

# Vitamin D<sub>3</sub> Supplementation and Childhood Diarrhea: A Randomized Controlled Trial



**WHAT'S KNOWN ON THIS SUBJECT:** Hypovitaminosis D is common among children. Although there is prolific biochemical literature linking vitamin D to enteric immunologic function, there is a paucity of prospective data exploring the role of supplementation in prevention of diarrheal illnesses.



**WHAT THIS STUDY ADDS:** In a high-risk population, quarterly supplementation with 100 000 IU of vitamin D<sub>3</sub> did not reduce the risk for first or recurrent diarrheal illnesses in a population of children aged 1 to 29 months in a low-income inner city setting.

## abstract

**OBJECTIVE:** To investigate the effect of vitamin D<sub>3</sub> supplementation on the incidence and risk for first and recurrent diarrheal illnesses among children in Kabul, Afghanistan.

**METHODS:** This double-blind placebo-controlled trial randomized 3046 high-risk 1- to 11-month-old infants to receive 6 quarterly doses of oral vitamin D<sub>3</sub> (cholecalciferol 100 000 IU) or placebo in inner city Kabul. Data on diarrheal episodes ( $\geq 3$  loose/liquid stools in 24 hours) was gathered through active and passive surveillance over 18 months of follow-up. Time to first diarrheal illness was analyzed by using Kaplan-Meier plots. Incidence rates and hazard ratios (HRs) were calculated by using recurrent event Poisson regression models.

**RESULTS:** No significant difference existed in survival time to first diarrheal illness (log rank  $P = .55$ ). The incidences of diarrheal episodes were 3.43 (95% confidence interval [CI], 3.28–3.59) and 3.59 per child-year (95% CI, 3.44–3.76) in the placebo and intervention arms, respectively. Vitamin D<sub>3</sub> supplementation was found to have no effect on the risk for recurrent diarrheal disease in either intention-to-treat (HR, 1.05; 95% CI, 0.98–1.17;  $P = .15$ ) or per protocol (HR, 1.05; 95% CI, 0.98–1.12;  $P = .14$ ) analyses. The lack of preventive benefit remained when the randomized population was stratified by age groups, nutritional status, and seasons.

**CONCLUSIONS:** Quarterly supplementation with vitamin D<sub>3</sub> conferred no reduction on time to first illness or on the risk for recurrent diarrheal disease in this study. Similar supplementation to comparable populations is not recommended. Additional research in alternative settings may be helpful in elucidating the role of vitamin D<sub>3</sub> supplementation for prevention of diarrheal diseases. *Pediatrics* 2013;132:e832–e840

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### KEY WORDS

diarrhea prevention, children, vitamin D supplementation, Afghanistan

### ABBREVIATIONS

CI—confidence interval  
HR—hazard ratio  
VDR—vitamin D receptor  
WAZ—weight-for-age z-score

Dr Aluisio designed the analysis plan, analyzed the data, and drafted the manuscript; Dr Manaseki-Holland conceptualized and designed the study, directed the study execution, and drafted the manuscript; Dr Chandramohan conceptualized and designed the study and drafted the manuscript; Dr Ensink designed the analysis plan, analyzed the data, and drafted the manuscript; Drs Bhutta and Mughal conceptualized and designed the study and drafted the manuscript; Dr Walraven conceptualized and designed the study and critically reviewed the manuscript; Ms Bruce analyzed the data and critically reviewed the manuscript; Dr Masher conceptualized and designed the study and critically reviewed the manuscript; Dr Maroof managed the study execution, analyzed the data, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

This trial has been registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (identifier NCT00548379).

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Globally 2.5 billion diarrheal illnesses occur in children <5 years of age, resulting in 1.8 million deaths annually.<sup>1,2</sup> Although reductions in incidence have been made in resource-limited settings,<sup>3</sup> the disease burden associated with recurrent enteric illnesses remains an immense public health problem,<sup>4</sup> resulting in malnutrition, disability, and mortality among children.<sup>5–7</sup>

To combat diarrheal illnesses the World Health Organization recommends improved child nutrition and micronutrient supplementation.<sup>8</sup> Zinc and vitamin A have been shown to reduce the incidence of diarrheal illnesses.<sup>9,10</sup> Stemming from the immunologic properties of vitamin D, it represents an additional micronutrient that may have a role in the prevention of childhood diarrheal diseases.

