

Steroid requirements and immune associations with vitamin D are stronger in children than adults with asthma

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Background: The effects of serum vitamin D status on atopy, steroid requirement, and functional responsiveness to corticosteroids in children versus adults with asthma have not been studied systematically.

Objective: We sought to explore the age-specific effects of vitamin D in asthmatic patients.

Methods: Serum vitamin D levels were examined in a prospective study of adults and children (102 healthy control subjects and 103 asthmatic patients). PBMCs were cultured for 3 hours with or without 100 nmol/L dexamethasone, and the expression of corticosteroid-regulated genes was detected by using real-time PCR. Serum IgE levels were measured, and information about asthmatic patients' steroid requirements was collected.

Results: Deficient serum vitamin D levels (<20 ng/mL) were found in 47.6% of asthmatic patients and 56.8% of healthy control subjects, with means $\pm$ SDs of 20.7 ± 9.8 and 19.2 ± 7.7 ng/mL, respectively. In multivariate regression models a significant positive correlation between serum vitamin D levels and the expression of vitamin D-regulated targets, cytochrome P450, family 24, subfamily a (cyp24a) expression by PBMCs ($P = .0084$, pediatric asthma group only) and serum LL-37 levels ($P = .0006$ in the pediatric group but $P = .0067$ in the adult asthma group), was found. An inverse association between vitamin D and serum IgE levels was observed in the pediatric ($P = .006$) asthma group. Serum vitamin D level ($P = .05$), as well as PBMC cyp24a expression ($P = .0312$), demonstrated a significant inverse relationship with daily inhaled corticosteroid dose in the pediatric asthma group only. Cyp24a expression in PBMCs correlated positively with *in vitro* suppression of TNF- α by dexamethasone ($P = .05$) and IL-13 ($P = .0094$) in PBMCs in the pediatric asthma group only.

Conclusions: This study demonstrated significant associations between serum vitamin D status and steroid requirement and

in vitro responsiveness to corticosteroids in the pediatric but not the adult asthma group. Vitamin D was also related to IgE levels in children but not in adults. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Vitamin D, asthma, corticosteroids

The biomedical literature has demonstrated an enormous burst of interest in vitamin D over the past 10 years. It has been reported that vitamin D insufficiency/deficiency is escalating in the general population both in the United States and globally.¹⁻⁴ A number of epidemiologic investigations have shown an association between low serum vitamin D levels and various diseases.⁵⁻⁷ Several articles have reported a potential role for vitamin D in asthma.⁸⁻¹²

The accumulated data showed an association of low serum vitamin D levels in asthmatic patients with increased airway obstruction and increased corticosteroid requirement.¹³ Several recent studies have suggested that low serum vitamin D levels can influence the severity of asthma, atopy, or both.¹⁴⁻¹⁶ The majority of these associations were reported for the pediatric asthma study groups. The study of Costa Rican children (age 6-14) with asthma by Brehm et al¹⁴ reported an inverse association between serum vitamin D levels and total IgE levels and eosinophil counts. Subjects with reduced serum vitamin D levels had increased airway hyperreactivity and were more likely to be hospitalized for asthma complications and to use anti-inflammatory medications, including inhaled corticosteroids (ICSs).¹⁴ In a follow-up study using samples from the Childhood Asthma Management Program study, Brehm et al¹⁷ reported that children with insufficient serum vitamin D levels have higher odds of hospitalization or emergency department visits. It was shown that study subjects are likely to have severe asthma exacerbations if they have insufficient vitamin D levels despite using ICSs.¹⁷ The study by Searing et al¹⁰ reported that asthmatic children receiving ICSs and oral corticosteroids have significantly lower serum vitamin D levels. From the analysis of the results of the National Health and Nutrition examination Survey 2005-2006 in US children and adults, it was reported that vitamin D deficiency is associated with higher levels of IgE sensitization in the pediatric and adolescent group (<21 years old).¹⁵ No associations between vitamin D levels and allergic sensitization were reported in adults.¹⁵ To our knowledge, this is the only study to compare the relationship between vitamin D levels and allergic sensitization in adults and children. The nature of the age-specific effects of vitamin D is unclear.

Recently, an Institute of Medicine committee conducted a comprehensive review of the evidence for both skeletal and extraskeletal outcomes of vitamin D and calcium supplementation.^{18,19} The committee concluded that available scientific

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Supported in part by a research grant from Diasorin, Inc, as well as National Institutes of Health grants AI070140 and HL37260. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases; the National Heart, Lung, and Blood Institute; or the National Institutes of Health.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication June 30, 2011; revised November 23, 2011; accepted for publication January 17, 2012.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2012.01.044

Abbreviations used

BMI: Body mass index
 Cyp24a: Cytochrome P450, family 24, subfamily a
 DEX: Dexamethasone
 ICS: Inhaled corticosteroid
 VDR: Vitamin D receptor

evidence supports a key role for calcium and vitamin D in skeletal health. However, for other outcomes, including cancer, cardiovascular disease, diabetes, and autoimmune disorders, the evidence was inconsistent, inconclusive as to causality, and insufficient to inform the composition of nutritional requirements. It was stated that randomized clinical trial evidence for these extraskeletal outcomes was limited and generally uninformative. The committee concluded that the prevalence of vitamin D inadequacy in North America has been overestimated because circulating levels of 20 ng/mL were determined to be sufficient to support bone health in the majority of the population. The Institute of Medicine panel concluded that more research and reassessment of laboratory ranges for serum vitamin D should be done to avoid problems of both undertreatment and overtreatment.^{18,19}

The goal of the current study was to examine the relationships between serum 25-hydroxyvitamin D levels, markers of activation of vitamin D receptor (VDR)-activated pathways, PBMC responsiveness to dexamethasone (DEX), and corticosteroid requirements in asthmatic patients and to explore whether these associations differed in adults and children. The knowledge about age-specific relationships between the abovementioned markers is of great importance to justify the potential benefits of vitamin D supplementation in different age groups of asthmatic patients.

