

ORIGINAL ARTICLE

EPIDEMIOLOGY AND GENETICS

Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study

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Abstract

Background: Vitamin D levels are known to be associated with atopic disease development; however, existing data are controversial. The aim of this study was to investigate whether corresponding maternal and cord blood vitamin D levels are associated with atopic outcomes in early infancy.

Methods: Within the LINA cohort study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk), 25(OH)D was measured in blood samples of 378 mother–child pairs during pregnancy and at birth. Information about children's atopic manifestations during the first 2 years of life was obtained from questionnaires filled out by the parents during pregnancy and annually thereafter. Cord blood regulatory T cells (Treg) were detected by methylation-specific PCR using a Treg-specific demethylated region in the *FOXP3* gene.

Results: The median maternal 25(OH)D₃ level was 22.19 ng/ml (IQR 14.40–31.19 ng/ml); the median cord blood 25(OH)D₃ 10.95 ng/ml (6.99–17.39 ng/ml). A high correlation was seen between maternal and cord blood 25(OH)D₃ levels, both showing a seasonal distribution. Maternal and cord blood 25(OH)D₃ was positively associated with children's risk for food allergy within the first 2 years. Further, higher maternal 25(OH)D₃ resulted in a higher risk for sensitization against food allergens at the age of two. Cord blood 25(OH)D₃ levels were negatively correlated with regulatory T cell numbers.

Conclusion: Our study demonstrates that high vitamin D levels in pregnancy and at birth may contribute to a higher risk for food allergy and therefore argues against vitamin D supplement to protect against allergy.

In recent years, there has been a worldwide increase in the prevalence of allergic diseases, which greatly disrupts the lives of those affected. Many risk factors for the development of atopic diseases, including genetic background, individual immune response as well as lifestyle and environmental conditions, have been identified (1). One factor that also seems to interfere with the pathology of atopy, next to its osseous endocrine function, is vitamin D. The discussion about

vitamin D as a risk factor for allergic diseases was raised following a publication, which showed an association between vitamin D supplementation due to rickets prevention with an increase in allergy prevalence throughout the 20th century (2). Thereafter, several birth cohort studies reported conflicting data on the risk and prevention of allergy following vitamin D exposure (3–5). On the one hand, *low* infantile vitamin D was associated with higher rates of atopic disease at the age

of 6 and 14 years (6) and enhanced eczema severity in children aged between 8 months and 12 years (7). Low maternal vitamin D levels during pregnancy were shown to increase the risk for infantile atopic dermatitis (8) and the severity of allergic rhinitis and asthma in the offspring (9). On the other hand, *high* vitamin D levels, especially as a consequence of vitamin D supplementation, were described to increase the risk of allergic disease (2, 10, 11). Children born to mothers with high (>30 ng/ml) vitamin D levels were more likely to develop offspring eczema compared to mothers with low (<10 ng/ml) vitamin D concentrations (12). Excessive vitamin D supplementation in early childhood has also been shown to increase the risk of sensitization to inhalant or food allergens, allergic rhinitis or asthma in children and adults (13–15).

To date, there are limited studies addressing the prenatal vitamin D status and its impact on the newborn immune status and later infantile atopic outcomes. Especially, the debate about the recommendation of a vitamin D supplementation during pregnancy or in early childhood to reduce the allergy risk of the child requires more and precise data on the role of vitamin D in the disease pathology. Therefore, the aim of this study was to investigate whether and how corresponding vitamin D levels of pregnant mothers and newborns are associated with the immune status at the time of birth and atopic outcomes in early infancy.

Methods

Study design and sample collection

Within the LINA cohort study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk), 629 mother–child pairs (622 mothers and 629 children; 7 twins) were recruited between May 2006 and December 2008 in Leipzig, Germany (51.4°N), to investigate the influence of lifestyle and environmental factors in the pre- and postnatal period on the immune system of the newborn and the child later in life, along with the risk of developing allergic disease. Mothers suffering from immune or infectious diseases during pregnancy were excluded from the study.

Blood samples were obtained from mothers at 34th week of pregnancy and cord blood at delivery. After birth, blood samples were collected annually from both the mother and child. During pregnancy and at children's first and second birthday, standardized questionnaires were recorded, collecting data about family history of atopy (FHA), housing and environmental conditions (first- or second-hand smoke, mould, traffic, noise, pets, renovation activities, personal lifestyle, etc.), as well as atopic outcomes of the children. All questionnaires were self-administered by the parents. Participation in the study was voluntary, and informed consent was obtained from all participants. The study was approved by the Ethics Committees of the University of Leipzig (046-2006, 160-2008).