Levels of 1,25(OH)<sub>2</sub>D, the active form of vitamin D, are increased by the enzymatic activities of CYP27B1-hydroxylase and reduced by CYP24A1-hydroxylase.<sup>11</sup> It is postulated that the kinetics of that equilibrium control tissue-specific paracrine activities of vitamin D.<sup>12</sup> Immunologic activation of toll-like receptors on macrophages by pathogens augments intracellular expression of CYP27B1-hydroxylase and vitamin D receptor (VDR) genes. Subsequently, with sufficient cytosolic concentrations of 25(OH)D, CYP27B1-hydroxylase produces 1,25(OH)<sub>2</sub>D. Binding of the 1,25(OH)<sub>2</sub>D-VDR complex to DNA response elements upregulates expression of the antimicrobial peptides cathelicidin and  $\beta$ -defensin, intracellular modulators that are ubiquitously expressed in the gastrointestinal tract.<sup>13–15</sup> Thus in vitamin D-deficient individuals innate immune activity may be impaired, thereby enhancing susceptibility to intracellular diarrhoeogenic pathogens. Additionally, in vitro research of adaptive immunity has shown that the 1,25(OH)<sub>2</sub>D-VDR complex induces CCR10 expression in

terminally differentiated B cells.<sup>16</sup> CCR10 functions in cellular homing of immunoglobulin A-secreting cells to enteric tissues.<sup>17</sup> Given the importance of immunoglobulin A in adaptive mucosal immunity, vitamin D deficiency could result in impaired host abilities to mount pathogen-focused immune responses to diarrhoeogenic microbes.

Numerous observational studies from adults and children reporting associations between vitamin D deficiency and increased risk for infectious diseases exist in the literature,<sup>18–20</sup> and a recent prospective cohort found that deficiency was associated with increased rates of diarrheal illnesses among school-aged children.<sup>21</sup> However, in the published literature only 1 small clinical trial exists that assesses the effects of vitamin D supplementation on diarrheal illnesses. In that study children hospitalized with acute diarrhea in Dhaka, Bangladesh were randomized to daily supplementation with 1000 IU vitamin D ( $n = 27$ ) or placebo ( $n = 15$ ). Supplementation was not found to reduce stool weight or time to clinical resolution.<sup>22</sup> Although a null study, the small sample size and short duration of follow-up (5 days) make it difficult to draw conclusions from this single work on the role of vitamin D supplementation.

Although appropriate levels for immunologic function have yet to be identified, vitamin D deficiency in relation to skeletal metabolism for children is defined as a serum 25(OH)D level <50 nmol/L.<sup>23,24</sup> It is estimated that more than a billion people worldwide are deficient,<sup>25</sup> and the prevalence of hypovitaminosis D is great among children in resource-limited settings.<sup>26,27</sup> A previous survey in Kabul, Afghanistan from a population similar to that in the current trial found that 73% of children were vitamin D deficient.<sup>28</sup>

Afghanistan has a high burden of diarrheal disease; in 2007 more than

82 000 child deaths were attributed to diarrhea.<sup>8</sup> The disease burden coupled with the high prevalence of childhood deficiency in Afghanistan provided an optimum setting to investigate the effects of supplementation on diarrheal illness. We aimed to assess in a randomized controlled trial the effects of quarterly supplementation with 100 000 IU of vitamin D<sub>3</sub> (cholecalciferol) on the risk for recurrent diarrheal illnesses among children in Kabul.

## METHODS

### Ethics Statement

This study was approved by the Ethics and Review Board of the Ministry of Public Health of Afghanistan (Reference: 422328) and the Ethics Committee of the London School of Hygiene and Tropical Medicine (Application no. 5117). Written informed consent was obtained from the mother and father or other head of household for all enrolled children.