In this study we examined age-specific associations between vitamin D levels and atopy, steroid requirement, and functional response to corticosteroids in pediatric (<18 years old) and adult (≥18 years old) asthma groups. In this study we used *in vitro* response of PBMCs to DEX (TNF- α and IL-13 mRNA suppression by DEX) as a readout of cellular functional response to corticosteroids. To our knowledge, there are no studies to date that looked at atopy, steroid requirement, and functional responses to corticosteroids in children versus adults with asthma in the same study in relationship to patients' vitamin D status. Considering that cellular responses to vitamin D are influenced by genetics,²⁰ another novel aspect of this study was that we explored the relationship between serum vitamin D levels and cytochrome P450, family 24, subfamily a (cyp24a) mRNA (a known target inducible by vitamin D) expression in PBMCs as a functional readout of vitamin D status. Vitamin D status was then analyzed in the context of laboratory biomarkers of corticosteroid response. A number of associations were found to be significant in the pediatric but not the adult asthma groups, suggesting that future vitamin D oral supplementation studies should compare vitamin D responses in children versus those in adults.

METHODS**Subjects**

25-Hydroxyvitamin D levels and clinical features were analyzed in a total of 102 healthy control subjects (51 adults and 51 children) and 103 asthmatic patients (50 adults and 53 children). Blood samples were collected between

January and March 2010. Approval was received from the National Jewish Health Institutional Review Board for the study.

Data collection

Serum levels of 25-hydroxyvitamin D (the inactive form of vitamin D) were analyzed by using the vitamin D 25-hydroxy chemiluminescent immunoassay performed by DiaSorin, Inc (Stillwater, Minn). This assay can measure both D₂ and D₃ derivatives of 25-hydroxyvitamin D. Values were reported in nanograms per milliliter. 25-Hydroxyvitamin D levels are the preferred marker of the body's vitamin D status because this form has a longer half-life (2-3 weeks) than 1,25-dihydroxyvitamin D (4 hours).²¹ Total IgE and specific IgE testing was performed by Advanced Diagnostics Laboratories (Denver, Colo) at National Jewish Health with the Phadia ImmunoCAP method. Medication use, vitamin supplementation, and dosage were recorded.

Laboratory studies were performed on purified PBMCs collected from the patients recruited into the study. Human PBMCs were isolated by means of Ficoll-Hypaque density gradient centrifugation. Freshly isolated PBMCs (2×10^6) were immediately preserved in RNA lysing solution (Qiagen, Hilden, Germany). PBMCs (2×10^6) were cultured in hormone-free medium with or without 100 nmol/L DEX for 3 hours and preserved in RLT buffer for RNA extraction. Total RNA was prepared (Qiagen), transcribed into cDNA, and analyzed by means of real-time PCR with the dual-labeled fluorogenic probe method on an ABI Prism 7300 Real Time PCR system (Applied Biosystems, Foster City, Calif). Cyp24a, TNF- α , IL-13, and 18s RNA expressions were determined.

Plasma cathelicidin expression was determined in serum samples from asthmatic patients by using a human LL-37 ELISA kit (Hycult Biotech, Uden, Netherlands), according to the manufacturer's instructions.

Statistical analysis

Population values for the variables examined are given in Table I. Multivariate linear regression modeling controlling for each subject's body mass index (BMI), sex, age, race, and vitamin D supplementation was used to assess the relationship between serum vitamin D levels and cyp24a mRNA expression by PBMCs and a number of clinical and laboratory variables that indicate the patient's steroid requirements and responsiveness to corticosteroids *in vitro*. Variables were considered statistically significant at *P* values of less than .05 by using 2-sided tests. Statistical analysis was performed with JMP 8.0.1 software (SAS Institute, Inc, Cary, NC).

RESULTS**Subjects' characteristics**

Serum 25-hydroxyvitamin D levels and clinical features were analyzed in 103 asthmatic patients and 102 healthy control subjects (Table I). The groups were balanced for age, sex, and race distributions. Asthmatic patients had significantly lower FEV₁ percent predicted values and FEV₁/forced vital capacity ratio percentages, as well as significantly increased serum IgE levels, compared with those seen in healthy control subjects. More than 75% of asthmatic patients in this study were taking ICSs. Blood samples were collected between January and March 2010. This corresponds to the winter season, when little vitamin D can be produced through sun exposure in latitudes above 35°N.³

In this study 47.6% of asthmatic patients and 56.8% of healthy control subjects had deficient serum vitamin D levels (<20 ng/mL; means $\pm$ SDs of serum vitamin D were 20.7 ± 9.8 and 19.2 ± 7.7 ng/mL for the asthmatic patients and healthy control subjects, respectively). The racial/ethnic breakdown of the study population and corresponding serum vitamin D levels are shown in Table II. Of note, adult African American subjects had the

TABLE I. Patients' characteristics*

Characteristic	Healthy control subjects		Asthmatic patients	
	Adults	Children	Adults	Children
Age (y)	31 (24-39)	12 (10-15)	32.5 (22-46)	10 (8.5-13)
Sex (M/F)	24/27	29/22	17/33	25/28
Patient race (white/other/African American)	15/20/16	4/25/22	18/13/19	9/18/26
BMI (kg/m ²)	27.8 (24.7-30.7)	20.9 (18.4-24.2)	29 (23.4-34.5)	20 (16.6-24.2)
IgE (kU/mL)	58 (25-148)	60 (24-182)	122 (48-422)	226 (89-423)
FEV ₁ (%)	100 (88-109)	101 (90-112)	88 (76-97)	93 (77-105)
FEV ₁ /FVC ratio	82 (77-84)	85 (81-88)	74 (69-81)	82 (72-88)
Patients taking ICSs (Y/N)			32/18	41/12
ICS dose (μg/d)			320 (228-500)	360 (180-500)

F, Female; FVC, forced vital capacity; M, male.

