Vitamin D over the first decade and susceptibility to childhood allergy and asthma

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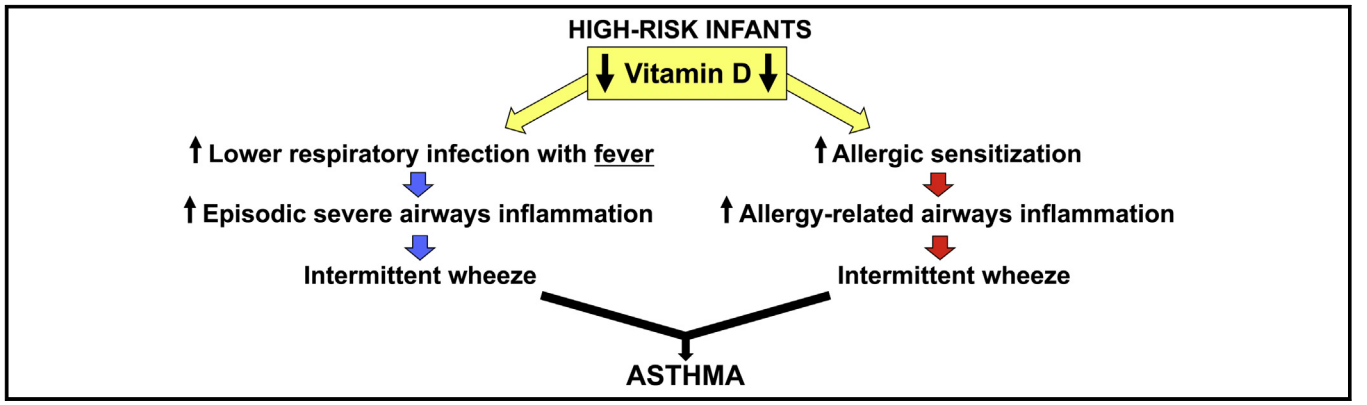
GRAPHICAL ABSTRACT



Background: Vitamin D (25(OH)D) deficiency has been implicated as a possible risk factor for asthma development, but studies at selected time points measuring 25(OH)D levels during childhood have yielded conflicting findings. Prospective studies tracking 25(OH)D levels during the initiation phase of asthma in early childhood have not been reported.

Objective: We sought to elucidate relationships between 25(OH)D levels from birth to age 10 years and susceptibility to allergic sensitization, respiratory tract infections, and asthma.

Methods: Asthma-, allergy-, and respiratory tract infection–associated phenotypes (including pathogen identification) were characterized in a high-risk birth cohort. Plasma 25(OH)D concentrations were quantified at birth and at clinical follow-ups at the ages of 0.5, 1, 2, 3, 4, 5, and 10 years, and relationships with clinical outcomes were examined.

Results: Cross-sectional analyses demonstrated inverse associations between 25(OH)D concentrations and the risk for concurrent sensitization at age 0.5, 2, and 3 years, and mixed-effects regression demonstrated inverse longitudinal associations of 25(OH)D levels with both sensitization and eczema. Multivariate regression modeling suggested that the number of 25(OH)D-deficient follow-ups was positively associated with risk for asthma/wheeze, eczema, and sensitization at 10 years; adjustment for sensitization (particularly by 2 years) in the asthma/wheeze models reduced 25(OH)D associations with these latter outcomes. 25(OH)D levels were also inversely associated with early nasopharyngeal colonization with *Streptococcus* species and age of first febrile lower respiratory illness, both of which are known asthma risk factors.

Conclusion: 25(OH)D deficiency in early childhood is associated with increased risk for persistent asthma, potentially through modulating susceptibility to early allergic sensitization, upper respiratory tract colonization with bacterial pathogens, or both. These relationships are only evident if 25(OH)D status is monitored prospectively and longitudinally. (J Allergy Clin Immunol 2017;139:472-81.)

Key words: *Vitamin D, asthma, allergy, respiratory infections, microbiome, Streptococcus, longitudinal birth cohort, childhood*

There is considerable interest in the role of vitamin D in asthma and allergy pathogenesis in light of its immune-modulating properties and its apparent role in lung development.[1,2] The active form of vitamin D ($1,25(OH)_2D$) controls expression of many genes in a tissue-specific manner, including within the immune system,[3,4] and experimental models have demonstrated vitamin D–mediated boosting of protection against infection and promotion of immune tolerance against allergens.[5] Circulating

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Abbreviations used
ARI: Acute respiratory tract infection
CAS: Childhood Asthma Study
IQR: Interquartile range
LRI: Lower respiratory tract infection
NPA: Nasopharyngeal aspirate
sLRI: Severe lower respiratory tract infection (with wheezing or fever)

concentration of 25(OH)D is currently accepted as the most reliable index of $1,25(OH)_2D$ availability. 25(OH)D undergoes enzymatic conversion to $1,25(OH)_2D$ in the kidney, in epithelial cells, and in immune cells, including dendritic cells, macrophages, and T cells.[1,6] Although it has been suggested that inadequate vitamin D might be a factor contributing to the surge in asthma rates over recent decades,[7] studies investigating the relationship between 25(OH)D levels and asthma or allergy risk have yielded conflicting results.[8,9] Much of this contradiction can arise from genetic and environmental differences between study populations[10-16] and the use of nonstandardized methods for assaying 25(OH)D.[17,18] In addition, it is unknown whether the requirement of vitamin D for normal immune and respiratory development varies with age; therefore it is difficult to compare studies with single 25(OH)D measures taken at differing ages.