### Participants, Randomization, and Intervention

This was an individually randomized placebo-controlled trial undertaken between November 2007 and June 2009 in 5 inner-city districts of Kabul. The trial was primarily designed to evaluate the impact of quarterly supplementation with 100 000 IU of vitamin D<sub>3</sub> on the incidence of childhood pneumonia. A secondary a priori end point planned in the original protocol was to evaluate the effect of supplementation on diarrheal illnesses. Infants residing in the study districts aged 1 to 11 months were recruited at home, randomized to receive either oral vitamin D<sub>3</sub> or placebo at 3-month intervals, and followed for 18 months. At the administration of each dose, study personnel crosschecked participant identification numbers and monitored for acute adverse events (integumentary and respiratory symptoms). The participants,

randomization, and intervention details are described in greater detail elsewhere.<sup>29</sup>

### Diarrheal Outcome and Follow-up

Diarrheal episodes were defined using the World Health Organization criterion of a child having 3 or more loose/liquid stools in a 24-hour period.<sup>8</sup> Active surveillance was achieved through 2-weekly home visits by fieldworkers trained in Integrated Management of Childhood Illness.<sup>30</sup> During visits, children underwent a physical examination and their health history since the previous evaluation was ascertained by caregiver report. Recall of child defecation history was based on the 24-hour period preceding each visit. Passive surveillance was undertaken at the Maiwind Teaching Hospital, the primary health center serving the study districts. At the hospital clinic, all presenting study children were examined and treated by experienced pediatricians trained in Integrated Management of Childhood Illness. During each clinical evaluation, standardized data forms assessing the number of loose/liquid stools during the 24 hours before presentation were completed. Nutritional status was assessed using weight-for-age z-scores (WAZ) calculated through data gathered at 4 time points during follow-up. Children found to have  $WAZ \leq -2$  to  $> -3$  were categorized as moderately malnourished, and those who had a  $WAZ \leq -3$  were deemed severely malnourished.<sup>31</sup>

### 25(OH)D Serological Assessment

To assess serum 25(OH)D levels, blood samples were collected from randomly selected subsets of participants at 5 time points. Baseline serum calcifediol concentrations were derived from samples drawn from children randomized to the placebo arm 1 week after the first round of trial

dosing. Samples were analyzed at the Manchester Royal Infirmary, Supra-Regional Vitamin D Reference Laboratories (accredited to ISO9001:2000 and ISO13485:2003, Vitamin D External Quality Assurance Scheme) where IDS-iSYS Multi-Discipline Automated Chemiluminescent assays for 25(OH)D were completed (Immunodiagnostic Systems Ltd, Bolton, UK).<sup>29</sup>

### Statistical Analysis

The trial sample size was based on the outcome measure of first episode of pneumonia.<sup>29</sup> In the current study, applying the estimated global diarrheal incidence of 2.9 episodes/child-year<sup>3</sup> to the 14 896 episodes observed over follow-up allowed for identification of a  $<4.5\%$  effect change between allocation arms. This estimate assumed 95% significance with 80% power in recurrent events analysis.

Robustness of randomization and participant loss-to-follow-up were assessed by using independent sample *t* and Pearson  $\chi^2$  tests. Child health records from home and clinic visits were triangulated with duplicate records (same date of follow-up) removed. In estimating child-time at risk, participants were censored 4 days after each episode to account for mean illness duration.<sup>32–34</sup> Diarrheal episodes with repeat visits were excluded. Children absent from surveillance for  $>45$  days were censored at the time of their last recorded contact, and if subsequently seen were re-entered into follow-up at that time.

The median time to first diarrheal episode was calculated for each study arm. Kaplan–Meier analyses were performed with differences in time to first events evaluated by using log rank tests. Cox proportional hazard models were used to assess the effect of vitamin D<sub>3</sub> supplementation on risk for first diarrheal illness.

A recurrent events intention-to-treat survival analysis, using all randomized children, was undertaken to investigate the effectiveness of vitamin D<sub>3</sub> supplementation in reducing the risk for diarrheal illnesses. An additional per-protocol recurrent events survival analysis was also performed to investigate the efficacy of supplementation. In the per-protocol analysis, children were censored 3 months after their last received dose.

Poisson regressions with random effects models accounting for participant heterogeneity were used in all recurrent events survival analyses. Child-years at risk and hazard ratios (HRs) were derived from the regression models. To account for changes in the incidence of diarrheal illness that occur with development, analyses were stratified by child age groups as:  $\leq 6$  months,  $>6$  months to  $\leq 12$  months, and  $>12$  months of age.<sup>35</sup> To control for the effect of climate variation on diarrheal disease, comparisons were stratified by season.<sup>36,37</sup> As malnutrition is a known risk factor for diarrheal mortality and persistent illness, the population was analyzed based on WAZ strata as:  $WAZ > -2$ ,  $WAZ \leq -2$  to  $> -3$ , and  $WAZ \leq -3$ .<sup>38,39</sup> The lowest calculated WAZ found during follow-up was used in analyses. To assess for effect in more severe cases, episodes diagnosed clinically were subanalyzed.

