterns in children up to the age of 4 years.

Comparison of main findings with other studies

Studies that explored the association of maternal 25-hydroxy vitamin D levels during pregnancy with childhood eczema reported inconsistent results (10, 14, 15). Two studies suggested that maternal levels of 25-hydroxyvitamin D measured at before pregnancy (14), some period in pregnancy (10), or at birth (14) were not associated with the risk of eczema at the age of 2 (14) or 7 (10) years, respectively. Other studies reported that higher maternal 25-hydroxyvitamin D levels in late pregnancy were associated with an increased risk of visible eczema at the age of 9 months (15) or with decreased risk of eczema at the age of 4 years (14). Differences in results might be explained by the period of pregnancy at which 25-hydroxyvitamin D was measured, method of eczema measurement such as having visible eczema on examination or parental reporting of eczema through questionnaires, or adjustment for different confounding factors. Additional to these studies, we observed that levels of 25-hydroxy vitamin D in mid-gestation were not associated with the risk of overall eczema, eczema per year, or eczema patterns in children up to

the age of 4 years, taking also 25-hydroxyvitamin D levels at birth into account.

We hypothesized that lower 25-dihydroxy vitamin D levels in early life lead to an increased risk of eczema at a young age, but did not find such associations. We observed that the levels of 25-hydroxy vitamin D levels at birth were not associated with the risk of childhood eczema. Our results are in contrast to a previous study that reported that a higher 25-hydroxy vitamin D3 levels at birth were associated with the decreased risk of eczema at the age of 6 months (5). The results with our study are difficult to explain due to differences in sample size and type of vitamin D level measurement. Therefore, further studies are needed to replicate our findings before any strong conclusions might be drawn. Several prospective cohort studies have examined associations of 25-hydroxy vitamin D levels at birth with the risk of childhood eczema at later ages and reported conflicting results (11–13). One study did not observe an association of 25-hydroxy vitamin D levels at birth with the risk of eczema, allergic sensitization, and total IgE levels until the age of 7 years (11), but others suggested that higher 25-hydroxy vitamin D levels at birth were associated with a lower risk of eczema in children until the age of 5 years (12, 13). Differences in results may be attributed to different sample sizes of the study (12, 13), use of 25-hydroxyvitamin D3 only (13), or different definitions of eczema patterns (12).

Table 3 Associations of 25-hydroxyvitamin D levels with childhood eczema patterns (n = 3019)

	Odds ratio (95% CI) for eczema patterns		
	Early n = 462	Late n = 586	Persistent n = 414
25-hydroxyvitamin D in mid-gestation			
Severely deficient, n = 155	0.99 (0.33, 2.92) 21/155	0.75 (0.28, 2.00) 32/155	0.90 (0.35, 2.26) 27/155
Deficient, n = 747	1.02 (0.37, 3.06) 134/747	1.12 (0.69, 1.84) 151/747	0.90 (0.54, 1.50) 99/747
Sufficient, n = 919	1.23 (0.78, 1.94) 142/919	0.88 (0.58, 1.34) 166/919	0.89 (0.58, 1.37) 116/919
Optimal, n = 1199	Reference 165/1199	Reference 237/1199	Reference 172/1199
p-value for trend	0.62	0.71	0.66
25-hydroxyvitamin D at birth			
Severely deficient, n = 684	1.00 (0.36, 2.80) 93/684	0.98 (0.35, 2.74) 148/684	0.63 (0.25, 1.56) 93/684
Deficient, n = 1401	0.92 (0.38, 2.22) 208/1401	0.96 (0.36, 2.30) 259/1401	0.67 (0.31, 1.43) 195/1401
Sufficient, n = 741	1.28 (0.54, 3.05) 127/741	1.21 (0.48, 3.01) 148/741	0.53 (0.25, 1.15) 96/741
Optimal, n = 193	Reference 34/193	Reference 31/193	Reference 30/193
p-value for trend	0.30	0.78	0.79