*Values are listed as medians (interquartile ranges).

TABLE II. Serum vitamin D levels by race and age in asthmatic patients and healthy control subjects (ng/mL [mean ± SD])

Study group	White	African American	Other§
Asthmatic patients, adults (≥18 y)	25.6 ± 10.8 (n = 18)	12.5 ± 6.2† (n = 19)	15.9 ± 6.8* (n = 13)
Asthmatic patients, children (<18 y)	27.6 ± 10.0 (n = 9)	22.0 ± 8.3 (n = 26)	22.6 ± 9.2 (n = 18)
Healthy control subjects, adults (≥18 y)	19.8 ± 8.4 (n = 15)	13.7 ± 7.3‡ (n = 16)	16.4 ± 6.3 (n = 20)
Healthy control subjects, children (<18 y)	24.8 ± 7.0 (n = 4)	21.9 ± 7.9 (n = 22)	21.3 ± 6.1 (n = 25)

*P < .01, white vs other.

†P < .0001, white vs African American.

‡P < .05, white vs African American.

§Other group consistent mainly of Hispanic/Latino ethnic category (100% for asthmatic adults, 72% for asthmatic children, 70% for healthy control adults, and 100% for healthy control children, respectively).

lowest serum vitamin D levels compared with other racial groups. Serum vitamin D levels of African American children were comparable to the serum vitamin D levels of children from 2 other racial groups (Table II). The proportions of adults and children (both the 6- to 12-year-old and 13- to 18-year-old groups) with sufficient (>30 ng/mL), insufficient (20-30 ng/mL), and deficient (<20 ng/mL) serum vitamin D levels are provided in the Table III.

Expression of vitamin D-regulated genes in PBMCs and sera from asthmatic patients

In this study the expression of vitamin D-regulated targets in sera (serum LL-37 levels) and by PBMCs (cyp24a) and their relationship to serum vitamin D levels was examined in the pediatric (<18 years old) and adult (≥18 years old) asthmatic groups. Multivariate regression models were developed controlling for BMI, sex, race, age, and vitamin D supplementation to explore the relationships between serum vitamin D levels and the expression of the vitamin D-inducible targets, which reflect an intact VDR-activated cellular pathway.

A positive correlation between serum vitamin D levels and serum LL-37 levels was found in the pediatric asthma group ($R^2 = 0.34$, $P = .0006$; Fig 1, A) and the adult asthma group ($R^2 = 0.35$, $P = .0067$; Fig 1, B). However, serum vitamin D levels had a greater effect on serum LL-37 levels in the pediatric asthma group (+0.09 increase in log picograms per milliliter of serum LL-37 for each nanogram per milliliter increase in serum vitamin D level) compared with the adult asthma group (+0.04 increase in log picograms per milliliter of serum LL-37 for each nanogram per milliliter increase in serum vitamin D, Fig 1). A positive

association between serum vitamin D levels and cyp24a expression by PBMCs was found only in the pediatric asthma group ($R^2 = 0.20$, $P = .0084$; Fig 1, C) and not in the adult asthma group (Fig 1, D).