Atopic outcomes

Atopic dermatitis was recorded as a parental report of a doctor-diagnosed dermatitis or of dermatitis symptoms during

the last 12 months. Children were classified as having dermatitis symptoms when parents reported an intermittent itchy skin rash, which affected places other than the nappy area and lasted at least 2 weeks, or appeared repeatedly in the last 12 months. Food allergy was recorded as a parental report of a doctor diagnosis.

Analyses of serum 25(OH)D

In a LINA subgroup of 378 mother–child pairs (374 mothers and 378 children; 4 twins; Fig. 1), serum 25 hydroxy-vitamin D (25(OH)D) levels were measured. Cord and maternal vitamin D concentrations were determined by liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) by the laboratory of the Institute of Agricultural and Nutrition Science, Martin-Luther-University Halle/Wittenberg (Halle/Saale, Germany) as previously described (16, 17). Briefly, 25(OH)D₃ and 25(OH)D₂ were obtained from standards, cord and maternal serum via protein precipitation using the HPLC-MS/MS *MassChrom* Reagent Kit (Chromsystems, Munich, Germany). Analysis was performed by Agilent 1100 HPLC (Agilent Technologies, Böblingen, Germany) with an API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany). A high-resolution analytical column was used for cord blood samples to eliminate the C3-Epimer of 25(OH)D₃. Subsequent to chromatographic separation, 25(OH)D₃ and 25(OH)D₂ molecules were ionized in an atmospheric-pressure chemical-ionization source, followed by nitrogen collision-induced dissociation. The generated mass-specific ion fragments were detected with *m/z* 395/269

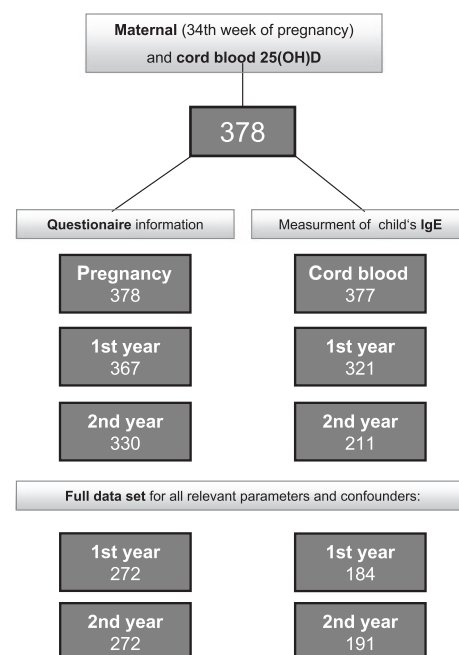


Fig. 1 Flow chart of the analysed LINA subcohort and resulting mother–child pairs after consideration of available measurements and questionnaire information.

for 25(OH)D₂, m/z 383/211 for 25(OH)D₃, m/z 383/211 for 3-Epi-25(OH)D₃ and m/z 389/211 for 25(OH)D₃-d₆ (internal standard).

The assay detection limit was defined as 6.7 ng/ml for maternal 25(OH)D₃ and 5.2 ng/ml for maternal 25(OH)D₂. Detection limit for cord blood 25(OH)D₃ and D₂ was 3 ng/ml. Samples with 25(OH)D values below detection limit are included in analyses using half of the defined detection limit (e.g. 1.5 ng/ml for cord blood 25(OH)D₃).

Analyses of IgE

Total IgE, as well as IgE specific for food allergens (fx5) or inhalative allergens (sx1), were determined at birth in the cord blood (only total IgE) and at one and two years of age by Phadia CAP System (Phadia Diagnostics, Freiburg, Germany). Total IgE levels > 0.7 kU/l in cord blood and > 3.8 kU/l at the age of one or two were classified as 'increased', as well as specific IgE (sx1 or fx5) > 0.35 kU/l. Values below the detection limit were included in analyses using half of the defined detection limit.

Analyses of regulatory T cells

Quantification of regulatory T cells was performed as described earlier (for details, see Hinz et al. (18)). Genomic DNA was isolated from whole blood using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) and bisulphite-treated using the EpiTect 96 Bisulfite Kit (Qiagen, Hilden, Germany). Quantification of demethylation in Treg-specific demethylated region (TSDR) was performed by real-time PCR by Epiontis (Berlin, Germany). In the total LINA cohort ($n = 629$), Treg numbers were analysed in 426 cord bloods. Only samples with a copy number >10 were considered. As previously described, the number of Tregs in cord blood is presented as percentage corresponding to the measured amount of TSDR demethylation in the *FOXP3* gene (19, 20).