We hypothesized that inadequate vitamin D levels during early childhood promotes development of asthma by increasing susceptibility to 2 major asthma risk factors: allergic sensitization and severe respiratory tract infection. We looked for relevant associations within the well-characterized Childhood Asthma Study (CAS) cohort of children at high risk of asthma and allergy, which has been followed from birth to age 10 years.[19,20] Previous examination of this cohort has shown that sensitization to inhaled allergens by age 2 years and severe lower respiratory tract infections (sLRIs) independently and (particularly) in combination increased the risk of persistent wheeze and asthma development by 5 and 10 years.[20,21] We assayed 25(OH)D concentrations in plasma samples that were archived during 8 age-designated CAS cohort follow-ups from birth to age 10 years. We then examined whether 25(OH)D concentration level was associated with risk for asthma and related conditions, allergic sensitization, or type/timing/frequency/severity of respiratory tract infections. Included among the latter was early postnatal colonization of the upper respiratory tract with known bacterial pathogens exemplified by *Streptococcus* species, which we have previously demonstrated to increase the risk for asthma development in this cohort.[22]

METHODS

Study population and sample collection

The CAS cohort was recruited prenatally, selecting 263 children with at least 1 parent with a doctor-diagnosed history of asthma, hay fever, or eczema[19]; 198 children participated up to age 5 years, and 147 children participated up to age 10 years. Parents kept a daily symptom diary, and observation of respiratory tract infection symptoms up to age 5 years initiated a home visit by the study physician for verification and nasopharyngeal aspirate (NPA) collection; additional NPAs were collected at "healthy" visits at around 2 and 6 months. Detailed viral and bacterial profiling of NPAs was undertaken by using PCR and 16S rRNA gene deep sequencing, as recently reported.[22] Plasma was collected at birth (cord) and scheduled follow-ups at ages 6 months and 1, 2, 3, 4, 5, and 10 years and stored at −20°C.

Clinical phenotyping

Respiratory (both wheeze- and infection-associated) and atopy-associated phenotypes were defined as previously described[19-21] and detailed in the Methods section in this article's Online Repository at www.jacionline.org. Titers of total and specific IgE to a panel of allergens relevant to the study location were measured from plasma archived from "healthy" follow-ups by using ImmunoCAP (Phadia AB, Uppsala, Sweden; see the Methods section in this article's Online Repository).

25(OH)D measurement

Plasma 25(OH)D concentrations were measured using a modified liquid chromatography tandem mass spectrometry method[23] by Metabolomics Australia (University of Western Australia). This assay separately quantifies 25(OH)D3, 25(OH)D2, and 3-epi-25(OH)D, with accuracy confirmed through the Centers for Disease Control and Prevention/National Institutes of Health Vitamin D Standardization Program.[23,24] Plasma samples were assayed blind in random order, with internal quality controls confirming the comparability of all analyses. There was no detectable 25(OH)D2 in any of the samples; all 25(OH)D measures analyzed in this study comprise 25(OH)D3 but not 3-epi-25(OH)D. Quality control experiments verified that recovery efficiency of 25(OH)D from cord blood was equivalent to that from peripheral blood.

Statistical analyses

Statistical analyses were performed with SPSS 22/23 (IBM, Armonk, NY), STATA 13 (StataCorp, College Stations, Tex), and R software, as detailed in the Methods section in this article's Online Repository. Deseasonalized 25(OH)D concentrations were calculated by using a sinusoidal model (see the Methods section in this article's Online Repository).[25] In addition to examining continuous 25(OH)D concentrations, we used commonly accepted cutoffs to define 25(OH)D status categories for statistical analyses: *deficient* was defined as less than 50 nmol/L, *insufficient* was defined as 50 to 75 nmol/L, and *sufficient* was defined as greater than 75 nmol/L.

RESULTS

Tracking 25(OH)D levels from birth to age 10 years

Fig 1, *A*, illustrates age-dependent fluctuations in circulating 25(OH)D concentration in individual CAS cohort children at any of the 8 follow-ups. Fig 1, *B*, shows the distribution of 25(OH)D at each follow-up: concentrations were positively skewed at birth and essentially normally distributed at subsequent follow-ups. Overall, 25(OH)D concentrations were lowest at birth and highest at age 5 years, followed by age 10 years; median 25(OH)D concentrations for each follow-up were 26.2 nmol/L (interquartile range [IQR] 20.3 to 36.9 nmol/L) at birth, 68.7 nmol/L (IQR 55.4 to 80.5 nmol/L) at 6 months, 62.1 nmol/L (IQR 50.1 to 74.6 nmol/L) at 1 year, 58.6 nmol/L (IQR 49.6 to 68.0 nmol/L) at 2 years, 58.6 nmol/L (IQR 46.5 to 67.5 nmol/L) at 3 years, 57.5 nmol/L (IQR 47.4 to 67.6 nmol/L) at 4 years, 89.0 nmol/L (IQR 71.5 to 97.4 nmol/L) at 5 years, and 76.2 nmol/L (IQR 64.1 to 87.6 nmol/L) at 10 years.

At birth, the majority of children had 25(OH)D concentrations of less than 50 nmol/L, which is commonly classified as vitamin D deficiency; this was substantially less prevalent at all later follow-ups. As expected from the literature relating sun exposure to 25(OH)D levels (including data on Australian children[26]), there was seasonal variation in the prevalence of vitamin D deficiency (Fig 1, *C-F*), which was most frequent in samples collected during winter (Fig 1, *F*); vitamin D insufficiency was common and showed similar seasonal variation. Deseasonalized 25(OH)D