

Letter to the Editor

The association between vitamin D status and the rate of exacerbations requiring oral corticosteroids in preschool children with recurrent wheezing

To the Editor:

Preschool children with recurrent yet intermittent wheezing experience substantial disease morbidity that is primarily related to acute and often severe exacerbations.¹ Recent epidemiologic data suggest that vitamin D status may modulate the risk of these wheezing exacerbations because vitamin D levels are inversely associated with adverse asthma-related outcomes among older children and adolescents.^{2,3} To the best of our knowledge, no study has evaluated whether vitamin D deficiency during early life is a risk factor for exacerbation of wheezing episodes among preschool children who have already developed the recurrent

wheezing phenotype. We conducted this *post hoc* analysis to investigate whether deficient serum vitamin D levels were associated with an increase in the rate of wheezing exacerbations requiring oral corticosteroids (OCS) among a well-defined cohort of preschool children with severe intermittent wheezing participating in the Maintenance Versus Intermittent Inhaled Steroids in Wheezing Toddler (MIST) clinical trial of the Childhood Asthma Research and Education Network.⁴

A detailed description of the MIST trial,⁴ study population, clinical outcome measurements, vitamin D measurements, analysis plan, sample size, and power calculations is given in this article's Methods section in the Online Repository at www.jacionline.org.

Briefly, MIST⁴ was a 1-year multicenter, double-blind, randomized trial comparing daily low-dose budesonide inhalation suspension to intermittent high-dose budesonide starting at the

TABLE I. Baseline characteristics of study population

Characteristic	All participants (n = 264)	Participants with baseline serum 25-OH-VitD level of <20 ng/mL (n = 18)	Participants with baseline serum 25-OH-VitD level of ≥20 ng/mL (n = 246)	P value
Younger children (12-32 mo)*	120 (45.5)	7 (38.9)	113 (45.9)	.56
Sex: male*	184 (69.7)	13 (72.2)	171 (69.5)	.81
Race (white)*	163 (61.7)	5 (27.8)	158 (64.2)	<.01
Height (cm)	94.2 ± 8.9	93.4 ± 8.9	94.3 ± 9	.75
Weight (kg)	15.3 ± 3.1	15.1 ± 3.3	15.3 ± 3.1	.60
Physician diagnosis of asthma*	188 (71.2)	15 (83.3)	173 (70.3)	.24
Rate of wheezing episodes per child in the past year	6.6 ± 5.4	6.1 ± 2.6	6.7 ± 5.6	.95
Rate of urgent/ED visits per child in the past year	4.9 ± 4.2	6.3 ± 4.5	4.8 ± 4.2	.08
Hospitalization in the past year*	50 (18.9)	5 (27.8)	45 (18.3)	.32
Tobacco smoke exposure*	113 (43.3)	13 (72.2)	100 (41.2)	.01
Any asthma controller use in the past year*	184 (69.7)	13 (72.2)	171 (69.5)	.81
Received OCS in the past year*	199 (75.4)	12 (66.7)	187 (76)	.37
≥1 positive food skin test result*	95 (36.5)	9 (52.9)	86 (35.4)	.15
≥1 positive aeroallergen skin test result*	153 (58.4)	13 (72.2)	140 (57.4)	.22
≥1 positive aeroallergen skin test result to outdoor allergen*	120 (45.5)	10 (55.6)	110 (44.7)	.38
≥1 positive aeroallergen skin test result to indoor allergen*	98 (37.1)	6 (33.3)	92 (37.4)	.73
Serum IgE (kU/L), median (Q1-Q3)	58.8 (21.5-183.7)	82.5 (39.1-343.2)	55.3 (20.2-166.8)	.06
Percent eosinophil in CBC, median (Q1-Q3)	3.1 (2-6)	3 (2-8)	3.1 (2-6)	.61
Child ever had eczema*	141 (53.4)	12 (66.7)	129 (52.4)	.24
Presence of allergic rhinitis*	101 (38.3)	9 (50)	92 (37.4)	.29
Parental history of asthma*	160 (63.5)	15 (83.3)	145 (62)	.07
FENO (ppb), median (Q1-Q3)	8.6 (5.7-14)	6.3 (3.9-19.5)	8.6 (5.7-14)	.54
Percent episode-free days	66.9 ± 29.6	77.3 ± 22.5	66.2 ± 29.9	.16
Randomized to intermittent treatment arm*	132 (50)	12 (66.7)	120 (48.8)	.14
Family keep a cat or a dog*	121 (45.8)	5 (27.8)	116 (47.2)	.11
Season during which serum was obtained (season at enrollment)*				
Winter (December-February)	57 (22)	6 (33)	51 (21)	.05
Spring (March-May)	76 (29)	2 (11)	74 (30)	
Summer (June-August)	66 (25)	2 (11)	64 (26)	
Fall (September-November)	65 (25)	8 (44)	57 (23)	
Sites*				
Albuquerque, NM	10 (4)	2 (11)	8 (3)	.02
Denver, Colo	43 (16)	1 (6)	42 (17)	
Madison, Wis	35 (13)	0 (0)	35 (13)	
San Diego, Calif	40 (15)	1 (6)	39 (16)	
St Louis, Mo	74 (28)	10 (56)	64 (26)	
Tucson, Ariz	62 (23)	4 (22)	58 (24)	