Statistical analyses

25(OH)D levels were not normally distributed; therefore, analyses were performed using nonparametric tests in general and are presented as medians with 25th–75th percentile. The chi-square test for cross-relationship was used to compare the analysed subcohort with the total cohort. Spearman's rank correlation test was performed to analyse the correlation between maternal and newborn 25(OH)D. Seasonal distribution of 25(OH)D concentration was calculated using ANOVA. The relationship between atopic outcomes and vitamin D levels (divided in quartiles) was addressed using the Mann–Whitney *U*-test. In addition, multivariate logistic regression models were used to consider possible confounding factors. Here, data are presented as odds ratios with 95% confidence interval adjusted for gender, number of siblings, increased cord blood IgE levels, FHA, cotinine levels during pregnancy, breast feeding, UV intensity at birth and vitamin D supplementation during the first year of life. The

UV intensity at birth was classified according to the German Federal Office for Radiation Protection (Bundesamt für Strahlenschutz) in *low* (category 0–2, which is measured from October to March) and *high* UV intensity (category 3–7, which is measured from April to September) (21). Vitamin D supplementation was recorded as a parental report of vitamin D supplementation ever within the child's first year of life.

All *P*-values < 0.05 were considered to be significant. Adjustments due to multiple testing were not made because our analyses were based on a *priori* hypothesis (22). Statistical analyses were performed with STATISTICA for Windows, version 10 (Statsoft Inc. (Europe), Hamburg, Germany). For more details, see methods in the supplements.

Results

Characteristics of the analysed LINA subcohort

Characteristics of the analysed subcohort compared to the total LINA cohort are presented in Table 1. There were no differences between the analysed subcohort ($n = 378$) and the total cohort ($n = 629$) for all listed characteristics.

Vitamin D levels

Measured 25(OH)D₂ values were below the detection limit in each sample and were not included in further analyses. The median cord blood 25(OH)D₃ level was 10.95 ng/ml (interquartile range 6.99–17.39 ng/ml), and the median maternal blood 25(OH)D₃ concentration was 22.19 ng/ml (14.40–31.19 ng/ml; Table 2).

A high correlation was seen between maternal and cord blood 25(OH)D₃ levels ($R = 0.812$, $P < 0.001$; Fig. 2). Furthermore, both maternal and cord blood 25(OH)D₃ levels followed a significant seasonal distribution ($P < 0.05$, Fig. 3).

Of all pregnant mothers, 44.4% had deficient (<20 ng/ml), 25.7% had insufficient (20–29.9 ng/ml), and 29.9% had optimal (>30 ng/ml) 25(OH)D₃ levels (cut-offs according to (4)). 50.0% of the newborns had deficient 25(OH)D₃ levels (<11 ng/ml, cut-offs according to (23)). Only seven mothers obtained vitamin D supplements during pregnancy. There were no significant differences between the 25(OH)D₃ levels from mothers with (median 27.4 ng/ml) or without (median 22.2 ng/ml; Mann–Whitney *U*-test $P = 0.411$) vitamin D supplementation.

Infantile outcomes and IgE levels

Frequencies of atopic outcomes within the first 24 months are shown in Table 3(a). Within the analysed subcohort, 18.8% of the children were positive for symptoms of an atopic dermatitis within the first 24 months, 14.0% for physician-diagnosed atopic dermatitis and 5.5% for food allergy. Levels of children's IgE are shown in Table 3(b). Within the first 24 months, 87.5% of the children had increased total IgE, 22.3% had sensitizations against food allergens and 6.0% against inhalant allergens.

Table 1 Characteristics of the analysed subcohort compared to the total LINA cohort

	Analysed subcohort <i>N</i> = 378 <i>n</i> (%)	Entire LINA cohort <i>N</i> = 629 <i>n</i> (%)	<i>P</i> -value*
Gender of the child			
Male	196 (51.9)	327 (52.0)	0.967
Female	182 (48.1)	302 (48.0)	
Number of siblings			
>0	258 (68.3)	418 (66.5)	0.748
>1	92 (24.3)	156 (24.8)	
>2	24 (6.3)	45 (7.2)	
>3	2 (0.5)	8 (1.3)	
≥ 4	2 (0.5)	2 (0.3)	
Parental history of atopy			
None	129 (34.1)	210 (33.4)	0.980
Single	176 (46.6)	294 (46.7)	
Double	72 (19.4)	121 (19.2)	
Breast feeding			
≤ 3 months	64 (16.9)	112 (17.8)	0.712
≥ 4 months	270 (71.4)	443 (70.4)	
Total IgE in cord serum			
Increased†	65 (17.2)	84 (13.4)	0.684
Not increased	312 (82.5)	434 (69.0)	
UV intensity of month of birth‡			
Low	162 (42.9)	258 (40.4)	0.632
High	205 (54.2)	348 (54.5)	
Vitamin D supplementation during the first year of life§			
Yes	337 (91.8)	560 (92.4)	0.761
No	30 (8.2)	46 (7.6)	
Cotinine (µg/g) 34th week of pregnancy, log median (IQR)	0.65 (−0.22–1.67)	0.72 (−0.27–1.76)	0.391¶

**P*-value from the chi-square test for cross-table relationship.

†IgE level > 0.7 kU/l.

