

Serum 25-Hydroxyvitamin D in Early Childhood Is Nonlinearly Associated with Allergy

Emma Merike Savilahti^a Outi Mäkitie^{a, c, d} Anna Kaarina Kukkonen^b
Sture Andersson^a Heli Viljakainen^a Erkki Savilahti^a Mikael Kuitunen^a

^aChildren's Hospital and ^bHospital for Allergic and Skin Diseases, Helsinki University Hospital and University of Helsinki, and ^cFolkhälsan Institute of Genetics, Helsinki, Finland; ^dDepartment of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Key Words

25-Hydroxyvitamin D · Allergy · Probiotics · Sensitization · Umbilical cord blood

Abstract

Background: Vitamin D has several immunological functions. Data on the relation of vitamin D status and allergy are controversial. **Methods:** We investigated the association between serum concentrations of 25-hydroxyvitamin D (25-OHD) and allergy in childhood. The study population (n = 819) was part of a randomized, double-blind, placebo-controlled trial where the mothers of offspring with a high risk for allergy received a mixture of probiotics (or placebo) for the last 4 weeks of pregnancy, and the child received this from birth to 6 months. Study subjects were followed for the emergence of sensitization and allergic symptoms for a period of 5 years, with medical examinations at the ages of 3 and 6 months, 2 and 5 years and also in the event of allergic symptoms. Levels of 25-OHD were measured in umbilical cord blood (UCB) samples (n = 724) and serum samples drawn at the age of 2 years (n = 369); the data were categorized into tertiles (T1–T3) and quartiles (Q1–Q4). The relation between 25-OHD levels and sensitization and allergy was analyzed with multivariable logistic regression analysis. **Re-**

sults: 25-OHD levels in T2 in UCB were associated with a higher risk for sensitization by the age of 2 years and allergic disorders by the age of 5 years. In the serum samples, at the age of 2 years, 25-OHD levels in Q3 were associated with a higher risk of sensitization and IgE-mediated allergies by the age of 5 years. **Conclusions:** The 25-OHD levels in early childhood are associated with the emergence of allergy, but the association appears to be nonlinear.

© 2016 S. Karger AG, Basel

Introduction

The rapid increase in allergy prevalence in developed countries has coincided with lifestyle and dietary changes that affect vitamin D status, namely decreased sunlight exposure and increased nutritional vitamin D supplementation. Several studies have suggested that the incidence and prevalence of allergy are inversely associated with the amount of sunlight exposure and thus possibly cutaneous vitamin D₃ synthesis [1–3], but this association may be independent of serum 25-hydroxyvitamin D (25-

Clinical trial registration No. NCT00298337.

OHD) levels [4]. Vitamin D supplementation in infancy, on the other hand, has been found to be associated with a higher prevalence of sensitization and allergic rhinitis in adulthood [5].

The accumulating evidence on the multiple immunological functions of vitamin D [6] has further spurred interest in the hypothesis that vitamin D status influences the emergence of allergy. Several studies have investigated the association between vitamin D status, most accurately estimated by measuring the 25-OHD levels in serum, at birth and the emergence of allergy in the child. They have found either no association with allergic disorders [7–9] or rather an association between low 25-OHD levels and an increased prevalence of eczema [10, 11]. A randomized controlled trial (n = 180) found that vitamin D supplementation during late pregnancy had no effect on the occurrence of wheezing, atopy, eczema or food allergy in the child by the age of 3 years [12]. Cross-sectional studies on children have either reported inverse associations with 25-OHD serum levels and atopic dermatitis [13] or food allergy [14], or no association [15].

Since prospective studies on the association between serum 25-OHD levels in early life and emergence of allergy in childhood are still scarce and conflicting, we investigated, in a prospectively followed cohort of 819 healthy, full-term infants with atopic heredity, whether serum 25-OHD levels at birth or at the age of 2 years was associated with the emergence of sensitization and allergic disorders during the first 5 years of life.

Material and Methods

Our study was based on a randomized, double-blind, allergy prevention trial on pro- and prebiotics. The trial included 1,223 pregnant women who carried fetuses with a high risk for allergy, i.e. one or both parents had doctor-diagnosed allergic disease (flow chart in the suppl. material, for all online suppl. material, see www.karger.com/doi/10.1159/000447636). The protocol and the main clinical findings have been published [16, 17]. The main results were that probiotic treatment was associated with reduced incidence of eczema and especially IgE-mediated (atopic) eczema at the age of 0–2 years [16] whereas by the age of 5 years, the treatment was associated with less frequent IgE-mediated allergy only in children delivered by caesarean section [17]. We also published the associations of environmental and hereditary factors and the development of allergic diseases by the ages of 2 and 5 years in the whole study group [18].

