

Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis

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Background: There is increasing evidence of the effect of maternal vitamin D intake during pregnancy on the risk of asthma and allergic outcomes in offspring. However, studies on the relationship between cord levels of 25-hydroxyvitamin D (25 [OH]D) and asthma and allergic diseases are very few.

Objective: Our aim was to investigate the associations between cord serum 25(OH)D levels and asthma, wheezing, allergic rhinitis, and atopic dermatitis in the offspring from birth to 5 years.

Methods: Cord blood samples were collected at birth and analyzed for 25(OH)D levels in 239 newborns from the Etude des Déterminants pré et post natus du développement et de la santé de l'Enfant (EDEN) birth cohort. The children were followed up until age 5 years by using International Study of Asthma and Allergies in Childhood–based symptom questionnaires.

Results: The median cord serum level of 25(OH)D was 17.8 ng/mL (interquartile range, 15.1 ng/mL). By using multivariable-adjusted logistic regression models, a significant inverse association was observed between cord serum 25(OH)D levels and risk of transient early wheezing and early- and late-onset atopic dermatitis, as well as atopic dermatitis, by the ages of 1, 2, 3, and 5 years. We found no association between cord serum 25(OH)D levels and asthma and allergic rhinitis at age 5 years.

Conclusions: Cord serum 25(OH)D levels were inversely associated with the risk of transient early wheezing and atopic

dermatitis by the age of 5 years, but no association was found with asthma and allergic rhinitis. (*J Allergy Clin Immunol* 2014;133:147-53.)

Key words: Vitamin D, 25-hydroxyvitamin D, cord blood, allergic diseases, wheezing, atopic dermatitis, mother-child cohort

Over the last decade, the prevalence of allergies and asthma in childhood has increased worldwide, particularly in westernized societies. There is increasing evidence that fetal life and early-life exposures, along with associated epigenetic changes,¹ are important determinants of the future development of asthma and allergies throughout life.² Among other environmental factors, prenatal and early-life dietary exposures, including vitamin D, have been suggested to play an important role in the development of allergies and asthma in childhood.³

Vitamin D deficiency, which is defined as a circulating level of less than 20 ng/mL 25-hydroxyvitamin D (25[OH]D), has become a public health issue.⁴ It has been largely attributed to dietary, lifestyle, and behavioral changes during recent decades,⁴ especially in Western countries. Both children and pregnant and lactating women are identified as groups vulnerable to vitamin D deficiency.⁵

Although the relationship of vitamin D deficiency and rickets is well established,⁶ its association with a wide variety of medical conditions, including microbial infections,⁷ cardiovascular diseases,⁸ cancers,⁹ autoimmune diseases,¹⁰ and diabetes,¹¹ is well documented; only more recently has the role of vitamin D deficiency and insufficiency in patients with asthma¹²⁻¹⁴ and allergic diseases¹⁵ beginning in infancy or childhood¹⁶⁻¹⁸ been debated. In the last couple of decades in the 20th century and early 21st century, the vitamin D receptor has been found in a variety of cells, including keratinocytes and numerous cells of the immune system (eg, dendritic cells [DCs] and natural killer cells).¹⁹ Several lines of evidence demonstrate the effects of vitamin D on proinflammatory cytokines, regulatory T cells, and immune responses.²⁰

Vitamin D is found naturally in very few dietary sources, with the only significant ones being fatty fish²¹ and vitamin D–fortified foods. The latter are scarce in France. The main source of vitamin D in human subjects is synthesis from 7-dehydrocholesterol in the skin on exposure to UVB solar radiation and conversion to a readily measurable circulating metabolite, 25(OH)D, in the liver.²¹ Circulating 25(OH)D is the best index of vitamin D nutritional status.²¹ Low intake of vitamin D and inadequate exposure to sunlight constitute the main causes of vitamin D deficiency. A number of factors might influence the cutaneous synthesis and dietary sources of vitamin D,²² especially in pregnant women.²³ These factors include season of the newborn's birth, maternal vitamin D intake, body mass index, constitutive skin pigmentation, latitude, clothing, sunscreen use, outdoor activities, age,^{22,23} and genetic factors.²⁴