‡According to the German Federal Office for Radiation Protection (www.bfs.de).

§23 mother–child pairs did not participate in the 1-year follow-up (entire LINA cohort *n* = 606, subcohort *n* = 367).

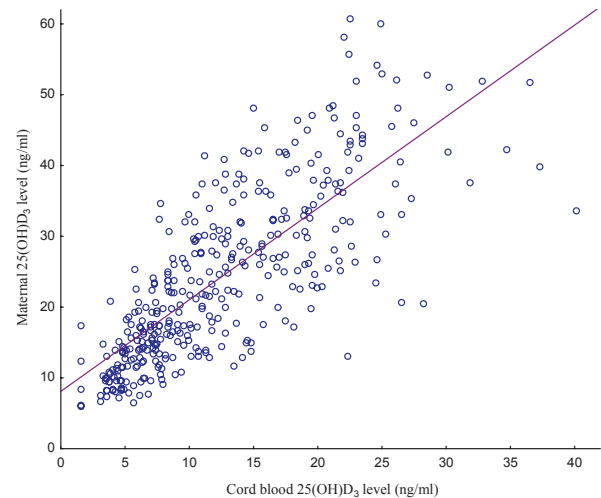
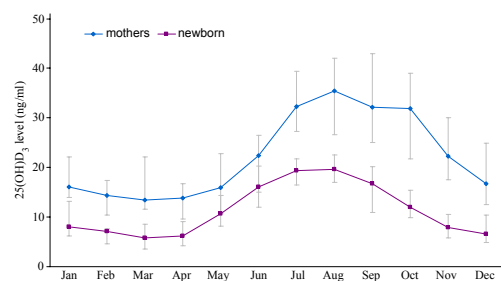
¶*P*-value from Student's *t*-test.

Vitamin D levels and outcomes

In a multivariate logistic regression model adjusted for known confounders (gender, number of siblings, increased cord blood total IgE levels, family atopy history, cotinine levels during pregnancy, breast feeding, UV intensity at birth and vitamin D supplementation within the first year of life), maternal 25(OH)D₃ levels were positively associated with questionnaire documented food allergy of the child (adj OR 3.66 [95% CI: 1.36–9.87] Table 4) within the second year of life or within the 2-year lifetime period (adj OR 1.91 [95% CI: 1.09–3.37]). Furthermore, maternal 25(OH)D₃ levels were positively associated with child's IgE specific to food

Table 2 Analysed maternal and cord blood 25(OH)D₃ concentrations

	Maternal 25(OH)D ₃		Cord blood 25(OH)D ₃	
	ng/ml (nM)	<i>N</i> = 374 <i>n</i> (%)	ng/ml (nM)	<i>N</i> = 378 <i>n</i> (%)
Median	22.2 (55.41)		10.95 (27.33)	
Quartiles				
1st	6.13–14.39	91 (24.3)	1.50–6.98	94 (24.9)
Quartile	(15.3–35.92)		(3.74–17.42)	
2nd	14.40–22.19	95 (25.4)	6.99–10.94	95 (25.1)
Quartile	(35.94–55.39)		(17.45–27.30)	
3rd	22.20–32.19	95 (25.4)	10.95–17.39	93 (24.6)
Quartile	(55.40–80.35)		(27.33–43.40)	
4th	32.20–60.80	93 (24.9)	17.40–40.10	96 (25.4)
Quartile	(80.37–151.76)		(43.43–100.10)	

**Fig. 2** Correlation between maternal and cord blood 25(OH)D₃ levels (ng/ml).**Fig. 3** Seasonal variation in 25(OH)D₃ levels (ng/ml) of mothers and newborns. Data are presented as medians with 25–75th percentile.

allergens (fx5) at the age of two (adj OR 1.59 [95% CI: 1.04–2.45]). Neither atopic eczema, symptomatic or physician diagnosed, nor total IgE levels and IgE levels specific to inhalant

Table 3 Prevalence of (a) atopic outcomes and (b) increased IgE levels within the first or second year of life or 2-year lifetime period of the child