The ethics committee of the Hospital for Children and Adolescents of Helsinki University gave its approval for the study protocol. Mothers signed a written informed consent.

Children came to the outpatient clinic for examination at the ages of 3 and 6 months and then at 2 and 5 years and also if par-

ents reported that their child showed allergic symptoms. Primary outcome measures were the cumulative incidence of any allergic disease and any IgE-mediated (atopic) allergic disease until the age of 2 or 5 years. Secondary outcome measures were eczema, food allergy and asthma with and without IgE sensitization, allergic rhinitis with sensitization and IgE sensitization alone. Eczema diagnosis was based on the UK Working Party's criteria [19], and eczema was designated to be IgE-associated when it was associated with sensitization. Food allergy diagnosis was based on an open food challenge [20], except in the case of doctor-diagnosed anaphylaxis. Diagnostic criteria for asthma were ≥ 2 doctor-diagnosed wheezy episodes coinciding with persistent cough or exercise-induced symptoms between episodes, or with bronchial hyper-responsiveness on oscillometry. Allergic rhinitis was diagnosed in the case of ≥ 2 recurrent symptoms (nasal discharge, blockage and sneeze/itch) during contact with a specific antigen to which the patient was sensitized [21]. Children underwent skin prick tests of a panel of food and inhalant allergens applied with a standard technique [22]. We measured in sera allergen-specific IgE sensitization to cow's milk, egg white, birch, Timothy grass, dog and cat dander and at 5 years also sensitization to wheat, fish, peanut, mugwort and *Dermatophagoides pteronyssinus* with an immunoassay (ImmunoCAP® system, Pharmacia Diagnostics, Uppsala, Sweden). Sensitization was defined as ≥ 1 positive skin prick test results (≥ 3 mm larger than the negative control) and/or a serum antigen-specific IgE level >0.7 kU/l to the allergens tested. In cases where allergic symptoms coincided with sensitization, the child was considered to have IgE-mediated allergy.

At each visit, the height and weight of the participants were measured. We also asked about the occurrence of infections and medications; the same information was collected by questionnaire at the ages of 1, 2, 3, 4 and 5 years.

Umbilical cord blood (UCB) samples were collected at the maternity hospitals and serum samples at the scheduled appointment when the children were 2 years old. Samples were kept frozen in -80°C until analysis. We included all available samples from the 819 individuals. UCB samples were available for 724 children and serum samples at the age of 2 years (2-year serum samples) were available for 369 children. Both UCB and 2-year serum samples were available for 275 individuals, only UCB was available for 450 individuals and only 2-year serum samples for 94 individuals (flow chart in suppl. material).

25-OHD Measurement

25-OHD was measured in UCB and serum samples with an automated IDS-iSYS analyzer (IDS Ltd., Boldon, UK). The measurements were performed in the Pediatric Research Centre Laboratory in Biomedicum (Helsinki, Finland); the intra-assay coefficient of variation was $<7\%$ and the interassay coefficient of variation was $<9\%$ for serum 25-OHD. A concentration below the detection limit (13 nmol/l) was recorded as 6 nmol/l and was included in the data analysis.

Reproducibility was ensured by adhering to the Vitamin D External Quality Assessment Scheme. Vitamin D status was defined as deficient when serum 25-OHD was ≤ 37.5 nmol/l, insufficient when serum 25-OHD was between 37.5 and 50 nmol/l and sufficient when serum 25-OHD was >50 nmol/l, according to the reference values for the pediatric population [23].

Table 1. The incidence of sensitization and allergic disorders in relation to potential confounding factors in the study population (n = 819)

		Sensitization at 0–2 years	Sensitization at 0–5 years	Allergic disorder at 0–5 years	IgE-mediated allergic disorder at 0–5 years	IgE-mediated eczema at 0–5 years
Treatment group	placebo	31.6%	42.0%	56.2%	33.8%	25.3%
	probiotic	27.5%	40.9%	53.3%	33.2%	23.7%
Sex	female	28.2%	38.9%	53.8%	31.4%	24.0%
	male	30.9%	44.1%	55.8%	35.6%	25.1%
Both parents allergic	no	25.7%	37.7%	48.5%	28.3%	20.1%
	yes	35.5%	47.3%	64.2%	41.3%	31.2%
Mode of delivery	vaginal	30.1%	42.2%	53.9%	33.8%	24.8%
	C-section	26.8%	37.3%	59.8%	31.7%	22.9%
Season of birth	dark ^a	31.1%	42.4%	56.8%	34.6%	24.9%
	sunny ^b	28.1%	40.6%	52.9%	32.4%	24.1%
Season when 2-year serum sample taken	dark ^a	31.7%	43.7%	58.6%	36.5%	28.1%
	sunny ^b	27.6%	39.6%	51.1%	30.8%	21.5%
Has siblings	no	27.3%	40.7%	51.1%	31.4%	23.8%
	yes	32.7%	42.6%	59.9%	36.5%	25.5%
Time exclusively breastfed, months	0–2.99	28.7%	41.4%	50.9%	32.5%	22.5%
	>3.0	30.8%	41.2%	58.9%	34.1%	26.1%
Total time breastfed, months	0–7.99	27.0%	39.9%	52.9%	29.9%	20.0%
	>8.0–24.0	32.4%	42.8%	56.6%	36.7%	28.2%
Smoking in the household (at age 0–2 years)	no	29.7%	42.0%	54.2%	33.9%	25.4%
	yes	29.3%	40.2%	56.3%	32.6%	22.6%
Cat or dog in the household (at age 0–2 years)	no	31.1%	42.5%	55.8%	34.7%	25.0%
	yes	22.9%	37.2%	50.3%	28.4%	22.3%