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Abbreviations used

DC: Dendritic cell

EDEN: Etude des Déterminants pré et post natus du développement et de la santé de l'Enfant

25(OH)D: 25-Hydroxyvitamin D

OR: Odds ratio

During pregnancy, the fetus is exposed to vitamin D through cord blood supply and the ability of 25(OH)D to cross the placenta.²⁵ Over the past 2 years, the number of studies, both cross-sectional and prospective, that have investigated the effects of maternal vitamin D intake on the inception and severity of asthma and allergies in offspring have increased significantly. Most studies have shown that low maternal vitamin D levels (assessed by food frequency questionnaires) increase the risk for asthma,²⁶ wheeze,^{18,27,28} allergic rhinitis,²⁶ and atopic dermatitis,¹⁸ but a few suggest an increased risk with high levels.^{29,30} However, the majority of these studies did not use objective measures of maternal vitamin D status. In addition, studies examining the associations between cord blood 25(OH)D concentrations and asthma and allergic diseases¹⁶ are still very scarce.

The purpose of the present study was to investigate whether the level of cord serum 25(OH)D, which is considered the best indicator of newborns' vitamin D status, is associated with risk of asthma, wheeze, allergic rhinitis, and atopic dermatitis in offspring. Our study was conducted in the French Etude des Déterminants pré et post natus du développement et de la santé de l'Enfant (EDEN) mother-child cohort.

METHODS**Study design and participants**

Mother-child pairs were recruited in the EDEN Prospective Birth Cohort Study (<http://eden.vjf.inserm.fr>). The primary aim of the EDEN cohort is to identify prenatal and early postnatal nutritional, environmental, and social determinants associated with children's health and normal and pathologic development. Pregnant women seen for a prenatal visit at the departments of Obstetrics and Gynecology of the University Hospital of Nancy and Poitiers before their 24th week of amenorrhea were invited to participate. Enrollment started in February 2003 in Poitiers and September 2003 in Nancy; it lasted 27 months in each center. Women with speaking and writing abilities in French who did not have type 2 diabetes diagnosed before pregnancy and did not plan to deliver outside the university hospital or move out of the region within 3 years were included in the study. Multiple pregnancies were excluded. Among eligible women, 55% (2002 women) agreed to participate (1034 women in Nancy and 968 in Poitiers). Of the 2002 mother-child pairs included in the EDEN study, 1140 of them had complete data by the child's age of 5 years. Among the 1140 participants, measurements of cord serum 25(OH)D levels performed at birth were available in 239 children. The final sample for analysis consisted of 239 mother-child pairs. This sample appeared representative of the overall study population.

Women were given an appointment with a study midwife, which was planned to take place between 24 and 28 gestational weeks, during which an interview on behavioral factors was conducted and biological samples were collected. Further information on the mothers and their newborns, including parity, mode of delivery, newborn's sex and birth weight (measured with electronic scales from Seca, Hamburg, Germany [Seca 737 in Nancy and Seca 335 in Poitiers with a 10-g precision]), gestational age, and season of birth, were either collected by using a questionnaire after birth or extracted from obstetric and pediatric records. All children were equally receiving vitamin D supplementation during the first 3 years of life.

Ethics statement

The ethical committees who approved the study include Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Le Kremlin-Bicêtre University hospital, and Commission Nationale de l'Informatique et des Libertés. The study was approved on December 12, 2002. Written consent was obtained from the mother for herself at the beginning of the study and from both parents for the newborn child after delivery.