	First year of life <i>N</i> = 272 % (<i>n</i>)	Second year of life <i>N</i> = 272 % (<i>n</i>)	Two-year lifetime period <i>N</i> = 272 % (<i>n</i>)
(a) Atopic outcomes			
Atopic eczema (symptoms)			
Positive	11.4 (31)	11.8 (32)	18.8 (51)
Negative	88.6 (241)	88.2 (240)	81.2 (221)
Atopic eczema (diagnosed)			
Positive	9.9 (27)	8.8 (24)	14.0 (38)
Negative	90.1 (245)	91.2 (248)	86.0 (234)
Food allergy (diagnosed)			
Positive	3.3 (9)	2.9 (8)	5.5 (15)
Negative	96.7 (263)	97.1 (264)	94.5 (257)
(b) IgE levels			
	First year of life <i>N</i> = 184 % (<i>n</i>)	Second year of life <i>N</i> = 191 % (<i>n</i>)	Two-year lifetime period <i>N</i> = 184 % (<i>n</i>)
Total IgE*			
Increased	78.3 (144)	82.2 (157)	87.5 (161)
Not increased	21.7 (40)	17.8 (34)	12.5 (23)
Food allergens (fx5) [†]			
Increased	15.8 (29)	13.1 (25)	22.3 (41)
Not increased	84.2 (155)	86.9 (166)	77.7 (143)
Inhalative allergens (sx1) [†]			
Increased	3.8 (7)	4.7 (9)	6.0 (11)
Not increased	96.2 (177)	95.3 (182)	94.0 (173)

*IgE cut-off: 3.8 kU/l.

[†]IgE cut-off: 0.35 kU/l (CAP-class >0).

allergens (sx1) showed a significant association with maternal vitamin D.

Similarly, cord blood 25(OH)D₃ was positively associated with questionnaire documented food allergy within the second year of life (adj OR 4.65 [95% CI: 1.50–14.48] Table 5). No association was found between cord blood vitamin D and infantile food allergy within the first year of life, as well as for atopic eczema, either symptomatic or physician diagnosed, total IgE or specific IgE (fx5 and sx1) levels at all times points.

Vitamin D level and cord blood regulatory T cells

Using samples from the LINA study, we have previously shown an association between the number of cord blood regulatory T cells and the occurrence of sensitizations against food allergens (fx5) later in life (18). We hypothesized that Treg may represent the mechanistic link between vitamin D and food allergy. Therefore, we have investigated in the present study whether maternal or cord blood 25(OH)D₃ was associated with cord blood Treg numbers. We observed a

negative correlation between cord blood vitamin D and cord blood Treg numbers ($R = -0.168$, $P = 0.031$; Fig. 4). For maternal vitamin D, this negative correlation was only seen in trend ($R = -0.143$, $P = 0.067$).

Discussion

In the present paper, we showed that maternal and cord blood 25(OH)D₃ levels are positively associated with children's risk for food allergy or sensitization against food allergens within the first 2 years of life. Furthermore, we found a negative correlation between cord blood 25(OH)D₃ levels and regulatory T cell numbers, which may be a contributing mechanism in the vitamin D-mediated allergy development.

In our study, the vitamin D status of pregnant mothers and their corresponding newborns was assessed in Leipzig, Germany. Majority of the pregnant mothers from our study had deficient (<20 ng/ml) or insufficient (20–29.9 ng/ml) 25(OH)D₃ levels, according to the suggested cut-offs (4). Also half of the newborns had deficient 25(OH)D₃ levels based on the cut-off level of <11 ng/ml for 25(OH)D₃ (23). Comparable data have already been shown in other nonequatorial regions such as United States (23, 24), New Zealand (25) and Australia (26, 27). Even in areas near the equator, characterized by high UV intensity, vitamin D deficiencies were reported, for example in Costa Rica (18.6°N) where 28% of children, between 6 and 14 years old, were found to have insufficient or deficient vitamin D levels (28).

In the LINA study, we found a high correlation between 25(OH)D₃ of pregnant mothers and their newborns, which was also observed in Pakistan (29) and Oakland (30) recently. This correlation is the result of the ability of 25(OH)D and 24,25(OH)D to cross the placenta, thereby exposing the foetus to vitamin D during pregnancy (31). Next to the seasonal variations, it was also obvious that absolute vitamin D concentrations of newborns are consistently lower than maternal levels, which confirms the observations published before (31).

In the present analyses, we also showed that higher maternal or cord blood 25(OH)D₃ levels were positively associated with a higher risk of developing food allergy or having increased food-specific IgE levels during the second year of life or within the 2-year lifetime period. Therefore, the risk for food allergy development resulting from cord blood 25(OH)D₃ levels was higher compared to that resulting from maternal levels. Thus, it seems that cord blood vitamin D level is more relevant for later food allergy than maternal vitamin D level. However, this could not be confirmed for IgE specific for food allergens.