Relationship between serum IgE and serum vitamin D levels

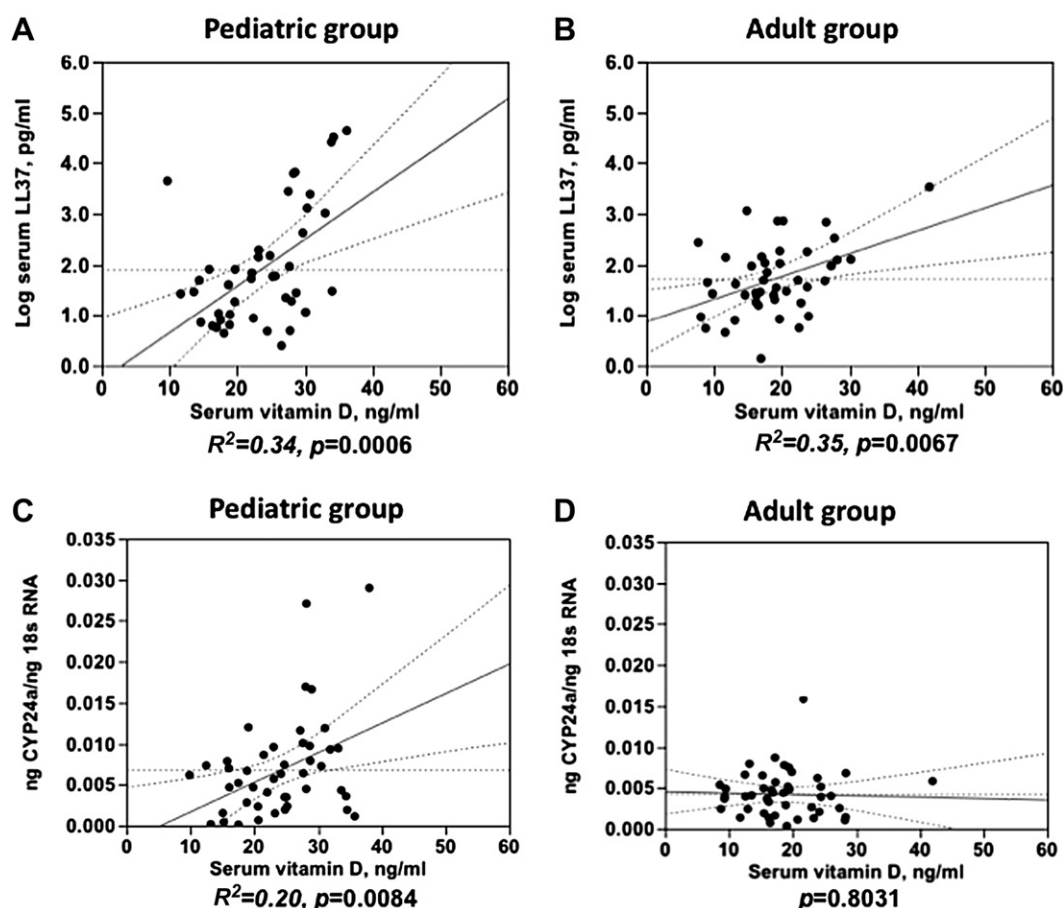
In a linear regression model controlling for the patient's age, BMI, sex, race, and vitamin D supplementation, it was found that there is a significant inverse correlation between serum vitamin D levels and the patient's IgE levels in the pediatric group only ($R^2 = 0.24$, $P = .006$, -23 kU/L IgE decrease for each nanogram per milliliter of serum vitamin D increase was observed; Fig 2, A). No relationship between serum vitamin D and serum IgE levels was found in the adult asthma group (Fig 2, B). In an adjusted regression model it was found that asthmatic children with positive IgE levels to dust mites (n = 13) had significantly lower serum vitamin D levels (serum vitamin D levels were 26.7 ± 1.3 vs 21.0 ± 2.1 ng/mL for the subjects with negative and positive IgE levels to *Dermatophagoides farinae* [$P = .0109$] and 27.3 ± 1.3 vs 20.5 ± 2.1 ng/mL for the subjects with negative and positive IgE to *Dermatophagoides pteronyssinus* [$P = .0037$]). No associations between positive IgE levels to cat, dog, *Alternaria* species, tree mix, grass mix, and weed mix and vitamin D levels were found.

Relationship between serum vitamin D levels and asthmatic patients' steroid requirements

In this study, to examine the relationship between serum vitamin D levels and patients' steroid requirements, the daily

TABLE III. Serum vitamin D levels in asthmatic patients and healthy control subjects of different age groups

Study group	Serum vitamin D levels		
	Deficient (<20 ng/mL)	Insufficient (20-30 ng/mL)	Sufficient (>30 ng/mL)
Asthmatic patients, adults (≥18 y)	28/50 (56%)	16/50 (32%)	6/50 (12%)
Asthmatic patients, children			
Total group (<18 y)	21/53 (40%)	20/53 (38%)	12/53 (22%)
6-12 y	10/37 (27%)*	15/37 (41%)	12/37 (32%)†
13-17 y	11/16 (69%)	5/16 (31%)	0/16 (0%)
Healthy control subjects, adults (≥18 y)	35/51 (69%)‡	12/51 (24%)	4/51 (7%)
Healthy control subjects, children			
Total group (<18 y)	23/51 (45%)	20/51 (39%)	8/51 (16%)
6-12 y	9/26 (35%)	11/26 (42%)	6/26 (23%)
13-17 y	14/25 (56%)	9/25 (36%)	2/25 (8%)

P* = .0064 compared with asthmatic patients, children (>12 years).†*P* = .0105 compared with asthmatic patients, children (>12 years).‡*P* = .0273 compared with healthy control subjects, children, total group (<18 years).FIG 1.** Associations between serum vitamin D levels and vitamin D-regulated targets (serum LL-37 levels [A and B] and cyp24a expression [C and D]) in the pediatric and adult asthma groups.

ICS dose for each patient was converted into budesonide equivalents. A significant inverse association between serum vitamin D level and ICS dose ($R^2 = 0.25$, $P = .05$, $-27 \mu\text{g}$ decrease in a daily ICS dose for each 1 ng/mL increase in serum vitamin D level) was found only in the pediatric asthma group (Fig 3, A), but there was no association between serum vitamin D levels and ICS doses in the adult asthma group (Fig 3, B). Similarly, a significant inverse relationship between cyp24a expression by PBMCs and the patient's daily ICS dose was observed in the

pediatric asthma group ($R^2 = 0.16$, $P = .0312$; Fig 3, C) but not in the adult asthma group (Fig 3, D).

Relationship between expression of vitamin D-regulated targets by PBMCs and cellular response to corticosteroids *in vitro*

A significant positive correlation between cyp24a expression (a vitamin D-inducible gene) by PBMCs and the degree of TNF- α

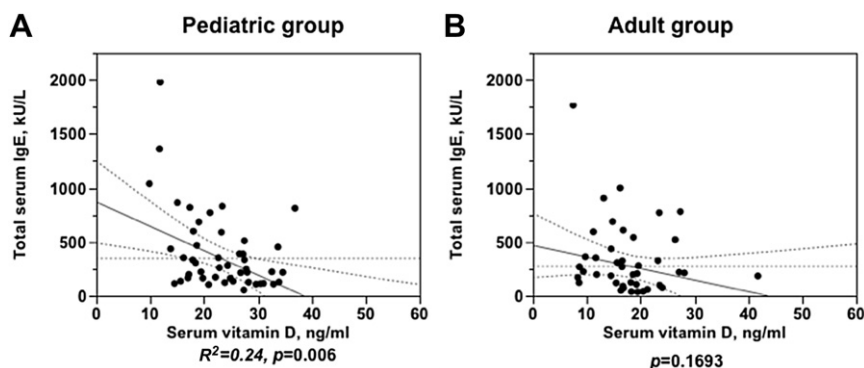


FIG 2. Significant inverse relationship between serum vitamin D and serum IgE levels in the pediatric asthma group (A) but not in the adult asthma group (B).