Data are expressed as mean ± SE, except as noted.

CBC, Complete blood cell count; ED, emergency department; FENO, fraction of exhaled nitric oxide.

*Data are expressed as number (%).

early signs of respiratory tract illness (RTI) for the prevention of severe respiratory exacerbations requiring OCS. Participants were children aged 12 to 53 months with a history of recurrent severe wheezing. All participants had risk factors for future asthma, as evidenced by a positive modified Asthma Predictive Index.⁵ Institutional review boards at all participating centers approved the MIST protocol, and parents provided written informed consent. The primary outcome measure of MIST, as well as this *post hoc* analysis, was the rate of severe respiratory exacerbations, requiring OCS (prednisolone), over the 1-year study period,⁴ which did not differ between the daily low-dose and intermittent high-dose regimens of inhaled budesonide.⁴

There is a lack of consensus as to the optimal levels of 25-hydroxyvitamin D (25-OH-VitD) to define vitamin D status for conditions other than for the maintenance of bone health, for which The Institute of Medicine recommends a serum 25-OH-VitD level of at least 20 ng/mL.⁶ Moreover, there is a lack of consensus concerning the normal or optimal vitamin D serum levels in various ethnic groups because it was recently reported that compared with whites, black adults had lower total serum vitamin D levels, but these black subjects had similar estimated concentrations of bioavailable vitamin D resulting from lower levels of vitamin D-binding protein.⁷ Previous asthma studies have detected associations between vitamin D levels and asthma outcomes using different serum vitamin D cutoff levels among older children.^{2,3} Because of these uncertainties in defining the appropriate vitamin D cutoffs for respiratory health, the lack of consensus concerning the normal vitamin D serum levels in various ethnic groups, and the absence of previous studies that investigated the relationships between serum vitamin D levels and asthma-related outcomes in preschool children, our primary analysis considered 25-OH-VitD level as a continuous variable, whereas secondary analyses were performed using 25-OH-VitD as a dichotomous variable, with a 25-OH-VitD cutoff of 20 ng/mL.

Baseline serum vitamin D levels were measured in 264 (95%) of the 278 children enrolled in the MIST trial. The mean age of the patients was 35 ± 11 months, 70% of the participants were males, and 62% were white (Table I). The median (Q1-Q3) 25-OH-VitD level was 33.5 ng/mL (26.4-43.7). Eighteen participants (7%) had 25-OH-VitD levels below 20 ng/mL (ie, vitamin D deficiency). Vitamin D-deficient participants were more often non-white (72% vs 36%; $P = .002$) and reported tobacco smoke exposure (72% vs 41%; $P = .010$) compared with the nondeficient participants (Table I). Vitamin D deficiency was more common in samples obtained in winter and fall seasons, although these differences were only marginally significant (Table I).