There is lack of consistent data addressing the association between vitamin D and food allergy or IgE. It was shown that vitamin D is able to both enhance (32) and inhibit (33, 34) the differentiation of plasma cells and IgE production. Nwaru et al. (35) demonstrated in a Finish cohort that dietary intake of vitamin D during pregnancy was inversely associated with IgE specific to food allergens. Liu et al. analysed the impact of certain risk genotypes involved in vitamin

Table 4 Association between maternal 25(OH)D₃ and atopic outcomes or IgE levels of the child

		Maternal 25(OH)D ₃ , quartiles							
		n (%)				OR (95% CI)			
	N	1st	2nd	3rd	4th	Unadjusted	P-value	Adjusted*	P-value
<i>First year of life</i>									
Atopic eczema (symptoms)	272	10 (15.2)	8 (11.0)	6 (9.5)	7 (10.0)	0.85 (0.60–1.19)	0.340	0.89 (0.63–1.32)	0.614
Atopic eczema (diagnosed)	272	4 (6.1)	9 (12.3)	7 (11.1)	7 (10.0)	1.12 (0.78–1.60)	0.538	1.16 (0.79–1.71)	0.451
Food allergy (diagnosed)	272	1 (1.5)	3 (4.1)	2 (3.2)	3 (4.3)	1.26 (0.69–2.30)	0.458	1.27 (0.67–2.40)	0.469
Increased total IgE [†]	184	35 (76.1)	34 (75.6)	41 (87.2)	34 (73.9)	1.03 (0.75–1.41)	0.846	1.06 (0.77–1.47)	0.715
Increased food allergens (fx5) [‡]	184	7 (15.2)	10 (22.2)	6 (12.8)	6 (13.0)	0.89 (0.62–1.27)	0.509	0.90 (0.61–1.32)	0.585
<i>Second year of life</i>									
Atopic eczema (symptoms)	272	6 (9.1)	10 (13.7)	10 (15.9)	6 (8.6)	1.00 (0.72–1.39)	0.984	1.02 (0.72–1.43)	0.928
Atopic eczema (diagnosed)	272	4 (6.1)	9 (12.3)	5 (7.9)	6 (8.6)	1.03 (0.71–1.50)	0.861	1.13 (0.74–1.72)	0.584
Food allergy (diagnosed)	272	0 (0.0)	0 (0.0)	4 (6.3)	4 (5.7)	2.79 (1.14–6.82)	0.024	3.66 (1.36–9.87)	0.010
Increased total IgE [†]	191	40 (81.6)	38 (82.6)	42 (84.0)	37 (80.4)	0.99 (0.71–1.38)	0.940	0.97 (0.69–1.36)	0.853
Increased food allergens (fx5) [‡]	191	4 (8.2)	4 (8.7)	8 (16.0)	9 (19.6)	1.46 (0.98–2.16)	0.063	1.59 (1.04–2.45)	0.033
<i>Two-year lifetime period</i>									
Atopic eczema (symptoms)	272	14 (21.1)	15 (20.5)	11 (17.5)	11 (15.7)	0.88 (0.67–1.16)	0.354	0.93 (0.70–1.24)	0.629
Atopic eczema (diagnosed)	272	6 (9.1)	14 (19.2)	8 (12.7)	10 (14.3)	1.07 (0.79–1.46)	0.655	1.14 (0.81–1.61)	0.444
Food allergy (diagnosed)	272	1 (1.5)	3 (4.1)	5 (7.9)	6 (8.6)	1.66 (1.00–2.76)	0.052	1.91 (1.09–3.37)	0.025
Increased total IgE [†]	184	39 (84.8)	38 (84.4)	43 (91.5)	41 (89.1)	1.20 (0.81–1.79)	0.358	1.19 (0.79–1.78)	0.401
Increased food allergens (fx5) [‡]	184	10 (21.7)	10 (22.2)	12 (25.5)	9 (19.6)	0.98 (0.72–1.34)	0.909	1.04 (0.75–1.45)	0.812

*confounders: gender of the child, number of siblings, cord blood total IgE, family history for atopy, log cotinine (34th week of pregnancy), breast feeding, UV intensity of month of birth and vitamin D supplementation during the first year of life.

[†]IgE level > 3.8 kU/l.

[‡]IgE level > 0.35 kU/l (CAP-class >0).

All P-values < 0.005, which is in bold, are significant.

D metabolism and IgE regulation on the association between vitamin D levels and food allergen-specific IgE. Comparable to the results from our LINA study, they showed that low-risk children (<2 risk genotypes) with deficient cord blood vitamin D levels (<11 ng/ml) had a lower food allergy frequency compared to children with higher cord blood levels (23). Another group showed a nonlinear (U-shaped) association between cord blood vitamin D and IgE levels through the age of 5 years. In that study, both high (>40 ng/ml) and low (<20 ng/ml) vitamin D levels were associated with high total IgE and IgE specific to certain inhalant allergens (36). Furthermore, they described an association between high, but not low, vitamin D levels and positive skin prick tests (36). A similar nonlinear relationship was also shown in adults (37). In our study, we could not reproduce this U-shaped relationship between IgE levels and vitamin D but confirmed the association between high vitamin D and an increased risk for food allergy up to the age of two.