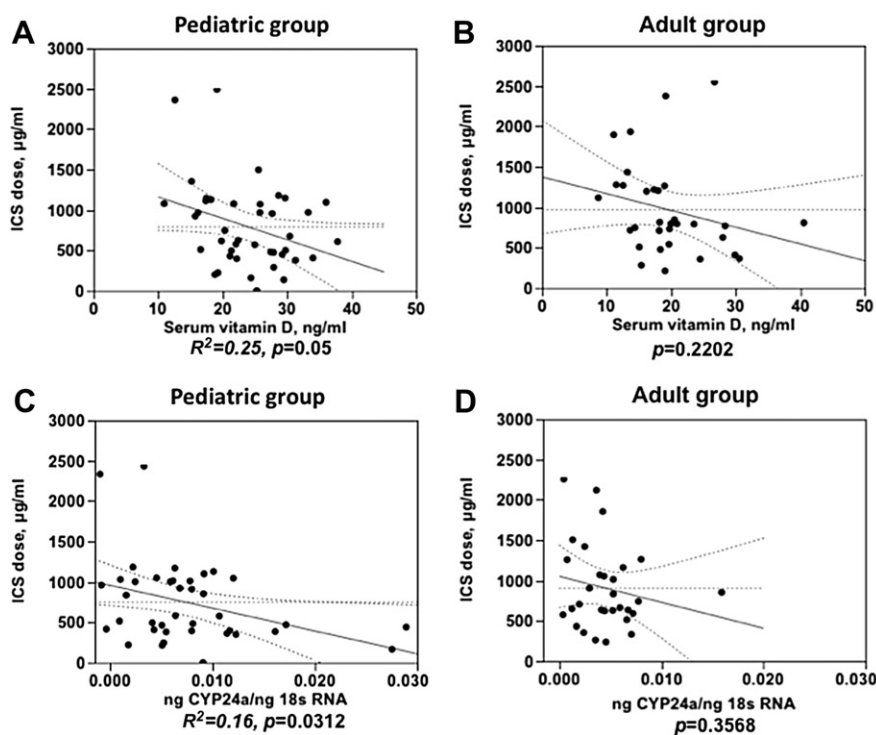


FIG 3. The pediatric asthma group (A and C) but not the adult asthma group (B and D) demonstrates a significant inverse relationship between serum vitamin D levels, PBMC expression of the vitamin D-regulated target cyp24a, and daily ICS dose (in budesonide equivalents).

and IL-13 suppression by DEX in PBMCs *in vitro* was found in the cells from the pediatric asthma group ($R^2 = 0.19$, $P = .05$ and $R^2 = 0.36$, $P = .0094$ for TNF- α and IL-13 suppression by DEX, respectively; Fig 4, A and C) but was not observed in the adult asthma group (Fig 4, B and D).

Can relationships between serum vitamin D levels in the pediatric group be driven by significantly higher proportion of patients with sufficient serum vitamin D levels?

The results of this study demonstrate a higher prevalence of vitamin D insufficiency/deficiency in adults than in children (Table III). Reported associations were re-examined after limiting serum vitamin D levels to less than 30 ng/mL. The associations

between serum vitamin D levels and serum LL-37 levels and between serum vitamin D levels and cyp24a expression by PBMCs were no longer significant in the pediatric asthma group with serum vitamin D levels of less than 30 ng/mL. The association between serum vitamin D and serum LL-37 levels remained significant in the adult asthma group with serum vitamin D levels of less than 30 ng/mL.

If restricted to serum vitamin D levels of less than 30 ng/mL, the following relationships remained significant in the pediatric asthma group: between serum vitamin D and serum IgE levels ($R^2 = 0.38$, $P = .001$), between serum vitamin D level and total ICS dose ($R^2 = 0.36$, $P = .011$), between cyp24 expression by PBMCs and total ICS dose ($R^2 = 0.16$, $P = .05$), and between cyp24 expression by PBMCs and IL-13 suppression by DEX in PBMCs ($R^2 = 0.43$, $P = .012$). The relationship between cyp24