Values are odds ratios (95% confidence interval) using multinomial logistic regression models. 'n = represents the number of cases per group.' Reference group is the 'optimal 25-hydroxy vitamin D group and never eczema'. Never eczema (n = 1557). Groups of 25-hydroxy vitamin D were defined as severely deficient <25.0 nmol/l, deficient 25.0–49.9 nmol/l, sufficient 50.0–74.9 nmol/l, and optimal ≥75.0 nmol/l. Models were adjusted for maternal age, pre-pregnancy body mass index, education, history of eczema, allergy and asthma, parity, smoking, pet keeping, psychological distress, folate level, mid-gestational season of blood sampling, child's sex, gestational age at birth, birth weight, breastfeeding, vitamin D supplementation at the age of 2 months, at birth season of blood sampling, and mutually for mid-gestational and cord blood 25-hydroxyvitamin D.

Furthermore, eczema measured at multiple time points during follow-up did not take repeated measurements of eczema within the same child into account, or adjustment for 25-hydroxy vitamin D during pregnancy (12).

Interpretation of results

Although the effects of 25-hydroxyvitamin D on the immune system have been well recognized (6), its effects do not seem to have a role in the occurrence and persistence of eczema through childhood. *In vivo* experiments in mice suggested that the treatment of eczema with vitamin D receptor agonists results in improvement of allergen-associated eczema (9). However, findings in animal models indicated that vitamin D3 or its analogues display different effects on expression of molecules in mouse skin as opposed to human or primate skin (27). Namely, expression of the thymic stromal lymphopoietin was seen after stimulation with vitamin D3 in mouse keratinocytes and not in human or primate keratinocytes (28). We observed no differences in results with eczema outcomes when 25-hydroxy vitamin D during pregnancy or at birth was under study. Levels of 25-hydroxy vitamin D in mid-gestation and at birth might have been highly correlated due to similar lifestyle and dietary habits of mother during pregnancy. However, we

observed a lower median 25-hydroxyvitamin D level at birth than in mid-gestation (39.3 vs. 65.5 nmol/l). This difference in 25-hydroxyvitamin D levels was not expected because in the Netherlands, pregnant women are advised to take 10 mg of vitamin D daily throughout pregnancy (25). Our study lacked information on adherence of vitamin D intake which could have explained the discrepancy in 25-hydroxyvitamin D levels at birth and mid-gestation. Placental function and vitamin D receptors, or genetic susceptibility, might have also affected the difference in 25-hydroxyvitamin D levels (29–32). We did not observe differences in results when we included or excluded supplementation of vitamin D in childhood (data not shown). The potential intermediating and protective role of child's vitamin D supplementation for the association of 25-hydroxy vitamin D levels during pregnancy and at birth with eczema is difficult to study in countries such as the Netherlands where most pre-school children use a vitamin D supplement because it is recommended (25). We did not observe associations of either maternal or birth 25-hydroxyvitamin D levels with any eczema outcome, and we therefore speculate that other factors, such as genetic or other environmental factors, might have a more important role in the onset of childhood eczema. Eczema appears to have a complex genetic background. Mutations of the filament-aggregating protein (filaggrin) gene have been

strongly associated with the development of eczema (33). Also, a recent study suggested that microRNAs influence the inhibition of innate immune response in chronic skin inflammation (27). For environmental factors, climate and housing conditions, urban or rural living, dietary and feeding habits, air pollution, microbial exposure, and socioeconomic status (34) have been suggested to be associated with childhood eczema. Also, exposure to higher ultraviolet (UV) levels seems associated with higher 25-hydroxy vitamin D levels (35) and lower prevalence of eczema compared with exposure to lower UV levels (36). Therefore, future studies are needed to explore the role of multiple genetic and environmental factors, and their interaction, in the development of eczema in childhood.