25-OH-VitD level (as a continuous variable) at the time of study randomization was not associated with the rate of exacerbations requiring OCS therapy over the 1-year trial (pseudo $r^2 = 0.006$; $P = .65$).

Vitamin D-deficient participants had a significantly higher mean rate of exacerbations requiring OCS compared with nondeficient participants (1.46 vs 0.93 exacerbations/child-year, $P = .035$; rate ratio, 1.56; 95% CI, 1.03-2.37). Because of the relatively small number of participants with vitamin D deficiency, adjustment for covariates that significantly differed between the vitamin D-deficient and nondeficient groups was performed for each covariate one at a time (one model included adjustment for race and an additional model included adjustment for tobacco smoke exposure) rather than simultaneously. The rate ratio for OCS treatment remained significant after adjustment for race

TABLE II. Rate ratios (95% CIs) of exacerbations requiring OCS among the children with 25-OH-VitD levels of less than 20 ng/mL relative to children with 25-OH-VitD levels of 20 ng/mL or more

Rate ratio	Rate ratio* of exacerbations requiring OCS	95% CI	P value
Unadjusted	1.56	1.03-2.37	.035
Adjusted for			
Race	1.68	1.09-2.58	.019
Tobacco smoke exposure	1.57	1.02-2.40	.038

*The rate ratio represents the ratio between the rate of exacerbations requiring OCS among the vitamin D-deficient group and the rate of exacerbations requiring OCS among the nondeficient group.

and smoke exposure (Table II). Multiple secondary outcomes did not differ between participants with vitamin D levels of less than 20 ng/mL and participants with vitamin D levels of 20 ng/mL or more (see Table E1 in this article's Online Repository at www.jacionline.org): the rate of RTIs, the rate of RTIs in which a viral etiology was detected by multiplex PCR in the nasal samples obtained during the acute episode (viral RTIs), the rates of emergency department or urgent care visits, and the proportion of episode-free days over the 12-month trial, defined as days without any respiratory symptoms and without use of albuterol. We did not detect interactions between MIST study treatment assignment or race and vitamin D deficiency status on the rate of exacerbations ($P = .3$ and $.6$, respectively). Stratification by race showed that both white and non-white participants who were deficient in vitamin D had numerically higher mean rates of exacerbations requiring OCS compared with nondeficient children; however, this difference was statistically significant only among non-whites (see Table E2 in this article's Online Repository at www.jacionline.org). The lack of statistical significance in the rate of exacerbations among white subjects is most likely a reflection of reduced statistical power to detect such a difference among white subjects, only 5 of whom were deficient in vitamin D. However, we cannot definitively exclude a differential effect of vitamin D deficiency on the basis of race because low serum vitamin D levels among black and white subjects might have different clinical significance resulting from different levels of vitamin D-binding proteins among these 2 ethnic groups.⁷

To the best of our knowledge, this is the first study to demonstrate an association between vitamin D deficiency and significant exacerbations among preschool children with severe but intermittent wheezing, corroborating the findings of increased asthma morbidity among vitamin D-deficient school-age children and adolescents with persistent asthma.^{2,3} Our findings demonstrate that the relationship between significant exacerbations and vitamin D status was evident when a level of 20 ng/mL of 25-OH-VitD was used as the cutoff, whereas no association was demonstrated using 25-OH-VitD as a continuous measure, suggesting a threshold effect of vitamin D level on the outcome of exacerbations in this age group in which serum vitamin D levels of at least 20 ng/mL may be adequate to attenuate the risk of exacerbations, while higher levels may not provide any additional benefits.