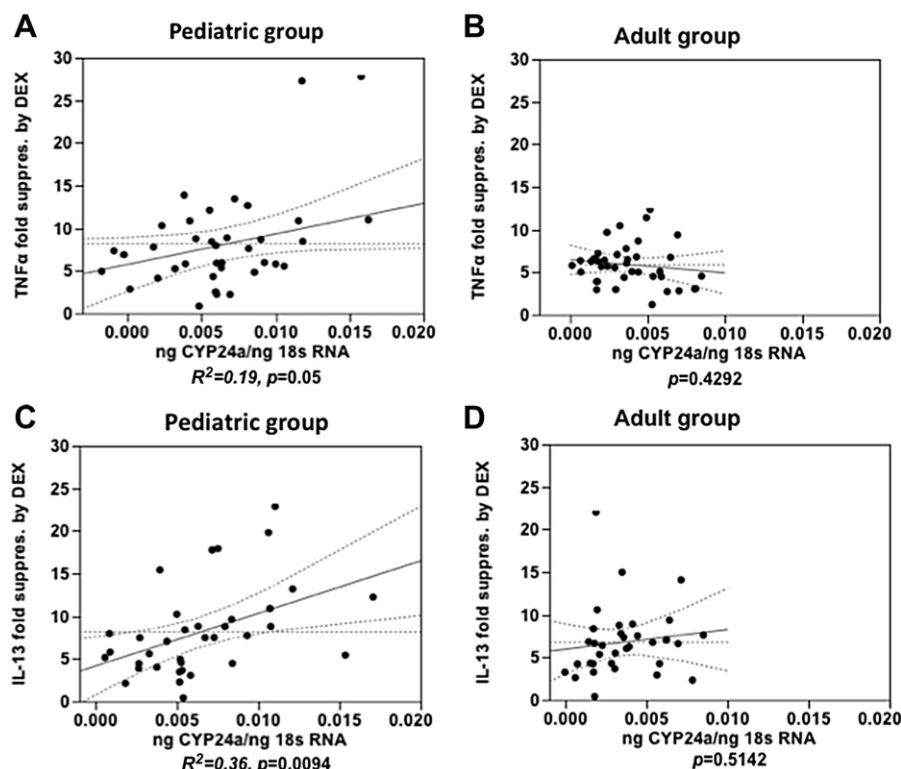


FIG 4. A significant positive correlation between cyp24a expression by PBMCs and the degree of TNF- α and IL-13 suppression by DEX in PBMCs of the pediatric asthma group (**A** and **C**) but not the adult asthma group (**B** and **D**) is shown.

expression by PBMCs and TNF- α suppression by DEX in PBMCs was no longer significant in the pediatric asthma group if the analysis were restricted to subjects with serum vitamin D levels of less than 30 ng/mL. As before, all of these relationships remained nonsignificant in the adult asthma group if the analysis was restricted only to the subjects with insufficient/deficient serum vitamin D levels (<30 ng/mL).

Is there a difference in reported associations in children 6-12 years old and >12 years old?

The data presented in Table III suggest that the prevalence of vitamin D insufficiency/deficiency in children older than 12 years is similar to that seen in adults. We further analyzed the relationships between serum vitamin D levels/PBMC expression of cyp24a and vitamin D response markers, steroid requirements, and *in vitro* responses of PBMCs to corticosteroids in children with asthma who are 6 to 12 years old and greater than 12 years old (Table IV). We found that associations between serum vitamin D and serum LL-37 levels and between serum vitamin D and serum IgE levels were still significant for the 6- to 12-year-old group and trended toward significance in the greater than 12-year-old group. Similarly, the association between PBMC expression of cyp24a with IL-13 suppression by DEX in PBMCs trended toward significance in both age groups of children. In contrast, the associations between serum vitamin D levels and cyp24a expression by PBMCs were only significant in the 6- to 12-year-old children. In addition, the relationship between serum vitamin D level/cyp24a expression by PBMCs and total daily ICS dose trended toward significance only in the younger pediatric age

group (6-12 years old). This was also true for the relationship between cyp24a expression by PBMCs and TNF- α suppression by DEX in PBMCs.

However, the associations between serum vitamin D and serum LL-37 levels, serum vitamin D and serum IgE levels, and PBMC expression of cyp24a and IL-13 fold suppression by DEX in PBMCs remained significant or trended toward significance in both pediatric age groups (Table IV).

DISCUSSION

The current prospective study in asthmatic patients assessed the age-specific relationship between serum vitamin D levels and atopy, steroid requirement, and cellular responsiveness to *in vitro* treatment with corticosteroids. Importantly, this study also assessed, for the first time, the relationship between PBMC expression of vitamin D-inducible cyp24a mRNA as a functional readout of VDR pathway activation, with clinical and laboratory variables designed to evaluate corticosteroid requirements and response to corticosteroids. These relationships were analyzed separately in the adult and pediatric asthma groups. A number of associations were found to be significant in the pediatric but not the adult asthma groups, suggesting that future vitamin D supplementation studies in asthmatic patients should include pediatric populations as a target group. Thus future findings from oral vitamin D supplementation in adult asthmatic patients might not necessarily be generalized to children with asthma.

In this study a significant positive correlation between serum vitamin D levels and the expression of vitamin D-regulated targets, cyp24a expression by PBMCs (pediatric asthma group

TABLE IV. Relationship between serum vitamin D levels/cyp24a expression by PBMCs and vitamin D/corticosteroid response markers in asthmatic children

Predictor	Outcome	Asthmatic patients, children		
		6-12 y (n = 37)		13-17 y (n = 16)
		R ²	P value	P value
Serum vitamin D (ng/mL)	Log serum LL-37 (pg/mL)	0.39	.006	.10
	Cyp24a expression by PBMCs (ng of cyp24a/ng of 18s RNA)	0.23	.016	.86
	IgE (U/mL)	0.21	.023	.056
	ICS dose (μg)		.11	.72
Cyp24a expression by PBMCs (ng of cyp24a/ng of 18s RNA)	ICS dose (μg)		.067	.21
	TNF-α fold suppression by DEX in PBMCs		.069	.28
	IL-13 fold suppression by DEX in PBMCs		.13	.052

only) and serum LL-37 levels (pediatric and adult asthma groups), was observed. Serum vitamin D levels had a greater effect on serum LL-37 levels in the pediatric asthma group compared with those in the adult asthma group. Significant inverse correlation between serum IgE and serum vitamin D levels was observed in the pediatric asthma group only. Serum vitamin D levels and cyp24a expression by PBMCs demonstrated a significant inverse association with the patient's daily ICS dose in the pediatric asthma group only. The degree of *in vitro* TNF-α and IL-13 suppression by DEX in PBMCs from children with asthma positively correlated with the expression of vitamin D-regulated cyp24a by these cells.