Strengths and limitations

Strength of this study is its population-based prospective design. Participants were followed up from fetal life onward, and a large number of potential confounding factors were recorded. Also, levels of 25-hydroxy vitamin D levels were objectively measured in maternal blood in mid-gestation and in cord blood at birth and categorized according to clinical cutoff values that would allow the comparison with other studies. However, selection bias in cohort studies arises from non-response or missing data of the exposure, and loss to follow-up. Subjects of our study were more affluent and healthy than those excluded. This could have led to biased effect estimates if the associations of mid-gestational and cord blood 25-hydroxy vitamin D levels with eczema would have been difference between those included and not included in the analyses. We have chosen cutoff levels of 25-hydroxy vitamin D based on those of adults because of the lack of studies showing possible differences in optimal 25-hydroxyvitamin D levels during pregnancy and at birth, and non-pregnant adults (≥ 75 nmol/l) (20). Information on eczema was collected by parents through questionnaires, and this method is commonly used in large epidemiological studies (37, 38). However, some misclassification of eczema

could still be present because of the lack of validation of eczema measured by questionnaire with medical records or physical examination in our study population. We aimed to take many covariates into account, but lacked data on potential modifying environmental factors such as UV exposure or dietary patterns of the children which could have affected our results. Our population for analysis comprised children of Dutch origin only and may limit applications of our findings to children of various ethnic origins or with heterogeneous skin types. Finally, we cannot exclude residual confounding from unmeasured exposures such as other vitamin or supplement use, as in any prospective cohort study.

In conclusion, our results suggest that levels of 25-hydroxy vitamin D in mid-gestation and at birth are not associated with the risk of overall eczema, eczema per year, or eczema patterns among children until the age of 4 years. Future studies focused on other risk factors, such as environmental and genetic factors, are needed to explore the development of eczema in childhood.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary methods.

Table S1. Observed and imputed characteristics of mothers and their children.

Table S2. Characteristics of subjects included and lost to follow-up.

Figure S1. Flowchart of participants.

**Associations of maternal and fetal 25-hydroxyvitamin D levels with
childhood eczema. The Generation R Study.**

Supplementary material

Supplementary Methods

Table E-1 Observed and imputed characteristics of mothers and their children

Table E-2 Characteristics of subjects included and lost to follow-up

Figure E-1 Flowchart of participants

Supplementary Methods

Total 25-hydroxyvitamin D level was reported as the sum of 25-hydroxyvitamin D2 and D3 species measured in plasma using a modification of a method previously described (25). Briefly, 50µL milli-Q water and 500µL of acetonitrile (ACN) containing 6,19,19-[2H3]-25-hydroxyvitamin D2 and 6,19,19-[2H3]-25-hydroxyvitamin D3 at 10 nmol/L each were added to 3µL plasma, sonicated, vortexed and centrifuged. The supernatant was filtered using a TiO₂/ZrO₂ filter plate (Glygen, USA) and evaporated to dryness. Samples were derivatised using 4-phenyl-1,2,4-triazoline-3,5-dione and reconstituted in ACN:H₂O (1:3) prior to analysis. Samples were quantified using isotope dilution liquid chromatography-tandem mass spectrometry. The analytical system was comprised of a Shimadzu Nexera UPLC coupled to an AbSciex 5500 QTRAP equipped with an APCI source. Chromatographic separation was achieved using a Kinetex XB-C18 column (50 × 2.1 mm, 1.7 µm; Phenomenex, USA), and 72% acetonitrile/32% aqueous 0.1% formic acid at a flow rate of 0.5 mL/min. Linearity was assessed using matrix-matched calibration standards, with R² values of >0.99 across the calibration range (10-125 nmol/L). Inter-assay inaccuracy and imprecision were assessed at four concentration levels for 25-hydroxyvitamin D3 (48.3, 49.4, 76.4 and 139.2 nmol/L) and a single level (32.3 nmol/L) for 25-hydroxyvitamin D2 using certified reference materials purchased from the National Institute of Standards and Technology (NIST) (NIST SRM 972a Levels 1-4), and were excellent at all concentration levels tested. Inter-assay inaccuracy and imprecision were both <10% for 25-hydroxyvitamin D3 and <17% for 25-hydroxyvitamin D2. Assay repeatability was assessed via replicate analysis of an independent reference material (NIST SRM1950, 61.9 nmol/L 25-hydroxyvitamin D3), with inter-assay inaccuracy and imprecision both <11% (n = 343). The limit of quantification was 1 and 5 nmol/L for 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 respectively.