### RESULTS

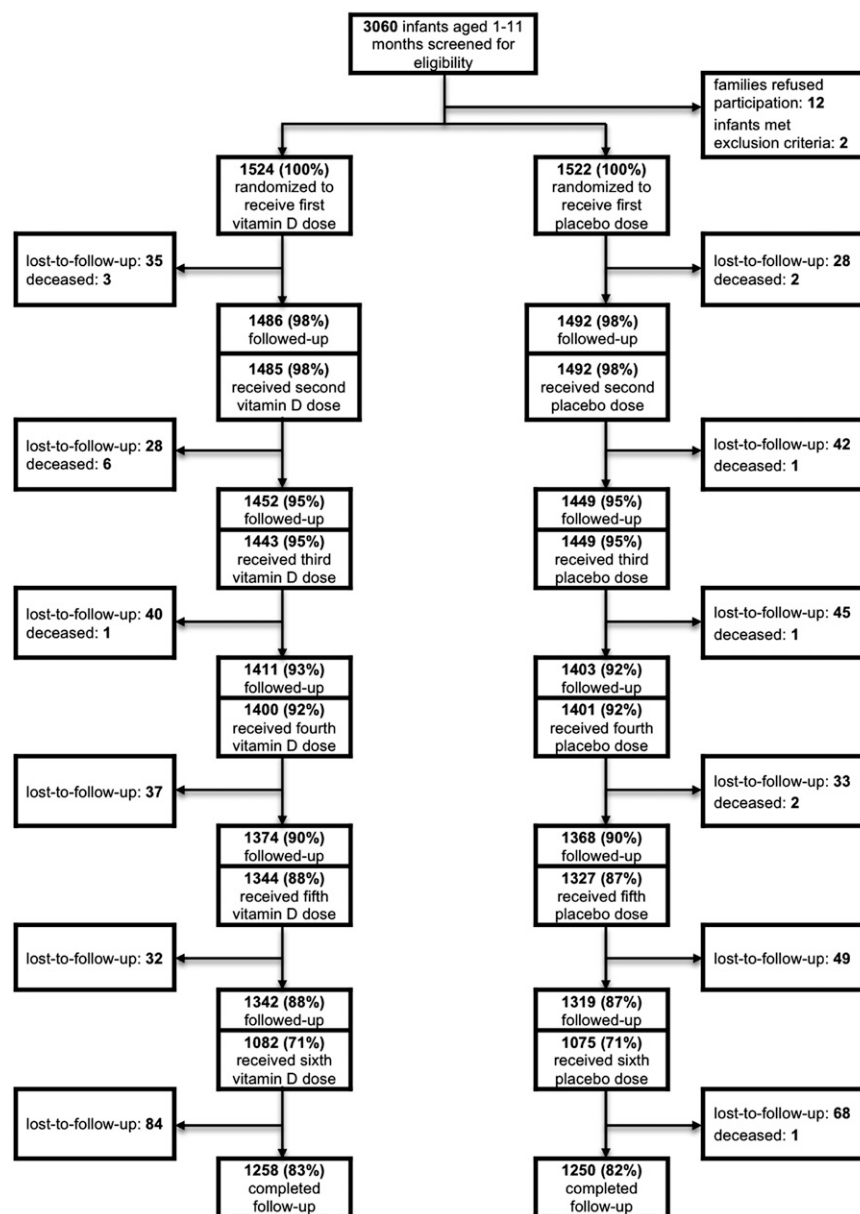
There were 1524 and 1522 infants randomized to vitamin D<sub>3</sub> supplementation or placebo, respectively. The proportion of children receiving all 6 doses of either vitamin D<sub>3</sub> or placebo was 71% in each study arm ( $P = .80$ ). At completion, 2507 (82.3%) children remained in follow-up with no significant difference in attrition between arms ( $P = .78$ ) (Fig 1). There were 17 child deaths, none of which were attributed to diarrheal disease or

vitamin D<sub>3</sub> supplementation. No acute side effects or adverse events were observed with supplementation during the study follow-up period.<sup>29</sup>