Cord serum 25(OH)D measurement

Immediately after delivery, cord blood serum samples were collected by research midwives. To prevent any contamination with maternal blood, the cord was doubly clamped immediately after birth (vaginal delivery) or after extraction of the fetus through the uterine incision (elective cesarean section); repeatedly rinsed and venous cord blood serum was sampled between the 2 clamps. Blood samples were centrifuged within 24 hours of collection. The serum was separated and stored at -80°C . Standard operating procedures for each assay were established. Serum 25(OH)D levels, which are considered indicators of vitamin D status,³¹ were measured by using immunochemiluminometric immunoassays performed on the LIAISON platform (DiaSorin, Saluggia, Italy). This assay measures both 25(OH)D₂ and 25(OH)D₃ levels and provides the sum of these 2 metabolites and does not cross-react with the C3 25(OH)D epimer, which has been shown to complicate the measurement of 25(OH)D levels in neonates. The intra-assay and interassay coefficients of variation were less than 10%, irrespective of the measured concentration.

Health outcomes

Children were followed up from birth to age 5 years. At the ages of 1, 2, 3, and 5 years, parents completed questionnaires including questions on asthma, wheeze, allergic rhinitis, and atopic dermatitis based on the validated International Study of Asthma and Allergies in Childhood phase I questionnaire.^{32,33} For each year, asthma was defined as parental report of a doctor's diagnosis of asthma plus either 1 or more attacks of wheeze or use of asthma medication in the last 12 months. Wheeze was defined as present if the parents answered yes to the following question: "Has your child had wheezing or whistling in the chest in the preceding 12 months?" Parental report of wheezing has been shown to be a sensitive and specific outcome when using physician's assessment as the criterion standard.³⁴ Allergic rhinitis was defined as sneezing, nasal congestion, or rhinitis other than that caused by respiratory tract infections accompanied by eye itching and tearing during the previous 12 months.³⁵ Atopic dermatitis was defined as atopic dermatitis diagnosed by a doctor in the last 12 months. Given the uncertainty of allergic rhinitis and asthma diagnoses in early childhood,³⁶ analyses were performed on allergic rhinitis and asthma from age 4 to 5 years (positive answers to the previous questions in the 5-year questionnaire). Lifetime prevalence of wheeze and atopic dermatitis at the ages of 1, 2, 3, and 5 years were analyzed.

Additionally, data on wheezing from 1 to 5 years of age were used to categorize the children as never wheezers, early transient wheezers, late-onset wheezers, or persistent wheezers, according to the method of Martinez et al.³⁷ Similarly, we distinguished between early- and late-onset atopic dermatitis (eg, atopic dermatitis up to age 2 years and thereafter).

Other variables

We collected information on potential confounders related to health outcomes of the offspring, including newborn sex, birth weight, gestational age, season of birth, number of older siblings at birth (0, 1-2, or ≥ 3), exclusive breast-feeding for 4 or more months (yes or no), maternal age at delivery (<25 years, 25-34 years, and >34 years), prepregnancy maternal body mass index (18.5-24.9, 25.0-29.9, 30.0-34.9, and 35.0-39.9 kg/m²), maternal and paternal history of allergies (yes or no), maternal and paternal educational level (primary or less, secondary, and university degree or higher), household income ($\leq \text{€}2300$ vs $> \text{€}2300$ per month, median income of the study population), city of residence (Nancy or Poitiers), any smoking during pregnancy (yes or no), environmental tobacco smoke exposure of the child from birth to age of 3 years (yes or no), dampness in housing (0-3 years, yes or no), and day

care attendance in the first year of life (yes or no). Maternal and paternal allergic histories were obtained regarding physician-diagnosed allergic diseases, such as asthma, rhinitis, eczema, and food allergies.

Statistical analyses

The sample was described by using frequencies of categorical variables and means and SDs of continuous variables. The characteristics of the mother-newborn pairs in our study sample ($n = 239$) were compared with those of the sample of mother-newborn pairs of children without measurement of cord serum 25(OH)D levels ($n = 901$). We used χ^2 tests for categorical data, t tests for normally distributed data, and Mann-Whitney U tests for skewed data.

We fitted logistic regression models to examine the associations between cord serum 25(OH)D level as a continuous variable and asthma and allergic rhinitis reported from 4 to 5 years and wheeze and atopic dermatitis by the age of 1, 2, 3, and 5 years, as well as wheezing and atopic dermatitis patterns.