Figures in bold type denote $p \leq 0.1$, Pearson's independent χ^2 test.

^a November to April; ^b May to October.

Statistical Analyses

The distribution of 25-OHD levels was skewed to the right, and therefore logarithmic transformation was used when the data were analyzed as a continuous variable. Since previous studies have reported several different patterns of association between 25-OHD levels and allergy, we tested the associations using 25-OHD level as a continuous variable and as categorized into tertiles (T1–T3) and quartiles (Q1–Q4), or <28 nmol/l or ≥ 28 nmol/l for UCB samples, or <50 nmol/l or ≥ 50 nmol/l for 2-year samples. The change in 25-OHD from birth to 2 years was analyzed as an absolute and as a proportional change and as a continuous and as a categorized change. Out of the ample data gathered in the original clinical trial, 18 clinical outcomes and 11 potential confounding factors (table 1) were chosen for statistical testing, based on previous research data. The clinical main outcomes were cumulative frequencies of sensitization, any allergic disorder and any IgE-mediated allergic disorder (allergic disorder with sensitization) as well as eczema, atopic eczema (eczema with sensitization), asthma, atopic asthma, food allergy, IgE-mediated food allergy at 0–2 and 0–5 years and, for the main outcomes, also at 2–5 years. The associations between the cumulative frequencies of clinical outcomes at the age of 0–2–5 and 2–5 years between the different 25-OHD categories as well as between the potential confounding factors were first screened with univariate analysis (Pearson's independent χ^2 test). If the univariate

test resulted in a p value ≤ 0.1 , the variable was included in the multivariable logistic regression analysis. Since the population was derived from a clinical trial concerning the use of probiotics, the treatment group was forced into all multivariate analyses. The results are given as adjusted ORs with 95% CIs.

We also compared the trajectory of 25-OHD levels with the ANOVA test for repeated measures. The association between 25-OHD levels and the following general health indicators were also tested: height (as standard deviation) and weight (as % for height) at the ages of 6 months, 2 years and 5 years, and the number of respiratory infections and the number of antibiotics regimens at 0–2 years. Analyses were performed with IBM SPSS Statistics for Windows (v22.0, IBM Corp., Armonk, N.Y., USA). For the final results, $p < 0.05$ was considered significant.

Results

The distributions of 25-OHD levels in UCB and in serum at the age of 2 years are shown in figure 1. The median was 25.0 nmol/l in the UCB samples and 51.6 nmol/l in sera drawn at 2 years. At birth, 83% of the 25-OHD levels were