Vitamin D deficiency in the United States was reported to be less common among young children than among older children and adolescents.⁸ Accordingly, the prevalence of vitamin D deficiency in our study was only slightly lower than the 12% prevalence

reported among 2 independent cohorts of preschool children in North America: 380 children in the United States⁹ and 508 children in Canada.¹⁰ Lower prevalence of vitamin D deficiency among toddlers in North America might be related to routine vitamin D supplementation among this age group and/or to the presence of vitamin D supplements in dairy products. Our study also revealed a substantially higher prevalence of vitamin D deficiency among non-whites, which is in agreement with the epidemiology of vitamin D deficiency.³ However, a recent report has questioned the clinical significance of low total serum vitamin D levels among black adults.⁷

Our study has the advantages of using a well-characterized cohort of preschool children with severe intermittent wheezing and positive modified Asthma Predictive Index, and of a direct measurement of vitamin D status in participants at study inception as opposed to previous studies that have estimated early life vitamin D status indirectly by measuring maternal serum or cord blood vitamin D levels.¹¹⁻¹³ These previous studies yielded conflicting results regarding the association between maternal vitamin D status and the development of the wheezing phenotype during early life.¹¹⁻¹³ Some study limitations exist. Because vitamin D deficiency was relatively infrequent in the MIST trial, we adjusted for the most relevant potential confounders (race and tobacco smoke exposure) one at a time using separate models rather than analyzing both confounders simultaneously in a single, unstable model. The low number of vitamin D-deficient participants at each Childhood Asthma Research and Education center precluded adjustment for study center because of multivariate model instability. Therefore, although unlikely, we cannot definitively exclude residual bias that contributes to the detection of a higher rate of exacerbations among the vitamin D-deficient children. Because our primary outcome was the rate of exacerbations assessed over the year of the study, which exposed all participants to seasonal variations in vitamin D levels, we did not adjust the rate of severe exacerbation by season at enrollment despite marginally significant variability in the prevalence of vitamin D deficiency by season of enrolment. Finally, on the basis of the cross-sectional nature of this analysis, we cannot determine whether the relationship between vitamin D deficiency and exacerbations noted in this study is causal, nor can we exclude the possible contributions of other factors, such as diet, activity, or other environmental exposures.

In summary, vitamin D deficiency in preschool children with severe intermittent wheezing treated with inhaled corticosteroid therapy was associated with a higher rate of exacerbations requiring OCS. While the association between vitamin D deficiency and exacerbations was statistically significant only among non-white children, the relevance of these ethnic differences remains uncertain because the use of a single reference value to discriminate vitamin D deficiency in white and black subjects may be inappropriate.⁷ The association between vitamin D levels and the risk of exacerbations was significant only among children with serum 25-OH-VitD levels of less than 20 ng/mL, suggesting that future studies of vitamin D supplementation as an intervention for the prevention of wheezing episodes might need to focus on this subgroup of children.

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METHODS

Study participants

The Childhood Asthma Research and Education Network performed a post hoc retrospective analysis of data from the MIST clinical trial.^{E1} Detailed descriptions of the screening, recruitment, design, outcomes, and statistical analysis for the MIST trial have been reported in detail elsewhere.^{E1} Briefly, MIST^{E1} was a 1-year multicenter, double-blind, randomized trial comparing daily low-dose budesonide inhalation suspension (0.5 mg nightly) to intermittent high-dose budesonide inhalation suspension (1 mg twice daily for 7 days) starting at the early signs of RTI for the prevention of severe respiratory exacerbations requiring OCS.

Participants were children aged 12 to 53 months with recurrent wheezing who experienced at least 4 episodes of wheezing in the year before randomization (or at least 3 episodes if treated with an asthma controller medication for at least 3 months), with at least 1 exacerbation requiring the use of systemic corticosteroids, urgent care visit, or hospitalization in the previous year. All participants had risk factors for future asthma, as evidenced by a positive modified Asthma Predictive Index.^{E2} Children with persistent asthma symptoms were ineligible to participate. The MIST study results showed that a daily low-dose regimen of inhaled budesonide was not superior to an intermittent high-dose regimen of inhaled budesonide in reducing exacerbations.^{E1}

Institutional review boards at all participating centers approved the MIST protocol, and parents provided written informed consent.