At enrollment, child age, gender, breastfeeding characteristics, parental education levels, familial socioeconomic status, and sun exposure were balanced between study arms. The proportion of children who had moderate

or severe malnutrition did not differ significantly between randomization arms ( $P = .59$ ). In the control group, 17.0% and 5.1% were found to be moderately or severely malnourished, respectively, versus 15.7% and 5.5% of participants in the intervention arm. Additionally, there were no significant differences between the 2 groups in maternal hand-washing before eating,

type of sanitation used by the household, or home water source (Table 1). The baseline serum calcifediol concentration among children randomized to the control arm was 18.1 nmol/L (95% confidence interval [CI], 15.6–20.7). Mean serum calcifediol concentrations were significantly higher in the intervention group at 1 week and 3 months post-administration of the



**FIGURE 1**  
Trial profile.

**TABLE 1** Household Water and Sanitation Characteristics

Characteristic	Vitamin D ( <i>n</i> = 1524)	Placebo ( <i>n</i> = 1522)	Loss to Follow-Up ( <i>n</i> = 534)
Maternal hand-washing with soap before eating			
No	577 (52.2)	559 (51.0)	100 (42.2)
Yes	528 (47.8)	537 (49.0)	137 (57.8)
Toilet type			
Open toilet/ground	1189 (88.2)	1191 (87.9)	223 (86.4)
Toilet (septic)	89 (6.6)	102 (7.5)	191 (7.1)
Toilet (canalized)	70 (5.2)	62 (4.6)	132 (4.9)
Water source			
Pipe	603 (46.8)	628 (48.2)	91 (36.6)
Open well	373 (29.0)	369 (28.3)	79 (31.7)
Tube well	312 (24.2)	306 (23.5)	79 (31.7)

All data are expressed as *n* (%).

initial trial dose (winter season) and at 2 weeks post-administration of the third dose (spring season).<sup>29</sup>

There were 14 896 episodes of diarrheal illnesses over 4200 child-years of follow-up. A total of 6090 (40.9%) episodes were from home-visit data and 8806 (59.1%) were diagnosed at the study hospital. No difference in diagnosis of episodes existed between randomization arms in either the home visit ( $P = .41$ ) or hospital ( $P = .23$ ) settings (Table 2). The median time to first diarrheal episode was 136 days (95% CI, 129–143) among children in the intervention arm and 143 days (95% CI, 134–149) for those in the control group. The difference in survival time was not significant (log rank  $P = .55$ ) (Fig 2). Cox regression analysis showed no difference between the groups in the risk for a first diarrheal episode (HR, 1.02; 95% CI, 0.95–1.11;  $P = .56$ ).

In intention-to-treat analysis among children randomized to the control arm, the incidence of diarrheal illness was 3.43 episodes/child-year (95% CI, 3.28–3.59) and among the intervention group was 3.59 (95% CI, 3.44–3.76). In

recurrent event analysis, the risk for diarrheal illness among children who received vitamin D<sub>3</sub> did not differ significantly from those who receive placebo (HR, 1.05; 95% CI, 0.98–1.17;  $P = .15$ ). This relationship was maintained when the analyses were stratified by child age group and nutritional status. Furthermore, vitamin D<sub>3</sub> supplementation showed no statistically significant benefit in prevention when the recurrent events were analyzed by seasons (Table 3). When analyzing illnesses diagnosed in the hospital setting there was no benefit found with supplementation (HR, 1.03; 95% CI, 0.98–1.09;  $P = .25$ ).

In the per-protocol analysis, the incidence of diarrheal episodes among the control and intervention groups was 3.46 (95% CI, 3.30–3.61) and 3.62 episodes/child-year (95% CI, 3.46–3.79), respectively, with no significant difference in disease risk (HR, 1.05; 95% CI, 0.98–1.12;  $P = .14$ ). No significant reduction in risk was found when analyzed by age groups, seasons, or nutritional status (Table 4). In sub-analysis, using only hospital-diagnosed

episodes, no preventive benefit was identified (HR, 1.03; 95% CI, 0.98–1.09;  $P = .26$ ).