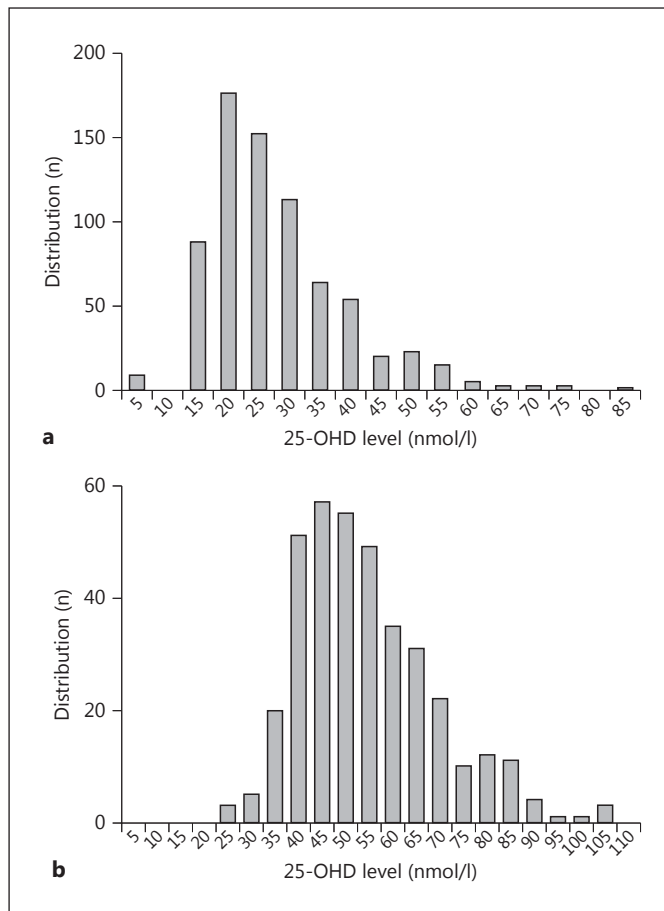


Fig. 1. Distribution (n) of 25-OHD levels in UCB (a) and serum (b) at the age of 2 years.

consistent with vitamin D deficiency (≤ 37.5 nmol/l) and 12% with vitamin D insufficiency (37.5–50.0 nmol/l), while the corresponding proportions at 2 years were 8 and 37%. Levels were >80 nmol/l in 0.1% at birth and in 7% at 2 years.

The incidences of sensitization and allergic disorders (%) in relation to potential confounding factors in the study population are shown in table 1.

Children in the study population were evenly born in the sunny (May until October; $n = 419$; 51%) and dark (November until April; $n = 400$; 49%) seasons. Seasonal variation was significant ($p < 0.001$, in the t test of independent samples) in the UCB samples: 25-OHD levels were higher in the sunny season (geometric mean 29.7 nmol/l, SD 12.3; $n = 366$) than in the dark season (geometric mean 22.0 nmol/l, SD 7.5; $n = 358$). Samples drawn at the age of 2 years were also evenly distributed across the 2 seasons, and showed a statistically nonsignificant ($p = 0.09$, in the t test of independent samples) seasonal difference in 25-OHD

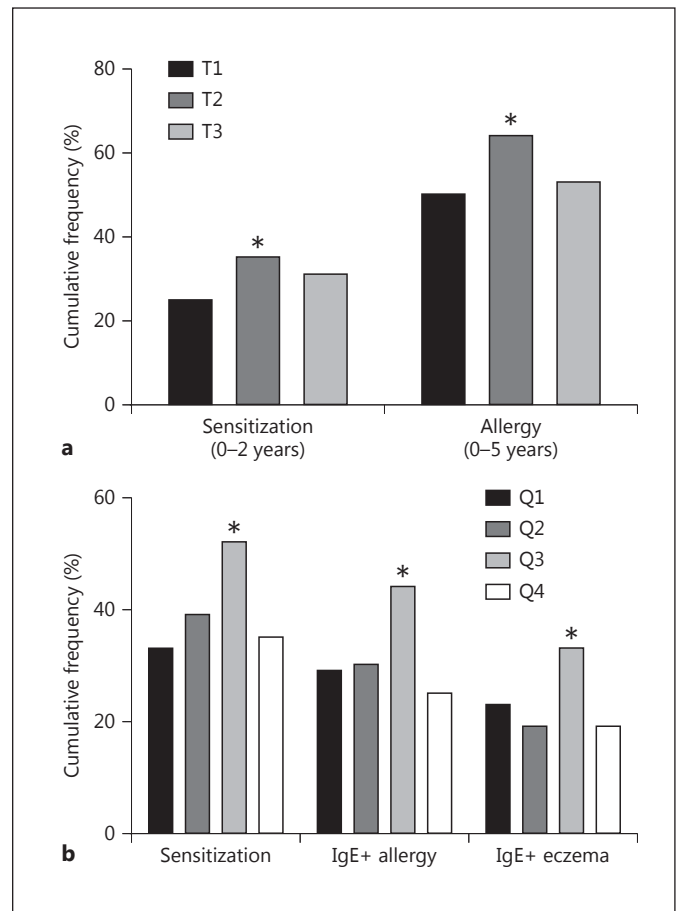


Fig. 2. a Cumulative frequency (%) of sensitization at the age of 0–2 years and any allergic disorder (allergy) at the age of 0–5 years in groups categorized as tertiles of 25-OHD levels in UCB. **b** Cumulative frequency (%) of sensitization, any IgE-associated (IgE+) allergic disorder (allergy) and IgE-associated eczema at the age of 0–5 years in groups categorized as quartiles of 25-OHD levels in serum at the age of 2 years. * $p < 0.05$, denotes a significant difference (χ^2 test).

levels: in the sunny season, the geometric mean was 53.7 nmol/l (SD 13.6; $n = 199$) and in the dark season, the geometric mean was 51.3 nmol/l (SD 15.6; $n = 171$). At the age of 2 years, 97% of the children used vitamin D supplementation according to their parents' reporting.