Bivariate analysis between potential confounders and each health outcome were performed to identify confounding factors. In a first step all variables associated with 1 health outcome with a P value of less than .30 were retained. In a second step confounders affecting at least 20% of the coefficient estimates of the investigated association between vitamin D levels and health outcomes were selected and included in the multivariate models. In addition to the inclusion of confounders based on their statistically significant association with health outcomes, we selected other adjustment variables on the basis of their known relationship to 25(OH)D concentrations independent of any association with exposures and regardless of whether they changed the effect estimates significantly. They included the following: child's sex,³⁸ prepregnancy body mass index,³⁹ and birth weight.⁴⁰ As a result, the adjustments included the following: city, maternal age at delivery, maternal prepregnancy body mass index, any smoking during pregnancy, any passive smoke exposure during the first 3 years of life, number of siblings, household income, newborn's sex and weight, season of birth (calendar based), and exclusive breast-feeding for 4 or more months. Multiple logistic regression analysis was used to adjust for confounding factors. Estimations of crude and adjusted odds ratios (ORs) and their 95% CIs of asthma, wheeze, allergic rhinitis, and atopic dermatitis at different time points per 5 ng/mL increase in cord serum 25(OH)D concentrations were made by means of logistic regression analysis.

To further explore the associations between asthma and allergic rhinitis and cord vitamin D levels, we examined effect modification of the association of these outcomes with cord serum 25(OH)D levels by stratifying the cohort according to maternal history of allergy and testing the interaction term (ie, continuous 25(OH)D * maternal history of allergy). All statistical analyses were performed with SAS statistical software, version 9.2 (SAS Institute, Cary, NC). P values of less than .05 were considered statistically significant for all analyses.

RESULTS

Characteristics of the mother-child pairs

Children with both cord serum 25(OH)D data and complete questionnaires from birth to 5 years of age were included in this analysis ($n = 239$). Included children did not differ significantly from other children with complete questionnaires up to 5 years of age but without measurement of cord serum 25(OH)D levels ($n = 901$) with respect to all characteristics, except for season of birth and city of residence (Table I).

In our study population no mothers reported type 2 diabetes diagnosed before pregnancy or chronic illnesses apart from asthma, eczema, and other allergic diseases, such as rhinitis and food allergies.

Health outcomes in offspring

By the ages of 1, 2, 3, and 5 years, 24.7%, 32.6%, 35.1%, and 38.9% of all children had experienced wheezing, respectively, and 10%, 13.4%, 14.2%, and 25% had atopic dermatitis, respectively.

From age 4 to 5 years, 8% of all children had asthma, and 7.5% had allergic rhinitis. The prevalences of early transient, late-onset, and persistent wheezing patterns were 28.5%, 11.7%, and 4.2%, respectively. The prevalences of early-onset and late-onset atopic dermatitis were 34.7% and 20.5%, respectively.

Newborns' cord serum 25(OH)D levels

Fig 1 shows the distribution of cord blood serum 25(OH)D levels. The mean concentration and interquartile range in cord blood serum were 17.8 ng/mL and 15.1 ng/mL.

Relationship of cord serum 25(OH)D levels and outcomes by age 5 years

The associations between cord serum 25(OH)D levels and risk of different wheezing patterns; atopic dermatitis by the age of 1, 3, and 5 years; different atopic dermatitis patterns; and asthma and allergic rhinitis at 5 years of age are presented in Table II. Without adjustment, a significant inverse association was observed between cord serum 25(OH)D levels (per 5 ng/mL increase) and the risk of early transient wheezing and atopic dermatitis by the age of 3 and 5 years and early- and late-onset of atopic dermatitis. After multivariable adjustment, the previously observed significant inverse associations persisted (OR for early transient wheezing, per 5 ng/mL increase of cord serum 25(OH)D concentration: 0.67 [95% CI, 0.54-0.81]; OR for atopic dermatitis by the age of 1, 3, and 5 years: 0.84 [95% CI, 0.71-1.00], 0.82 [95% CI, 0.68-0.97], and 0.75 [95% CI, 0.63-0.88], respectively; and OR for early- and late-onset atopic dermatitis: 0.73 [95% CI, 0.62-0.90] and 0.75 [95% CI, 0.60-0.94], respectively). The adjusted inverse association along with the CIs and regression line between cord serum 25(OH)D level and risk of atopic dermatitis by the age of 5 years is shown in Fig 2. The graph represents the predicted probability of atopic dermatitis by the age of 5 years for each observed value of cord serum 25(OH)D in the multivariate model.