The majority of subjects in the current study had serum vitamin D levels of less than 30 ng/mL. Deficient serum vitamin D levels (<20 ng/mL) were present in 47.6% of asthmatic patients and 56.8% of healthy control subjects, with means ± SDs of 20.7 ± 9.8 and 19.2 ± 7.7 ng/mL, respectively. Overall, the prevalence of vitamin D insufficiency/deficiency in subjects with asthma in our study population appeared similar to that in the healthy control subjects who participated in this study. Given these data, the contribution of vitamin D insufficiency as a risk factor for the development of asthma requires further evaluation.

We recently reported on associations between a number of clinical variables and serum vitamin D levels in a pediatric asthma group.¹⁰ The retrospective nature of the data collection made it difficult to create an efficient multivariate model to examine the strength of the correlations noted in univariate analyses. All of these limitations were accounted for in the current prospective study. Race, BMI, age and supplement use (multivitamins, vitamin D supplements, and/or calcium supplements) were identified as significant confounding factors that affect subjects' serum vitamin D levels. The multivariate regression models accounting for these variables in this prospective study continued to show significant relationships between ICS doses and vitamin D levels in children.

To our knowledge, this is the first study that concurrently compared the relationship between steroid requirements and serum vitamin D levels both in children and adults with asthma. Previously, several pediatric studies reported that subjects with deficient serum vitamin D levels are more likely to use anti-inflammatory medications¹⁴ and are likely to have asthma exacerbations despite using ICSs.¹⁷ The study by Searing et al¹⁰ in an unadjusted linear regression model demonstrated that children with asthma receiving ICSs or oral corticosteroids have

significantly lower serum vitamin D levels. The study by Sutherland et al⁹ reported that reduced vitamin D levels in adults with asthma are associated with impaired lung function, increased airway hyperreactivity, and reduced *in vitro* response to corticosteroids in PBMCs. We are not aware of a study that examined the relationship between serum vitamin D levels and ICS doses in adults with asthma. Importantly, our study demonstrated a significant inverse association between patients' ICS doses and serum vitamin D levels. Patients with the lowest serum vitamin D levels required the use of significantly higher doses of ICSs to control their asthma symptoms. One explanation for our findings is that lower vitamin D levels contribute to increasing asthma severity, resulting in a concomitant need to escalate pharmacologic intervention with ICSs. We recently demonstrated that *in vitro* vitamin D has steroid-sparing effects on the cellular *in vitro* response to corticosteroids¹⁰ in healthy subjects, thus presenting the novel concept that vitamin D pathways demonstrate cross-talk with glucocorticoid pathways. This is clinically important because vitamin D insufficiency could promote the need for higher doses of glucocorticoids to achieve treatment effects.

The physiologic effects of the active form of vitamin D (1,25 [OH]₂D₃) are mediated through the VDR. The binding of the biologically active form of vitamin D to the VDR enhances the interaction of the VDR with the retinoid X receptor. The vitamin D-bound VDR-retinoid X receptor heterodimers interact with vitamin D response elements in the promoters of vitamin D-regulated genes. Levels of 1,25(OH)₂D₃ are tightly controlled by a feedback regulation that is designed to rapidly metabolize its excess. According to previous studies, the gene encoding 25-dihydroxyvitamin D₃ 24-hydroxylase, CYP24A, is the strongest known 1,25(OH)₂D₃-responsive gene; it has multiple vitamin D response elements in its promoter. CYP24A belongs to the cytochrome P450 (CYP) family, which encodes a variety of enzymes that are needed in the oxidative metabolism of many endogenous and exogenous compounds. Cyp24a converts the active form of vitamin D into its inactive form.⁶

Considering the high likelihood that vitamin D responses are subject to genetic influence,²⁰ we not only examined the relationship between serum vitamin D levels and a number of clinical and laboratory variables but studied their relationships to a vitamin D-inducible gene (ie, cyp24a mRNA expression) as a functional readout of vitamin D cellular response. In this study we demonstrate that cyp24a expression by PBMCs correlates significantly with serum vitamin D levels in the pediatric asthma group.

A significant positive correlation was found both for serum vitamin D levels and cyp24a PBMC mRNA expression and serum levels of LL-37, another highly vitamin D–inducible target. Importantly, both serum vitamin D levels and cyp24a mRNA expression by PBMCs showed inverse correlation with total ICS requirements of the pediatric asthma group in the current study. The functional effects of these associations were supported by the observation that vitamin D–induced cyp24a mRNA positively correlated with TNF- α and IL-13 mRNA fold suppression in PBMCs *in vitro* by DEX in the pediatric asthma group.

No information about cyp24a polymorphisms were collected in this study, although it has been reported that cyp24a might influence serum vitamin D levels.²⁰ Future studies examining cyp24a as a diagnostic or clinical marker in relationship to vitamin D–mediated responses should take this into consideration.

Serum LL-37 was used as another vitamin D–regulated target in this study. We did not examine the relationship between serum LL-37 levels and atopy markers, PBMC steroid responses, and patients' steroid requirements because serum LL-37 is known to be produced by multiple cell types and a number of vitamin D–independent pathways control LL-37 production by the cells.²² Of interest, the association between serum vitamin D and serum LL-37 levels remained significant in the adult asthma group with serum vitamin D levels of less than 30 ng/mL. The study by Bhan et al²³ also recently reported a significant relationship between serum vitamin D and serum LL-37 levels in healthy adults but only to serum vitamin D levels of less than 32 ng/mL; at greater than this serum vitamin D level, no relationship to serum LL-37 levels was observed. Importantly, Bhan et al²³ reported that a significant change in LL-37 levels was only seen in subjects with the greatest increase in serum vitamin D levels after vitamin D supplementation; in addition, these subjects appeared to be the most deficient in serum vitamin D levels before supplementation with vitamin D.