25-OHD levels in UCB and in serum at the age of 2 years were nonlinearly associated with sensitization and allergy in childhood. Figure 2 shows the associations that were statistically significant in multivariate analyses. Levels of 25-OHD in UCB were categorized as T1: 6.0–21.4 nmol/l ($n = 232$), T2: 21.5–29.5 nmol/l ($n = 233$) and T3: 29.6–84.8 nmol/l ($n = 234$; fig. 2a). Levels of 25-OHD in serum at the age of 2 years were categorized as Q1: 26.0–43.4 nmol/l

Table 2. Multivariable logistic regression analysis for allergy outcomes

Allergy outcome	Explanatory variables ^a	OR	95% CI	p
Sensitization at 0–2 years	25-OHD in UCB ^b			0.08
	T2 (21.5–29.5 nmol/l)	1.59	1.06–2.39	0.03
	T3 (29.6–84.8 nmol/l)	1.36	0.90–2.06	0.14
	probiotic treatment group	0.89	0.64–1.24	0.50
	both parents allergic	1.59	1.14–2.22	0.01
	has siblings	1.30	0.94–1.82	0.12
	total breastfeeding ≥8 months	1.19	0.85–1.66	0.31
	cat or dog ownership at 0–2 years	0.79	0.50–1.25	0.31
Allergic disorder at 0–5 years	25-OHD in UCB ^b			0.01
	T2 (21.5–29.5 nmol/l)	1.85	1.25–2.73	0.002
	T3 (29.6–84.8 nmol/l)	1.30	0.87–1.94	0.20
	probiotic treatment group	0.98	0.71–1.34	0.88
	both parents allergic	1.94	1.40–2.67	<0.001
	has siblings	1.50	1.09–2.06	0.01
	exclusive breastfeeding >3 months	1.32	0.96–1.82	0.08
	sample taken at age 2 years ^d	0.70	0.51–0.98	0.03
Sensitization at 0–5 years	25-OHD at 2 years ^c			0.04
	Q2 (43.6–51.5 nmol/l)	1.31	0.70–2.46	0.39
	Q3 (51.7–62.6 nmol/l)	2.23	1.21–4.12	0.01
	Q4 (62.8–106.8 nmol/l)	1.07	0.56–2.01	0.84
	both parents allergic	1.82	1.16–2.84	0.01
	probiotic treatment group	0.91	0.59–1.41	0.68
IgE-associated allergic disorder at 0–5 years	25-OHD at 2 years ^c			0.02
	Q2 (43.6–51.5 nmol/l)	1.29	0.65–2.56	0.46
	Q3 (51.7–62.6 nmol/l)	2.35	1.22–4.52	0.01
	Q4 (62.8–106.8 nmol/l)	0.96	0.48–1.93	0.91
	both parents allergic	2.32	1.44–3.75	0.001
	probiotic treatment group	1.01	0.63–1.62	0.96
	total breastfeeding ≥8 months	1.18	0.73–1.90	0.50
IgE-associated eczema at 0–5 years	sample taken at age 2 years ^d	0.53	0.33–0.86	0.01
	25-OHD at 2 years ^c			0.06
	Q2 (43.6–51.5 nmol/l)	0.99	0.46–2.13	0.98
	Q3 (51.7–62.6 nmol/l)	2.06	1.02–4.17	0.04
	Q4 (62.8–106.8 nmol/l)	0.90	0.42–1.94	0.78
	both parents allergic	2.51	1.49–4.24	0.001
	probiotic treatment group	0.84	0.50–1.42	0.52
	total breastfeeding ≥8 months	1.41	0.83–2.40	0.21
	sample taken at age 2 years ^d	0.47	0.28–0.80	0.01

The 25-OHD levels and background characteristics were considered as explanatory variables. The 25-OHD level in UCB was divided into 3 categories (T1–T3, each containing a third of the population) and the 2-year serum levels into 4 categories (Q1–Q4, each containing a quarter of the population). Figures in bold type denote statistically significant differences.

^a Variables with a p value <0.10 in the univariate analysis were included in the model.

^b The lowest-concentration category T1 (6.0–21.4 nmol/l) is the reference group with an OR of 1.00.

^c The lowest-concentration category Q1 (26.0–43.4 nmol/l) is the reference group with an OR of 1.00.

^d In the sunny season, i.e. May to October.

(n = 87), Q2: 43.6–51.5 nmol/l (n = 88), Q3: 51.7–62.6 nmol/l (n = 92) and Q4: 62.8–106.8 nmol/l (n = 86; fig. 2b).