In contrast, no significant association was observed between cord serum 25(OH)D levels and asthma and allergic rhinitis prevalence. To further investigate this null finding, we stratified each of these outcomes according to maternal history of allergy. In all models cord serum 25(OH)D levels had no association according to either atopic or nonatopic maternal status (all $P > .20$).

DISCUSSION

Main findings

In this prospective birth cohort study of French mother-child pairs, we found that cord serum 25(OH)D levels were negatively associated with early transient wheezing; atopic dermatitis by the age of 1, 3, and 5 years; and early- and late-onset atopic dermatitis. However, cord serum 25(OH)D levels were not associated with risk of other wheezing phenotypes, asthma, and allergic rhinitis at 5 years of age.

Literature

To date, there is sparse information on vitamin D status and wheezing and atopic dermatitis. The few studies that have investigated the association between maternal vitamin D status and risk of wheezing⁴¹ and atopic dermatitis¹⁸ in childhood are limited by the fact that vitamin D intake was based only on

TABLE I. Comparison of basic characteristics of mother-newborn pairs with (n = 239) and without (n = 901) measurement of cord serum 25(OH)D levels

	Study sample with measurement (n = 239)	Sample without measurement (n = 901)	P value
City (%)			
Poitiers	47.7	57.3	
Nancy	52.3	42.7	.008
Maternal age (y), mean $\pm$ SD	30.8 $\pm$ 4.6	30.5 $\pm$ 4.7	.6
<25 (%)	9.2	11.6	
25-34 (%)	69.5	71.1	
>34 (%)	21.3	17.4	.3
BMI (kg/m ²), mean $\pm$ SD	26.4 $\pm$ 4.4	26.3 $\pm$ 4.5	.6
Normal (%)	46.8	45.9	
Overweight (%)	36.7	37.1	
Moderate obesity (%)	11.4	11.6	
Severe obesity (%)	5.1	5.4	.9
Maternal allergies (%)	31.4	31.0	.9
Paternal allergies (%)	23.0	22.3	.8
Maternal educational level (%)			
Primary school or less	3.3	4.9	
Secondary school	56.2	59.3	
University degree or higher	40.5	35.9	.1
Paternal educational level (%)			
Primary school or less	8.2	7.2	
Secondary school	64.3	65.0	
University degree or higher	27.5	27.8	.7
Smoking during pregnancy (active, passive [%])	31.9	31.8	.5
Environmental tobacco smoke exposure of the child (0-3 y [%])	44.8	48.5	.3
Dampness in housing (0-3 y [%])	5.9	7.8	.3
Attending day care (0-1 y [%])	15.9	12.0	.1
Household income (€ [%])			
$\leq$ 2300	36.0	43.0	
>2300	64.0	57.0	.06
Older siblings (%)			
0	45.6	49.0	
1-2	48.1	47.5	
$\geq$ 3	6.3	3.6	.1
Birth weight (kg), mean $\pm$ SD	3.4 $\pm$ 0.5	3.3 $\pm$ 0.5	.06
Gestational age at birth (wk), mean $\pm$ SD	39.5 $\pm$ 1.5	39.2 $\pm$ 1.8	.3
Sex of the newborn (%)			
Female	41.8	48.0	
Male	58.2	52.1	.1
Season of birth (%)			
Summer	23.4	26.9	
Autumn	20.5	23.1	
Winter	16.7	23.8	
Spring	39.3	23.3	.0008
Exclusively breast-fed for $\geq$ 4 mo (%)	16.3	14.8	.6

BMI, Body mass index.

food frequency questionnaires.^{18,28} Although such methods have been validated and are representative of intake over a period of time, most of these observational studies did not have direct serologic measures of 25(OH)D levels to accurately confirm vitamin D status. However, all studies adjusted for total energy intake and for other nutrients associated with healthy diets.