Among those receiving supplementation, the risk for recurrent diarrheal illnesses was higher during the autumn months (September to November). This was found in both the intention-to-treat (HR, 1.11; 95% CI, 1.01–1.22;  $P = .03$ ) and per protocol survival analyses (HR, 1.11; 95% CI, 1.01–1.22;  $P = .03$ ) (Tables 3 and 4).

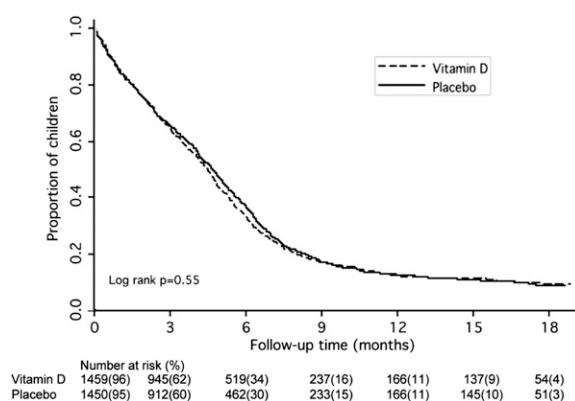
## DISCUSSION

This is the first report of a sufficiently powered randomized controlled trial investigating the effect of vitamin D supplementation on diarrheal incidence in young children. Our findings demonstrate that among children at high risk, 1 to 29 months of age, quarterly supplementation with 100 000 IU of vitamin D<sub>3</sub> has no effect on the risk for diarrheal illnesses. The lack of an effect with supplementation was consistent in intention-to-treat and per-protocol analyses, and furthermore was maintained when the population was stratified by age groups, nutritional status, and seasons.

This study has several strengths. The sample size was large, randomization was robust, and loss to follow-up was low. Disease surveillance from the trial resulted in overall and age-specific incidences that are concordant with child diarrheal disease estimates for resource-limited settings,<sup>3</sup> and the large number of recurrent events facilitated high power to detect differences between groups. Recall of defecation frequency was limited to 24 hours before assessment, which should have enhanced disease identification and provided validity in the results,<sup>40</sup> as shorter recall periods have been shown to improve accuracy in diarrheal reporting.<sup>41,42</sup> Supplementation was demonstrated to be successful in increasing serum 25(OH)

**TABLE 2** Diarrheal Episodes

	<i>N</i>	Vitamin D, <i>n</i> (%)	Placebo, <i>n</i> (%)
Overall population	14 896	7629 (51.2)	7267 (48.8)
In-hospital	8806	4537 (51.5)	4269 (48.5)
Home visit	6090	3092 (50.8)	2998 (49.2)

**FIGURE 2**

Survival time to the first diarrheal episode.

D levels in the intervention arm as compared with the control arm, suggesting that the lack of effect was not likely attributable to methodologic errors or ineffective supplementation in the trial.<sup>29</sup>

There are limitations to this study. Continuous active surveillance for diarrheal illnesses was not possible and, as a result, some episodes may have been missed. Although a limitation for accuracy of population incident rate estimates, such nondifferential error would equally reduce outcome surveillance in both randomization arms and not bias the intergroup comparison.<sup>43</sup>

Additionally, all enrolled children had continuous access to hospital services and families were encouraged to seek care, which should have functioned to improve identification of episodes. Samples to identify microbiologic causes were not collected in this trial and therefore the effect of supplementation on differing etiologies of childhood diarrheal illnesses could not be explored. Stemming from the multiple immunologic mechanisms associated with vitamin D<sup>13–17</sup> it is feasible that microbes eliciting differing immune responses (ie, an innate versus adaptive

predominance) could have differential outcomes with supplementation, and further research is required to investigate this possibility. Although no differences in feeding parameters existed between groups,<sup>29</sup> the data are inadequate to assess temporal changes in breastfeeding status throughout follow-up. It is possible that differences in feeding known to alter the risk profile of diarrheal illness in children existed, but in light of the robust randomization, differences were likely to have been equal between the 2 arms of the trial.<sup>44</sup>