In the UCB samples, T2 was associated with a higher risk of sensitization by the age of 2 years and of allergic disorder by the age of 5 years (table 2). Moreover, 25-

OHD levels >28 nmol/l were associated with a higher risk for IgE-associated allergic disorder by the age of 2 years (OR 1.68, 95% CI 1.12–2.51; p = 0.012).

Q3 at 2 years was associated with a higher risk for sensitization as well as IgE-mediated allergic disorders and

also IgE-mediated (atopic) eczema alone by the age of 5 years (table 2).

25-OHD levels at birth and at 2 years were not associated with height or weight at the ages of 6 months, 2 years and 5 years (data not shown). Nor were they associated with the number of respiratory infections or of antibiotic regimens at 0–2 years (data not shown).

Both UCB and 2-year serum samples were available for 275 children. Pearson's correlation between 25-OHD levels at these 2 time points was 0.14 ($p < 0.02$). Data on the emergence of allergy by the age of 5 years were available for 263 of these children. A change in 25-OHD levels between birth and 2 years was not significantly associated with any of the clinical outcomes and nor was the trajectory (data not shown).

Discussion

This prospective study shows that 25-OHD levels at birth and at the age of 2 years were nonlinearly associated with the incidence of sensitization and allergic disorders up to the age of 5 years, even when potential confounding factors were taken into consideration. The highest risk for allergy was associated with T2 and Q3 in the 2-year samples.

Our study has an advantage over previous studies of not only a considerable size but, notably, 25-OHD measurements at 2 time points. Furthermore, diagnostics were based not only on a questionnaire and laboratory tests but on a doctor's examination at the ages of 3 and 6 months and 2 and 5 years. Since all the participants enrolled in the trial had at least 1 allergic parent, the incidence of sensitization and allergic disorders was relatively high, thus yielding high statistical power. This may, on the other hand, limit the application of our results to the general population. We were able to take into account numerous potential confounding factors that had mostly been recorded with little or no delay. A limitation of the study is that detailed information on the vitamin D intake of the mother and child was not available. On the other hand, maternal concentration of 25-OHD at delivery correlates strongly with 25-OHD in UCB [24]. The seasonal variation and low 25-OHD levels in UCB suggests that vitamin D supplementation was not widely used by the mothers during pregnancy. Their children were more frequently supplemented, as reported by their parents, and going by the improved 25-OHD levels and lack of clear seasonal variation in serum 25-OHD in the 2-year-old study participants. The Finnish national guidelines at the

time recommended daily supplementation with 10 µg ergocalciferol orally to all children from the age of 2 weeks to 2 years. According to a Finnish study that took place in the same period as our data was collected, 86% of the children followed the national recommendation at the age of 1 year and 70% at the age of 2 years, with an average daily vitamin D intake of 12.2 and 8.8 µg, respectively [25]. At the time, there was no general recommendation of vitamin D supplementation for pregnant women.

The result that the highest risk for sensitization and allergic disorders was associated with UCB 25-OHD levels in T2 differs from previous studies. Several studies have found no association between UCB 25-OHD levels and sensitization or food allergy in infancy [10], or asthma [7], eczema [25] or asthma and allergic rhinitis [8, 11] during the first 5 years of life. Some studies have reported an association between low UCB 25-OHD levels and eczema during early childhood [10, 11]. One reported a positive association between UCB 25-OHD levels and food allergy in infancy [25]. Another reported that high levels of vitamin D in mothers in late pregnancy were associated with a higher incidence of eczema and asthma in children than lower levels [26]. One study found a nonlinear association between UCB 25-OHD levels and detectable inhalant allergen-specific IgE [8].

Our observation that serum 25-OHD levels at the age of 2 years are nonlinearly associated with the incidence of sensitization and IgE-mediated allergic disorders by the age of 5 years is difficult to compare with previous research data since prospective data on serum 25-OHD levels after birth and the emergence of allergy are scarce. A recent prospective study investigated the association between food sensitization and serum 25-OHD levels at several time points in early childhood and reported no association at any single time point, but persistently low serum 25-OHD levels were associated with an elevated risk for food sensitization [27]. Cross-sectional studies have reported variable associations [28–30].

Several factors, such as heterogeneity in the diagnostics, the timing of 25-OHD analysis and variation in the length of follow-up may explain some of the discrepancies between studies. Genotype can play a decisive role [27, 30]. Our study population was at a high risk for allergy. This could also explain the finding that having siblings conferred an increased risk for allergic disorder at the age of 0–5 years, contrary to the findings of studies on more general populations [31]. The association between 25-OHD levels and allergy may also depend on whether vitamin D intake is mainly through UVB radiation, diet or supplementation. It is also possible that statistical anal-

yses in some studies have been based on the assumption of a linear association and may have missed significant nonlinear associations. The variation in the distribution of serum 25-OHD levels complicates comparisons between studies. In our pediatric study population, 25-OHD levels were low overall compared with the aforementioned studies on allergy and with more recent reports on serum 25-OHD levels in Finnish pediatric populations [32, 33]. The latter is most likely due to improved maternal vitamin D supplementation during pregnancy and the fortification of dairy products including infant milks with vitamin D since 2003.