Additionally, a study in 2008 found that vitamin D supplementation significantly improved skin symptoms in children with winter-related atopic dermatitis.⁴² In adults Oren et al⁴³ also found a protective effect of vitamin D on atopic dermatitis. These results raise interesting questions about the potential role of vitamin D in the pathogenesis of atopic dermatitis.

Regarding the relationship between early transient wheezing and vitamin D deficit, Martinez et al³⁷ found evidence that in most

cases infant wheeze is a transient condition not associated with increased risk of asthma or allergies later in life. In infants with transient wheeze, a reduced airway caliber seems to be the predisposing factor to wheeze in association with viral infections. In our study the inverse association between cord serum 25(OH)D levels and early transient wheezing is consistent with the findings of Camargo et al¹⁶ of an inverse relationship between cord blood 25(OH)D levels and respiratory tract infections.

Potential mechanisms

Cord vitamin D might play a protective role against transient wheezing through its effects on immune function.^{44,45} The molecular effects of vitamin D in the skin have been well documented in

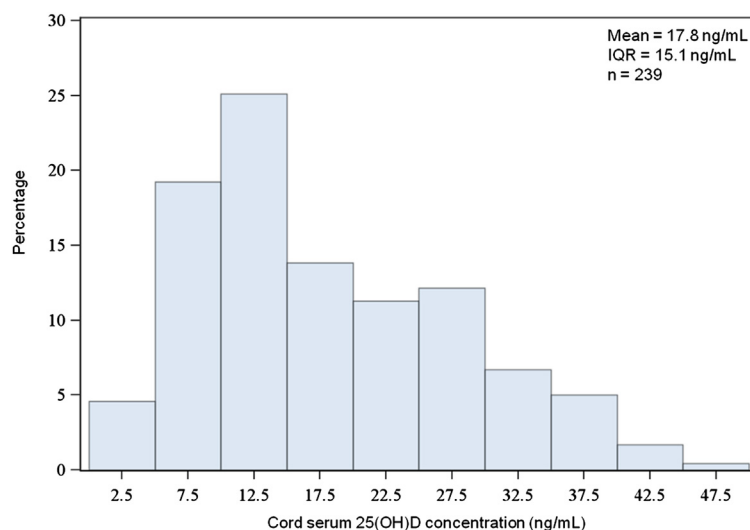


FIG 1. Distribution of vitamin D levels in cord blood (in nanograms per milliliter). Vitamin D levels are indicated on the x-axis, and percentages of newborns are indicated on the y-axis. Multiply nanograms per milliliter by 2.496 to convert nanograms per milliliter of 25(OH)D to nanomoles per liter. IQR, Interquartile range.

TABLE II. Association between cord blood 25(OH)D levels (per 5 ng/mL increase) and risk of wheeze and atopic dermatitis outcomes and asthma and allergic rhinitis (n = 239)

Outcome	OR (95% CI)	
	Unadjusted	Adjusted*
Wheeze		
Early transient	0.77 (0.69-0.95), P = .01	0.67 (0.54-0.81), P = .0002
Late onset	0.94 (0.77-1.16), P = .58	0.86 (0.67-1.11), P = .24
Persistent	1.10 (0.70-0.95), P = .50	1.15 (0.79-1.691), P = .46
Atopic dermatitis		
By the age of 1 y	0.88 (0.76-1.00), P = .08	0.84 (0.71-1.00), P = .05
By the age of 2 y	0.86 (0.77-1.00), P = .05	0.82 (0.70-0.95), P = .02
By the age of 3 y	0.87 (0.76-1.00), P = .05	0.82 (0.68-0.97), P = .02
By the age of 5 y	0.82 (0.71-0.91), P = .003	0.75 (0.63-0.88), P = .0005
Early onset	0.82 (0.70-0.95), P = .01	0.73 (0.62-0.90), P = .002
Late onset	0.82 (0.68-0.98), P = .03	0.75 (0.60-0.94), P = .01
Asthma at 5 y	1.09 (0.87-1.38), P = .42	1.07 (0.78-1.45), P = .69
Allergic rhinitis at 5 y	1.06 (0.84-1.34), P = .62	0.99 (0.72-1.38), P = .98