Outcome measures

The primary outcome measure of the MIST trial and the outcome of this *post hoc* analysis was the rate of severe respiratory exacerbations over the 1-year study period. A severe exacerbation was defined as an episode of lower respiratory tract symptoms for which an oral glucocorticoid (prednisolone) was started after consultation with a study physician (by telephone or in person) according to a specific protocol.^{E1,E3,E4}

The Institute of Medicine concluded that serum 25-OH-VitD levels of at least 20 ng/mL are sufficient to maintain appropriate bone health.^{E5} However, there is a lack of consensus as to the optimal levels of 25-OH-VitD to define vitamin D status for conditions other than bone health. Previous asthma studies among older children have detected associations with asthma outcomes using serum vitamin D cutoff levels of 20 ng/mL (vitamin D deficiency),^{E6} 30 ng/mL,^{E7-E9} or while considering vitamin D as a continuous variable.^{E10} Because of these uncertainties in determining the appropriate vitamin D cutoffs, along with the absence of previous studies that investigated serum vitamin D levels on asthma-related outcomes in preschool children, our primary analysis investigated the association between 25-OH-VitD level as a continuous variable and the study outcomes. In addition, given the possibility of a threshold effect of 25-OH-VitD levels on respiratory outcomes, we then performed analyses using 25-OH-VitD as a dichotomous variable, with a 25-OH-VitD cutoff of 20 ng/mL.

Vitamin D level measurements

Vitamin D levels were measured in serum samples obtained on enrollment using a direct competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25OHD Total assay.^{E11,E12}

Analysis plan, sample size, and power calculations

Regression models were used to examine potential relationships between baseline vitamin D levels and the study outcomes. Log-linear regression models based on the Poisson distribution were used for the frequency of exacerbations. The length of follow-up time from randomization to study termination was used as an offset so that model results could be interpreted as rates per child-year. Strength of association was quantified by the R^2 statistics for the ordinary linear regression models and by the pseudo R^2 statistics for the Poisson regression models.^{E13} Secondary analyses examining vitamin D as a dichotomous variable used Poisson regression models for frequency outcome and ANOVA for continuous outcomes. The total sample size was fixed by availability of serum samples. Power calculations based on the observed rate of exacerbations in the MIST trial indicated that a sample size of 264 would provide 90% power to detect a relative rate of less than 0.83 or greater than 1.2 per 20 ng/mL change in vitamin D level. All analyses were carried out using the SAS statistical software system version 9.2 (SAS, Inc, Cary, NC).

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TABLE E1. Secondary outcomes during the trial based on baseline 25-OH-VitD level

Outcome	Participants with baseline 25-OH-VitD levels of <20 ng/mL (n = 18)	Participants with baseline 25-OH-VitD levels of ≥20 ng/mL (n = 246)	P value
Rate (95% CI) of RTI*	3.15 (2.41-4.11)	3.55 (3.3-3.81)	.4
Rate (95% CI) of viral RTI*†	1.81 (1.27-2.57)	2.5 (2.29-2.72)	.08
Rate (95% CI) of urgent/ED visits	0.53 (0.27-1.01)	0.34 (0.27-0.43)	.21
Proportion (95% CI) of EFDs	0.83 (0.75-0.92)	0.78 (0.76-0.81)	.29

ED, Emergency department; EFD, episode-free day.

*Measured as the number of illnesses per child-year.

†Viral RTI: RTI in which viral etiology was detected by multiplex PCR.

TABLE E2. Rate of exacerbations (95% CIs) requiring OCS among children with 25-OH-VitD levels of less than 20 ng/mL relative to children with 25-OH-VitD levels of 20 ng/mL or more, stratified by race

Participants' race	Participants with baseline 25-OH-VitD levels of <20 ng/mL		Participants with baseline 25-OH-VitD levels of ≥20 ng/mL		P value
	No. of participants	Rate (95% CI)	No. of participants	Rate (95% CI)	
Entire cohort (n = 264)	18	1.46 (0.99-2.16)	246	0.93 (0.81-1.07)	.035
White subjects (n = 163)	5	1.40 (0.63-3.11)	158	0.99 (0.84-1.18)	.416
Non-white subjects (n = 101)	13	1.48 (0.94-2.32)	88	0.82 (0.63-1.05)	.024