The association between serum 25-OHD levels at birth as well as at the age of 2 years and the incidence of sensitization and allergic disorders by the age of 5 years was nonlinear: the highest incidence was associated with T2 in the UCB samples and Q3 in the 2-year serum samples. This could reflect the fact that vitamin D can promote tolerogenic processes [34–36], but also induce a shift to Th2-type reactions [37] and reduce the proliferation of T regulatory cells [38]. Reports on mouse studies suggest that topically applied vitamin D at high concentrations induces atopic eczema [39] but that at lower concentrations it rather inhibits the proinflammatory responses of mast cells [36]. The vitamin D dose required for the activation of different immunological functions plausibly varies,

and the balance of these functions could determine whether an allergic reaction occurs, thus resulting in a nonlinear association. Different phenotypes' response to vitamin D is also a possible explanation. Discrepancies between studies on the association of vitamin D status and allergy may, on the other hand, indicate that despite, or rather because of, the variety in the immunological functions of vitamin D, no direct causal link exists between vitamin D and the emergence of sensitization and allergy.

Our results infer that serum 25-OHD levels at birth and in early childhood are associated with the emergence of sensitization and allergy, and that the association is nonlinear. The possibility of a nonlinear association between vitamin D status and allergy should be considered in further studies.

Acknowledgements

We are grateful to Professor Tari Haahtela from the Skin and Allergy Hospital, University of Helsinki, for his valuable contribution in the clinical trial. We warmly thank Sari Lindén for her excellent technical assistance and Tuija Poussa for performing the statistical analysis. We thank Helsinki University Central Hospital Research Funds, the Academy of Finland, the Sigrid Juselius Foundation, the Foundation for Pediatric Research and Valio Ltd., Helsinki, Finland, for funding the study.

References

- ▶ 1 Camargo CA Jr, Clark S, Kaplan MS, Lieberman P, Wood RA: Regional differences in EpiPen prescriptions in the United States: the potential role of vitamin D. *J Allergy Clin Immunol* 2007;120:131–136.
- ▶ 2 Sheehan WJ, Graham D, Ma L, Baxi S, Phipatanakul W: Higher incidence of pediatric anaphylaxis in northern areas of the United States. *J Allergy Clin Immunol* 2009;124:850–2.e2.
- ▶ 3 Wjst M, Dharmage S, Andre E, Norback D, Raherison C, Villani S, et al: Latitude, birth date, and allergy. *PLoS Med* 2005;2:e294.
- ▶ 4 Kemp AS, Ponsonby AL, Pezic A, Cochrane JA, Dwyer T, Jones G: The influence of sun exposure in childhood and adolescence on atopic disease at adolescence. *Pediatr Allergy Immunol* 2013;24:493–500.
- ▶ 5 Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, et al: Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. *Ann NY Acad Sci* 2004;1037:84–95.
- ▶ 6 Priel B, Treiber G, Pieber TR, Amrein K: Vitamin D and immune function. *Nutrients* 2013;5:2502–2521.
- ▶ 7 Camargo CA Jr, Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, et al: Cord blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011;127:e180–e187.
- ▶ 8 Rothers J, Wright AL, Stern DA, Halonen M, Camargo CA Jr: Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J Allergy Clin Immunol* 2011;128:1093–9.e1–5.
- ▶ 9 Chawes BL, Bonnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H: Cord blood 25(OH)-vitamin D deficiency and childhood asthma, allergy and eczema: the COP-SAC2000 birth cohort study. *PLoS One* 2014;9:e99856.
- ▶ 10 Jones AP, Palmer D, Zhang G, Prescott SL: Cord blood 25-hydroxyvitamin D₃ and allergic disease during infancy. *Pediatrics* 2012;130:e1128–e1135.
- ▶ 11 Baiz N, Dargent-Molina P, Wark JD, Souberbielle JC, Annesi-Maesano I; EDEN Mother-Child Cohort Study Group: Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *J Allergy Clin Immunol* 2014;133:147–153.
- ▶ 12 Goldring ST, Griffiths CJ, Martineau AR, Robinson S, Yu C, Poulton S, et al: Prenatal vitamin D supplementation and child respiratory health: a randomised controlled trial. *PLoS One* 2013;8:e66627.
- ▶ 13 Wang SS, Hon KL, Kong AP, Pong HN, Wong GW, Leung TF: Vitamin D deficiency is associated with diagnosis and severity of childhood atopic dermatitis. *Pediatr Allergy Immunol* 2014;25:30–35.
- ▶ 14 Allen KJ, Koplin JJ, Ponsonby AL, Gurrin LC, Wake M, Vuillermin P, et al: Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013;131:1109–1116, 1116.e1–e6.