*Adjusted for city, mother's age, maternal history of allergy, prepregnancy body mass index, any smoking during pregnancy, any passive smoke exposure during the first 3 years of life, number of siblings, household income, newborn's sex and weight, season of birth, and exclusive breast-feeding for 4 months or longer.

experimental studies. Vitamin D receptor expression in the skin was first confirmed after rats injected with radiolabeled 1,25-dihydroxyvitamin D (1,25[OH]2D), the active form of 25(OH)D, demonstrated radioactivity concentrated in the nuclei of the epidermis along with a variety of other tissues.⁴⁶ 1,25(OH)D enhances keratinocyte differentiation, as well as having either stimulatory or suppressive effects on keratinocyte growth that are concentration dependent.⁴⁷

In addition, there is evidence to suggest that vitamin D decreases inflammatory responses in epidermal keratinocytes.⁴⁸⁻⁵⁰ *In vitro*

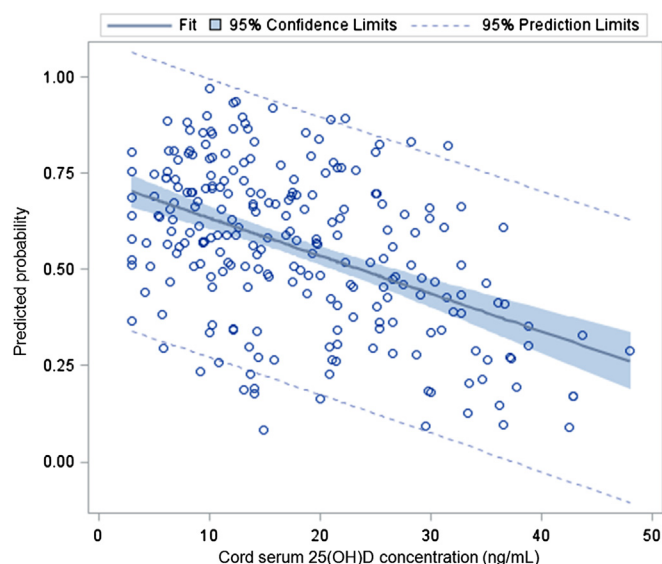


FIG 2. Adjusted associations between cord serum 25(OH)D levels and predicted probabilities of atopic dermatitis by age of 5 years.

treatment of DCs with 1,25(OH)2D or vitamin D analogs led to decreased IL-12 and enhanced IL-10. These cytokine effects, along with inhibitory effects on DC maturation, promote tolerogenic properties and suppressor T-cell induction.⁵¹ A short treatment course of 1,25(OH)2D in mice induced tolerogenic DCs and increased regulatory T-cell numbers.⁵² As a result, vitamin D might promote regulatory T cells, which are known to play an important role in the prevention of atopic dermatitis development⁵³ by playing a pivotal role in immune suppression and being crucial to the control of allergic responses.

Vitamin D also has a beneficial effect on the permeability barrier in the epidermis. Bikle et al⁵⁴ examined mice null for the expression of 25(OH)D-1 α -hydroxylase. Lower levels of multiple proteins necessary for formation of the stratum corneum,

including filaggrin, were observed in the null mice compared with those seen in the wild-type control animals.⁵⁴ After skin barrier disruption, null mice had a significantly delayed barrier recovery compared with wild-type mice.⁵⁴

Another potential role for vitamin D is suggested by the work of Schaubert and Gallo,⁵⁵ who found that 1,25(OH)₂D enables keratinocytes to recognize and respond to microbes through action on the Toll-like receptor 2 and leads to upregulation of cathelicidin, an antimicrobial peptide. As a result, vitamin D might promote the production of antimicrobial peptides that have the ability to enhance immunity and protect skin from bacterial infections.