Data from the present study provide a mechanistic understanding for the increased food allergy risk resulting from high cord blood vitamin D levels. We observed a correlation between high cord blood vitamin D levels and low numbers of cord blood Treg, which are responsible for maintaining immune tolerance. Within our LINA study, we already found that low cord blood Treg numbers are associated with a higher risk to develop atopic outcomes later in life (18). Because a deficient Treg response at birth consequently

increases the risk for the development of an allergic immune response, our data suggest that vitamin D may modify the immune tolerance by affecting regulatory T cells. Our results are supported by an earlier birth cohort study that also showed cord blood vitamin D is negatively correlated with Treg numbers (38). Within the PASTURE study, a possible allergy-protective tolerance-inducing effect of vitamin D was suggested (39). Rochat et al. showed that prenatal vitamin D supplementation induces a tolerogenic phenotype of dendritic cells characterized by the expression of immunoglobulin-like transcripts (ILT)3 and ILT4. However, whether cord blood ILT3 and ILT4 mRNA levels are of relevance for the allergy risk later in life is unclear.

The strength of our study lies in the fact that the analysis of vitamin D was performed in corresponding mother–child pairs which are well characterized regarding immune parameters as well as for atopic outcomes and exposure variables. By coupling vitamin D measurements with immune parameters and disease outcomes, we were able to address the question of possible mechanisms responsible for the allergy-mediating effect of vitamin D. A weakness of the LINA study in general is the potential bias by high rates of participating atopic parents (65.9%) resulting in higher frequencies of children with atopic manifestations. We have considered this point by including family history of atopy as confounding variable in the regression models. One other limitation of the study is the low number of cases in certain

Table 5 Association between cord blood 25(OH)D₃ and atopic outcomes or IgE levels of the child

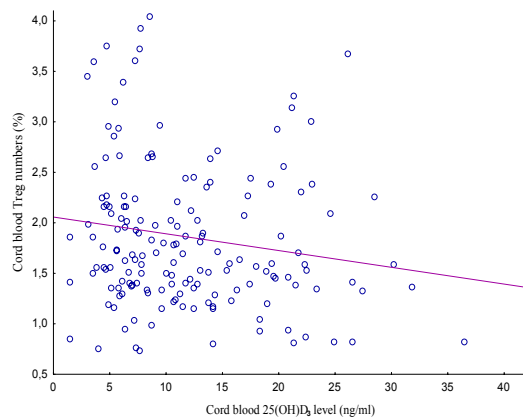
		Cord blood 25(OH)D ₃ , quartiles							
		<i>n</i> (%)				OR (95% CI)			
	<i>N</i>	1st	2nd	3rd	4th	Unadjusted	<i>P</i> -value	Adjusted*	<i>P</i> -value
<i>First year of life</i>									
Atopic eczema (symptoms)	272	12 (17.1)	6 (9.0)	5 (6.8)	8 (12.9)	0.85 (0.60–1.19)	0.346	0.94 (0.62–1.41)	0.756
Atopic eczema (diagnosed)	272	7 (10.0)	6 (9.0)	5 (6.8)	9 (14.5)	1.12 (0.78–1.61)	0.534	1.17 (0.76–1.81)	0.472
Food allergy (diagnosed)	272	3 (4.3)	1 (1.5)	3 (4.1)	2 (3.2)	0.98 (0.54–1.79)	0.950	0.92 (0.45–1.85)	0.812
Increased total IgE †	184	33 (73.3)	39 (84.8)	37 (77.1)	35 (77.8)	1.03 (0.75–1.42)	0.844	1.07 (0.74–1.54)	0.713
Increased food allergens (fx5) ‡	184	11 (24.4)	7 (15.2)	4 (8.3)	7 (15.6)	0.77 (0.54–1.11)	0.165	0.76 (0.49–1.18)	0.224
<i>Second year of life</i>									
Atopic eczema (symptoms)	272	7 (10.0)	11 (16.4)	6 (8.2)	8 (12.9)	1.00 (0.72–1.40)	0.992	0.98 (0.67–1.44)	0.926
Atopic eczema (diagnosed)	272	6 (8.6)	4 (6.0)	7 (9.6)	7 (11.3)	1.15 (0.79–1.69)	0.464	1.26 (0.78–2.02)	0.344
Food allergy (diagnosed)	272	0 (0.0)	1 (1.5)	3 (4.1)	4 (6.5)	2.49 (1.08–5.75)	0.032	4.65 (1.50–14.48)	0.008
Increased total IgE †	191	36 (76.6)	43 (87.8)	40 (80.0)	38 (84.4)	1.11 (0.79–1.56)	0.542	1.07 (0.73–1.58)	0.728
Increased food allergens (fx5) ‡	191	5 (10.6)	5 (10.2)	6 (12.0)	9 (20.0)	1.30 (0.88–1.92)	0.187	1.60 (0.99–2.57)	0.055
<i>Two-year lifetime period</i>									
Atopic eczema (symptoms)	272	17 (24.3)	14 (20.9)	8 (11.0)	12 (19.4)	0.84 (0.63–1.11)	0.217	0.91 (0.66–1.26)	0.569
Atopic eczema (diagnosed)	272	10 (14.3)	8 (11.9)	9 (12.3)	11 (17.7)	1.09 (0.80–1.48)	0.606	1.20 (0.81–1.76)	0.365
Food allergy (diagnosed)	272	3 (4.3)	2 (3.0)	5 (6.8)	5 (8.1)	1.34 (0.83–2.18)	0.234	1.70 (0.92–3.14)	0.089
Increased total IgE †	184	37 (82.2)	42 (91.3)	42 (87.5)	40 (88.9)	1.16 (0.78–1.72)	0.467	1.21 (0.77–1.91)	0.412
Increased food allergens (fx5) ‡	184	13 (28.9)	10 (21.7)	8 (16.7)	10 (22.2)	0.86 (0.63–1.18)	0.361	0.94 (0.65–1.37)	0.755