36 **Supplementary Table E-1.** Observed and imputed characteristics of mothers and their children

	Observed	Imputed
Maternal characteristics		
Age (years)	30.5 (7.6)	-
<i>Missing</i>	-	-
Pre-pregnancy body mass index (kg/m ²)	22.9 (4.0)	23.2 (4.2)
<i>Missing</i>	0.5 (16)	-
Educational level (%)		
Higher	64.0 (1,909)	63.4 (1,915)
Secondary	33.7 (1,006)	33.6 (1,016)
Primary	2.3 (69)	2.9 (88)
<i>Missing</i>	1.2 (35)	-
<u>History of eczema, allergy and asthma (%)</u>		
No	65.3 (1,793)	65.4 (1,975)
Yes	34.7 (951)	34.6 (1,044)
<i>Missing</i>	9.1 (275)	-
Parity (%)		
0	59.8 (1,804)	59.9 (1,808)
≥1	40.2 (1,209)	40.1 (1,211)
<i>Missing</i>	0.2 (6)	-
Smoking during pregnancy (%)		
No	76.3 (2,113)	76.1 (2,298)
Yes	23.7.0 (656)	23.9 (721)
<i>Missing</i>	8.3 (250)	-
Pet keeping (%)		
No	58.4 (1,578)	58.3 (1,759)
Yes	41.6 (1,122)	41.7 (1,260)
<i>Missing</i>	10.6 (319)	-
Psychological distress	0.2 (0.2)	0.2 (0.2)
<i>Missing</i>	31.4 (947)	-
Folate level in 1 st trimester (nmol/L)	19.9 (13.7)	19.6 (13.4)
<i>Missing</i>	22.2 (671)	-
Mid-gestational blood sampling season (%)		
Spring	28.4 (787)	28.5 (859)
Summer	23.3 (646)	23.2 (702)
Autumn	23.4 (649)	23.1 (698)
Winter	24.9 (691)	25.2 (760)
<i>Missing</i>	8.1 (246)	-
25-hydroxyvitamin D level in mid-gestation (nmol/L)	65.5 (43.7)	66.3 (42.8)
<i>Missing</i>	8.2 (248)	-
Child characteristics		
Sex (%)		
Male	50.9 (1,514)	-
Female	49.1 (1,505)	

<i>Missing</i>	-	
Gestational age at birth (weeks)	40.2 (1.7)	-
<i>Missing</i>	-	
Birth weight (grams)	3,550 (655)	-
<i>Missing</i>	-	
Breastfeeding (%)		
Never	11.0 (283)	10.7 (323)
Partial until the age of 4 months	61.8 (1,582)	59.9 (1,809)
Exclusive until the age of 4 months	27.2 (696)	29.4 (887)
<i>Missing</i>	15.2 (458)	-
<u>Vitamin D supplementation at the age of 2 months (%)</u>		
<u>No</u>	<u>29.0 (757)</u>	<u>30.4 (918)</u>
<u>Yes</u>	<u>71.0 (1,850)</u>	<u>69.6 (2,101)</u>
<i>Missing</i>	<u>13.6 (412)</u>	-
At birth blood sampling season (%)		
Spring	24.1 (727)	-
Summer	26.4 (797)	
Autumn	27.9 (842)	
Winter	21.6 (653)	
<i>Missing</i>	-	
25-hydroxyvitamin D level at birth (nmol/L)	39.3 (28.8)	38.3 (29.2)
<i>Missing</i>	30.9 (932)	-
Eczema at the age of 6 months (%)		
No	84.9 (1,794)	77.3 (2,331)
Yes	15.1 (319)	22.7 (688)
<i>Missing</i>	30.0 (906)	-
Eczema at the age of 1 year (%)		
No	81.8 (2,323)	79.7 (2,405)
Yes	18.2 (518)	20.3 (614)
<i>Missing</i>	5.9 (178)	-
Eczema at the age of 2 years (%)		
No	87.2 (2,321)	82.6 (2,491)
Yes	12.8 (340)	17.4 (528)
<i>Missing</i>	11.9 (358)	-
Eczema at the age of 3 years (%)		
No	91.9 (2,277)	87.7 (2,648)
Yes	8.1 (201)	12.3 (371)
<i>Missing</i>	17.9 (541)	-
Eczema at the age of 4 years (%)		
No	92.8 (2,298)	87.3 (2,635)
Yes	7.2 (177)	12.7 (384)
<i>Missing</i>	18.0 (544)	-
Eczema patterns (%)		
Never	61.9 (1,066)	51.6 (1,557)
Early	12.1 (210)	15.3 (462)