Consequently, given the fact that the pathogenesis of atopic dermatitis involves both epidermal barrier and immunologic dysfunction, vitamin D might have a protective role against atopic dermatitis and the development of a variety of other skin disorders as well.

Strengths and limitations

First, this is one of the few birth cohort studies to have measured vitamin D levels in cord blood as opposed to estimating infant levels based on vitamin D levels in pregnant women³⁰ or maternal intake of vitamin D during pregnancy.^{18,26,28,41} Additionally, newborns' vitamin D status was assessed by measuring the serum 25(OH)D level, which is considered the best circulating biomarker of vitamin D metabolic status. The analyses were performed on the continuous 25(OH)D variable because the often used categorization of vitamin D levels is still arbitrary and without consensus. An additional strength of our prospective investigation consists in the robustness of the results because children were drawn from a cohort and their parents were able to provide information on possible confounders, thus diminishing the probability of biased or confounded results. In addition, we collected outcome data at different ages, providing more robust measures of potential health effects than single time point outcomes could provide. The definition of health outcomes was based on the validated International Study of Asthma and Allergies in Childhood questions.

Our study also has some limitations. Information on offspring health outcomes was obtained from parental report through self-administered questionnaires, which could result in misclassification. We could not obtain vitamin D data from all eligible newborns with complete information at age 5 years, which might have introduced selection bias. Because of the sample size of our study, although the change-in-estimate criterion of 10% or greater is a conventional one,⁵⁶ a more conservative criterion of 20% or greater was used to select potential confounders. In addition, the absence of significant associations between asthma, allergic rhinitis, and 25(OH)D concentrations might be due to the low prevalences of these outcomes, consequently limiting the power to detect a relationship between 25(OH)D and the outcome. An often-cited limitation of serologic studies is the reliance on a single 25(OH)D measurement per subject. It would have been interesting to measure serum 25(OH)D levels during childhood. The half-life of serum 25(OH)D is approximately 2 to 3 weeks,⁵⁷ which suggests that the measured concentrations reflect maternal-fetal status during the final months of pregnancy and first months of life. All children in our study were receiving vitamin D supplementation during the first 3 years of life, and therefore the difference between children's vitamin D levels might depend on both diet and food diversification, as well sun-related

behaviors, despite heightened awareness of the carcinogenic effect of sun exposure.⁵⁸ Finally, subjects were followed only to age 5 years, at which age asthma might be evolving from being infection related to being predominantly related to allergy. Similarly, wheezing in the preschool years is not necessarily indicative of allergic disease because we know that wheeze can be induced by viral infection.⁵⁹

Conclusion

In summary, our data indicate an inverse association between cord serum 25(OH)D levels and the risk of early transient wheezing and atopic dermatitis in young children. In contrast, we found no apparent association between cord serum 25(OH)D concentrations and other wheezing phenotypes, asthma, and allergic rhinitis at 5 years of age.

If these associations are causal, interventions to increase vitamin D levels *in utero* and in early life could reduce the risk of transient wheezing and atopic dermatitis in early childhood. Increased exposure to sunlight and increased intake of foods high in vitamin D (not necessarily supplementation) in both pregnant women and infants are potential public health interventions.

In light of recent findings on the role of vitamin D and its effects on asthma and allergic diseases, studies of vitamin D can enhance the understanding, prevention, and treatment of these increasingly common conditions in childhood. Further research is required to establish the optimal level (or range) of vitamin D that decreases both the risk for development and the severity of these disorders, as well as the timing and route of exposure to vitamin D.

Clinical implications: The risk of transient wheezing and atopic dermatitis in childhood is inversely associated with cord 25(OH)D levels. Interventions to increase vitamin D levels *in utero* and in early life are potential public health interventions to reduce transient wheezing and atopic dermatitis in young children.

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