Biochemical research has demonstrated that vitamin D has innate and adaptive paracrine immune functions, and observational studies have shown that deficiency may be associated with an increased risk for infectious diseases.<sup>13–20</sup> A recent prospective cohort of school-age children (mean age, 8.9 years) from Bogota, Colombia found a twofold increased risk for gastrointestinal illnesses among subjects who were vitamin D deficient, suggesting that supplementation may be beneficial in older children.<sup>21</sup> In this work, quarterly supplementation to children 1 to 29 months of age with 100 000 IU of

**TABLE 3** Effect of Vitamin D<sub>3</sub> Supplementation on Repeat Episodes of Diarrheal Illnesses<sup>a</sup>

	Vitamin D			Placebo			HR (95% CI)	P Value
	Events	Child-Years	Incidence per Child-Year (95% CI)	Events	Child-Years	Incidence per Child-Year (95% CI)		
Overall population	7629	2100.0	3.43 (3.28–3.59)	7267	2100.0	3.59 (3.44–3.76)	1.05 (0.98–1.17)	.15
Age group <sup>a</sup>								
≤6 mo	280	114.1	2.51 (2.18–2.89)	283	110.6	2.59 (2.45–2.98)	0.97 (0.80–1.18)	.76
>6 to ≤12 mo	2020	493.9	3.97 (3.73–4.22)	1893	498.7	3.68 (3.45–3.92)	1.08 (0.99–1.18)	.10
>12 mo	5329	1500.0	3.53 (3.36–3.71)	5091	1500.0	3.34 (3.20–3.54)	1.05 (0.98–1.13)	.19
Season <sup>b,c</sup>								
Summer	1989	338.1	5.89 (5.57–6.22)	1950	342.5	5.73 (5.41–6.05)	1.03 (0.95–1.11)	.48
Autumn	1492	389.4	3.86 (3.62–4.12)	1330	384.5	3.49 (3.26–3.73)	1.11 (1.01–1.22)	.03
Winter	1536	709.1	2.17 (2.03–2.33)	1480	708.9	2.10 (1.96–2.25)	1.04 (0.94–1.14)	.48
Spring	2612	669.6	3.93 (3.73–4.15)	2507	667.6	3.76 (3.56–3.98)	1.04 (0.97–1.13)	.26
Nutritional status <sup>d</sup>								
WAZ > -2	5543	1600.0	3.44 (3.27–3.63)	5210	1600.0	3.29 (3.12–3.47)	1.05 (0.97–1.13)	.23
WAZ ≤ -2 to > -3	1421	346.4	4.09 (3.67–4.55)	1467	377.6	3.88 (3.50–4.30)	1.05 (0.91–1.22)	.49
WAZ ≤ -3	523	120.9	4.32 (3.58–5.20)	450	114.1	3.94 (3.25–4.79)	1.09 (0.84–1.43)	.51

<sup>a</sup> Effectiveness represents intention-to-treat analyses and used all available data regardless of protocol violations.

<sup>b</sup> Children contributed data to all age groups or season during which they were followed.

<sup>c</sup> Seasons were defined as summer: June, July, and August; autumn: September, October, and November; winter: December, January, and February; and spring: March, April, and May.

<sup>d</sup> Nutritional status was based on WAZ calculated by using WHO child growth standards.<sup>51</sup>