The regression models performed in this study showed a relationship between the *in vitro* responsiveness to corticosteroids by PBMCs and PBMC expression of the vitamin D–regulated cyp24a mRNA but not serum vitamin D levels. A significant positive correlation between cyp24a expression by PBMCs and the degree of TNF- α and IL-13 suppression by DEX in PBMCs *in vitro* was found in the pediatric asthma group. As for serum vitamin D, a significant positive correlation was found only between serum vitamin D levels and the degree of TNF- α suppression by DEX when the total asthma group was analyzed ($R^2 = 0.14$, $P = .0256$, data not shown). This relationship was no longer significant if the data were subgrouped for the pediatric and adult asthma groups. No relationship between serum vitamin D levels and the degree of IL-13 suppression by DEX was found. These data suggest that measuring cyp24a expression by PBMCs might provide further insights over measuring serum vitamin D levels for the assessment of patients' functional responsiveness to vitamin D and evaluation of patients' PBMCs *in vitro* responsiveness to corticosteroids. These data emphasized that variations in the genes regulating steps in the VDR-activated pathways could obscure the relationship between serum vitamin D levels and the outcomes of interest measured. In addition, it points out that while evaluating the epidemiologic role of vitamin D, it might be worth examining not only patients' serum vitamin D levels but also the cellular responsiveness to vitamin D based on the expression of genes in the VDR-activated pathways.

The results of this study demonstrate a higher prevalence of vitamin D insufficiency/deficiency in adults than in children

(Table III). Importantly, the prevalence of vitamin D insufficiency/deficiency in children older than 12 years is similar to that seen in adults. Because all samples in this study were collected during winter/early spring, when skin production of vitamin D is minimal, the findings emphasize the importance of nutrition and lifestyle factors that determine higher serum vitamin D levels in children. No information about subjects' socioeconomic status was collected in this study.

When the pediatric group was further subgrouped into 2 age groups (ie, 6–12 years old and >12 years old), we found that some of the reported associations remained significant only in the younger age group (6–12 years old, Table IV). Therefore it is possible that in the number of reported associations in children, the significance of the association is driven by the greater proportion of serum vitamin D levels of greater than 30 ng/mL in the 6- to 12-year-old age group.

The fact that the associations between serum vitamin D and serum LL-37 levels and serum vitamin D and serum IgE levels, PBMC expression of cyp24a and IL-13 fold suppression by DEX in PBMCs remained significant or trended toward significance in both pediatric age groups (ie, 6–12 years old and >12 years old), which perhaps points to the potential plasticity of the vitamin D–mediated responses in children because LL-37 and atopy markers are more responsive to serum vitamin D levels in children than in adults. On the basis of these data, we can speculate that vitamin D supplementation in children with asthma could reduce atopy and boost protection from seasonal respiratory tract viruses (serum LL-37 levels).

Several recent studies have suggested that low serum vitamin D levels can influence the severity of asthma, atopy, or both,^{14–16} particularly in the pediatric group. From an analysis of the NHANES 2005–2006 data, it was reported that vitamin D deficiency is associated with higher levels of IgE sensitization in children and adolescents¹⁵ compared with those seen in adults. In a retrospective analysis of the associations between asthma phenotypes and serum vitamin D levels in the cohort of patients from Perth, Australia, it was noted that serum vitamin D levels at age 6 years were significant predictors of subsequent atopy/asthma-associated phenotypes at age 14 years.²⁴ In a nonselected community setting children (particularly boys) with inadequate vitamin D levels were determined to be at increased risk of atopy and, subsequently, bronchial hyperresponsiveness and asthma.²² A recent prospective study demonstrated that the control of newly diagnosed asthma in children can be enhanced by vitamin D oral supplementation.¹⁶ In our study a significant inverse correlation between serum IgE and serum vitamin D levels was observed in the pediatric asthma group but not in the adult groups, also suggesting that vitamin D supplementation might be beneficial in the pediatric group to downregulate allergic responses.

In the current study we also made the novel finding that the associations between serum vitamin D levels and vitamin D–regulated targets and patients' steroid responses *in vitro* and steroid requirements were mainly significant in the pediatric but not the adult asthma group. The basis for these differences in children versus adults are unknown but might have clinical significance because it suggests children might respond better than adults to oral vitamin D supplementation. As stated,¹⁵ because most allergies start in childhood, vitamin D insufficiency in childhood might influence initiation of allergy. On the contrary, adult vitamin D status has no relationship to the patient's IgE level because it is no longer reflective of the patient's vitamin D status in

childhood at the time of allergic sensitization. Further studies are needed to determine whether the VDR and the molecules activated after receptor binding function differently in children versus adults.

We thank Maureen Sandoval and Shih-Yun Lyman for help in preparing this manuscript. We also thank Dr James Murphy and Dr Doug Everett from the Division of Biostatistics at National Jewish Health for consultations and assistance with statistical data analysis. We would like to acknowledge Julie A. Henley, Cristina Campos, Pascuala Pinedo-Estrada, Hollis Hainey, and Gayle Spears for their help with clinical characterization of the study participants.

Clinical implications: The results of this study suggest that vitamin D supplementation in children might enhance corticosteroid responses and control atopy and could thereby improve asthma control.

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