- ▶ 15 Yao TC, Tu YL, Chang SW, Tsai HJ, Gu PW, Ning HC, et al: Suboptimal vitamin D status in a population-based study of Asian children: prevalence and relation to allergic diseases and atopy. *PLoS One* 2014;9:e99105.
- ▶ 16 Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al: Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:192–198.
- ▶ 17 Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al: Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;123:335–341.
- ▶ 18 Sandini U, Kukkonen AK, Poussa T, Sandini L, Savilahti E, Kuitunen M: Protective and risk factors for allergic diseases in high-risk children at the ages of two and five years. *Int Arch Allergy Immunol* 2011;156:339–348.
- ▶ 19 Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, et al: The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994;131:383–396.
- ▶ 20 Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, et al: Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. *Allergy* 1995;50:623–635.
- ▶ 21 International Rhinitis Managing Working Group: International consensus report on the diagnosis and management of rhinitis. *Allergy* 1994;49(suppl 19):1–34.
- ▶ 22 The European Academy of Allergology and Clinical Immunology: Position paper: allergen standardization and skin tests. *Allergy* 1993;48(suppl 14):48–82.
- ▶ 23 Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society: Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics* 2008;122:398–417.
- ▶ 24 Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, et al: Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* 2013;68:220–228.
- ▶ 25 Kyttälä P, Erkkola M, Kronberg-Kippilä C, Tapanainen H, Veijola R, Simell O, et al: Food consumption and nutrient intake in Finnish 1–6-year-old children. *Public Health Nutr* 2010;13:947–956.
- ▶ 26 Gale CR, Robinson SM, Harvey NC, et al: Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008;62:68–77.
- ▶ 27 Liu X, Arguelles L, Zhou Y, Wang G, Chen Q, Tsai HJ, et al: Longitudinal trajectory of vitamin D status from birth to early childhood in the development of food sensitization. *Pediatr Res* 2013;74:321–326.
- ▶ 28 Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML: Vitamin D levels and food and environmental allergies in the United States: results from the national health and nutrition examination survey 2005–2006. *J Allergy Clin Immunol* 2011;127:1195–1202.
- ▶ 29 Heimbeck I, Wjst M, Apfelbacher CJ: Low vitamin D serum level is inversely associated with eczema in children and adolescents in Germany. *Allergy* 2013;68:906–910.
- ▶ 30 Wang SS, Hon KL, Kong AP, Tang MF, Sy HY, Chan JC, et al: Eczema phenotypes are associated with multiple vitamin D pathway genes in Chinese children. *Allergy* 2014;69:118–124.
- ▶ 31 Karmaus W, Botezan C: Does a higher number of siblings protect against the development of allergy and asthma? A review. *J Epidemiol Community Health* 2002;56:209–217.
- ▶ 32 Viljakainen HT, Saarnio E, Hytinen T, Miettinen M, Surcel H, Makitie O, et al: Maternal vitamin D status determines bone variables in the newborn. *J Clin Endocrinol Metab* 2010;95:1749–1757.
- ▶ 33 Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Makitie O: Vitamin D is a major determinant of bone mineral density at school age. *PLoS One* 2012;7:e40090.
- ▶ 34 Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R: Dendritic cell modulation by 1 α ,25 dihydroxyvitamin D₃ and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. *Proc Natl Acad Sci USA* 2001;98:6800–6805.
- ▶ 35 Zhang Y, Leung DY, Goleva E: Anti-inflammatory and corticosteroid-enhancing actions of vitamin D in monocytes of patients with steroid-resistant and those with steroid-sensitive asthma. *J Allergy Clin Immunol* 2014;133:1744–1752.e1.
- ▶ 36 Yip KH, Kolesnikoff N, Yu C, Hauschild N, Taing H, Biggs L, et al: Mechanisms of vitamin D(3) metabolite repression of IgE-dependent mast cell activation. *J Allergy Clin Immunol* 2014;133:1356–1364, 1364.e1–e14.
- ▶ 37 Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A: 1 α ,25-dihydroxyvitamin D₃ has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* 2001;167:4974–4980.
- ▶ 38 Khoo AL, Joosten I, Michels M, Woestenink R, Preijers F, He XH, et al: 1,25-dihydroxyvitamin D₃ inhibits proliferation but not the suppressive function of regulatory T cells in the absence of antigen-presenting cells. *Immunology* 2011;134:459–468.
- ▶ 39 Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P: Topical vitamin D₃ and low-calceic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc Natl Acad Sci USA* 2006;103:11736–11741.