**TABLE 4** Efficacy of Vitamin D<sub>3</sub> Supplementation for Prevention of Repeat Diarrheal Illnesses<sup>a</sup>

	Vitamin D			Placebo			HR (95% CI)	P Value
	Events	Child-Years	Incidence per Child-Year (95% CI)	Events	Child-Years	Incidence per Child-Year (95% CI)		
Overall population	7570	2100	3.62 (3.46–3.79)	7198	2100	3.46 (3.30–3.61)	1.05 (0.98–1.12)	.14
Age group <sup>b</sup>								
≤6 mo	280	114.1	2.51 (2.18–2.89)	283	110.6	2.59 (2.25–2.98)	0.97 (0.80–1.18)	.77
>6 to ≤12 mo	2019	492.9	3.97 (3.73–4.23)	1890	497.6	3.68 (3.45–3.92)	1.08 (0.99–1.18)	.09
>12 mo	5271	1500	3.57 (3.40–3.75)	5025	1500	3.41 (3.24–3.58)	1.05 (0.98–1.12)	.20
Season <sup>b,c</sup>								
Summer	1988	337.9	5.89 (5.57–6.23)	1945	340.5	5.74 (5.43–6.07)	1.03 (0.95–1.11)	.52
Autumn	1491	388.4	3.87 (3.63–4.13)	1329	383.8	3.49 (3.27–3.74)	1.11 (1.01–1.22)	.03
Winter	1535	707.7	2.18 (2.03–2.33)	1479	706.5	2.10 (1.96–2.25)	1.04 (0.94–1.14)	.49
Spring	2556	640.7	4.03 (3.81–4.25)	2445	636.1	3.86 (3.65–4.07)	1.04 (0.97–1.13)	.27
Nutritional status <sup>d</sup>								
WAZ > -2	5501	1500.0	3.47 (3.30–3.66)	5163	1600.0	3.32 (3.15–3.49)	1.05 (0.97–1.13)	.22
WAZ ≤ -2 to > -3	1407	341.6	4.10 (3.69–4.57)	1453	371.4	3.91 (3.53–4.34)	1.04 (0.90–1.22)	.54
WAZ ≤ -3	522	119.3	4.36 (3.62–5.24)	445	112.1	3.96 (3.27–4.81)	1.10 (0.84–1.44)	.49

<sup>a</sup> Efficacy represents the per-protocol analyses in which children were censored 3 months post receiving their last study dose or at completion of follow-up.

<sup>b</sup> Children contributed data to all age groups or season during which they were followed.

<sup>c</sup> Seasons were defined as summer: June, July, and August; autumn: September, October, and November; winter: December, January, and February; and spring: March, April, and May.

<sup>d</sup> Nutritional status was based on WAZ calculated by using WHO child growth standards.<sup>51</sup>

vitamin D<sub>3</sub> had no effect in reducing the risk for diarrheal illnesses, and does not concur with the preceding non-controlled trial literature. However, in the absence of additional research for validation and exploration of effects at varying developmental states, this single trial cannot definitively rule out an effect of vitamin D supplementation on diarrheal prevention.<sup>45</sup> Furthermore, although the findings could be inferred to other populations, they are mainly applicable to children younger than 3 years of age from low-income urban settings similar to the Kabul population studied.

The lack of risk reduction in diarrheal diseases may be attributable to inappropriate serum concentrations of 25(OH)D for effective immunologic activity. Levels recommended in the literature pertain to skeletal physiology, and optimal concentrations of serum 25(OH)D are not known for any immunologic functions of vitamin D.<sup>23</sup> Alternatively, it is possible that the high-dose quarterly supplementation failed to achieve consistently elevated serum levels of 1,25(OH)<sub>2</sub>D and subsequently insufficient associated

immune modulation. It has been hypothesized that because of enzymatic feedback kinetics, frequent supplementation to prevent serum fluctuations is superior to higher-dose regimes administered over longer intervals.<sup>12</sup> However, this steady-state serum hypothesis has only been explored in vitro and there is no supportive evidence from clinical research. The immunologic roles of vitamin D are incompletely understood and may be interrelated to physiologic and pathogenic factors yet to be identified in the setting of effective supplementation; this too, however, is speculative. Congruent with a recent systematic review, the findings of this study underscore the need for further research to explore vitamin D serologic levels in relation to immune function and physiologic responses to infections.<sup>46</sup>

Although a null study, the implications from this trial are substantial. The pragmatic quarterly supplementation regimen of 100 000 IU was effective in raising serum 25(OH)D concentrations without resulting in any substantial adverse events during follow-up and can therefore be used to guide future study designs. Furthermore, if subsequent

research finds beneficial outcomes with supplementation, a feasible community-based model for delivery and dosing can be derived from the methods employed in this trial. As no public health policies exist regarding supplementation in relation to diarrheal diseases, this work provides the first randomized controlled data on the topic and may be used to direct future policies pertaining to child health programs.

## CONCLUSIONS

Quarterly supplementation with 100 000 IU of vitamin D<sub>3</sub> (cholecalciferol) was found to have no benefit on prevention of diarrheal illness among children aged 1 to 29 months in Kabul, Afghanistan. Similar supplementation to populations comparable to the one studied here is not recommended. The methods and results of this trial should be used to guide further research to identify appropriate serological levels and immune mechanisms associated with the effects of vitamin D on diarrheal diseases, and if beneficial, to determine optimal supplementation regimes for public health interventions.

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