Late	12.9 (222)	19.3 (586)
Persistent	13.1 (225)	13.7 (414)
<i>Missing</i>	42.9 (1,296)	-

Values for categorical variables are expressed as percentages (absolute numbers) and values for continuous variables are expressed as medians (interquartile range)

Supplementary Table E-2. Characteristics of subjects included and lost to follow-up

	Included n = 3,019	Not included n = 105	p-value for difference
Maternal characteristics			
Age (years)	30.5 (7.6)	28.8 (5.9)	0.001
Pre-pregnancy body mass index (kg/m ²)	22.9 (4.0)	25.1 (4.9)	0.157
Educational level (%)			0.001
Higher	64.0 (1,909)	34.0 (35)	
Secondary	33.7 (1,006)	47.6 (49)	
Primary	2.3 (69)	18.4 (19)	
<u>History of eczema, allergy and asthma (%)</u>			0.743
No	65.3 (1,793)	63.8 (60)	
Yes	34.7 (951)	36.2 (34)	
Parity (%)			0.008
0	59.8 (1,804)	46.7 (49)	
≥1	40.2 (1,209)	53.3 (56)	
Smoking during pregnancy (%)			0.001
No	76.3 (2,113)	53.7 (51)	
Yes	23.7 (656)	46.3 (44)	
Pet keeping (%)			0.017
No	58.4 (1,578)	43.6 (41)	
Yes	41.6 (1,122)	56.4 (53)	
Psychological distress	0.2 (0.2)	0.3 (0.3)	0.066
Folate level in 1 st trimester (nmol/L)	19.9 (13.7)	12.4 (17.9)	0.001
Mid-gestational blood sampling season (%)			0.751
Spring	28.4 (787)	32.6 (29)	
Summer	23.3 (646)	24.7 (22)	
Autumn	23.4 (649)	21.3 (19)	
Winter	24.9 (691)	21.3 (19)	
Child characteristics			
Female sex (%)	49.1 (1,505)	47.6 (50)	0.692
Gestational age at birth (weeks)	40.2 (1.7)	39.6 (1.9)	0.022
Birth weight (grams)	3,550 (655)	3,378 (560)	0.008
Breastfeeding (%)			0.001
Never	11.0 (283)	43.5 (20)	
Partial until the age of 4 months	61.8 (1,582)	56.5 (26)	
Exclusive until the age of 4 months	27.2 (696)	-	
<u>Vitamin D supplementation at the age of 2 months (%)</u>			
<u>No</u>	<u>29.0 (757)</u>	<u>58.7 (27)</u>	<u>0.001</u>
<u>Yes</u>	<u>71.0 (1,850)</u>	<u>41.3 (19)</u>	
At birth blood sampling season (%)			0.680
Spring	24.1 (727)	26.3 (17)	
Summer	26.4 (797)	27.7 (18)	
Autumn	27.9 (842)	27.7 (180)	

Winter

21.6 (653)

18.5 (12)

Values for categorical variables are expressed as percentages (absolute numbers) and values for continuous variables are expressed as medians (interquartile range). P-values for difference were calculated by Mann-Whitney U test for 2 independent samples and Chi-square test for categorical variables

Supplementary Figure E-1. Flowchart of participants