*Confounders: gender of the child, number of siblings, cord blood total IgE, family history for atopy, log cotinine (34th week of pregnancy), breast feeding, UV intensity of month of birth and vitamin D supplementation during the first year of life.

†IgE level > 3.8 kU/L.

‡IgE level > 0.35 kU/L.

All P-values < 0.005, which is in bold, are significant.

**Fig. 4** Correlation between cord blood 25(OH)D₃ levels (ng/ml) and cord blood Treg numbers (%)

outcomes. Main results in this study were obtained regarding food allergy. For this outcome, we only use the questionnaire information regarding a physician-diagnosed food allergy without having data on provocation tests. This might reduce the strengths of the reported results. Therefore, the presented results have to be interpreted with caution and need further validation. However, by including

immune and IgE data, we may overcome this limitation at least in part.

Conclusion

Many pregnant mothers worldwide were shown to have insufficient or deficient 25(OH)D concentrations, which subsequently result in insufficient or deficient vitamin D levels in the newborn. Currently, results addressing the impact of vitamin D deficiency on newborns' immune status and the risk of developing allergic diseases in early infancy have been controversial and lacking. The present analyses showed a positive association between maternal or cord blood vitamin D level and risk for food allergy in infants up to 2 years of age, suggesting that a high vitamin D level is a risk for the development of allergic diseases in early infancy. Furthermore, our data demonstrate that this association could be explained with an inhibition of regulatory T cell numbers at birth. As a clinical conclusion, we would not recommend to supplement with vitamin D to protect against allergy development because the results from our study indicate that low vitamin D levels are not necessarily associated with an increased risk for outcome development. Another fact pointing against vitamin D supplementation is its failing impact on blood 25(OH)D levels in the present analyses. In any case, it is of high relevance to clarify how vitamin D in pre- or neonatal period is

able to modify programming of future immune function and therefore atopic outcomes later on.

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Author contribution

Each named author has substantially contributed to this paper. Gunda Herberth, Mario Bauer, Stefan Röder, Michael Borte, Ulrike Diez and Irina Lehmann were involved in the development of the study design and the field work. Denise Hinz, Gunda Herberth, Mario Bauer and Sven Olek performed the *FOXP3* TSDR demethylation measurement. Frank Hirche, Sophie Winkler and Gabriele Stangl performed

the vitamin D measurements, and Ulrike Rolle-Kampczyk and Martin von Bergen performed the cotinine analyses. Kristin Weisse, Sophie Winkler, Stefan Röder, Gunda Herberth and Irina Lehmann contributed to the statistical analysis. Ulrich Sack contributed with IgE measurements and discussion of the IgE data. Ulrike Diez, Thomas Richter, Frank Hirche and Gabriele Stangl contributed to the discussion of vitamin D levels in mothers and newborns. Kristin Weisse, Sophie Winkler and Irina Lehmann wrote the paper; all authors were involved in the revision of the final text.

Conflicts of interest

All authors have declared that they have no conflict of interest.

Supporting Information

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

Data S1. Methods.

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Supplements

Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study

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METHODS:

Analyses of urinary cotinine concentration

Cotinine was analyzed in the urine of pregnant mothers (34th week). After dichloromethane extraction and chromatographic separation with a Chromolith Speed ROD column (Merck, Darmstadt, Germany) cotinine was determined using turbo ionspray ionization on the LC/MS/MS device API 4000 (Applied Biosystems). Analysis of creatinine as a measure for individual urinary dilution was used for standardization of the metabolite.