Randomized Trial of Vitamin D Supplementation and Risk of Acute Respiratory Infection in Mongolia

WHAT'S KNOWN ON THIS SUBJECT: A growing number of epidemiologic studies suggest that individuals with lower vitamin D levels are at higher risk of acute respiratory infection. Randomized controlled trials are needed to determine if vitamin D supplementation would decrease this risk.

WHAT THIS STUDY ADDS: In a randomized controlled trial of 247 Mongolian children with vitamin D deficiency in winter, with double-blinding and 99% follow-up, vitamin D supplementation significantly halved the risk of acute respiratory infections.

abstract

OBJECTIVE: Observational studies suggest that serum levels of 25-hydroxyvitamin D (25(OH)D) are inversely associated with acute respiratory infections (ARIs). We hypothesized that vitamin D supplementation of children with vitamin D deficiency would lower the risk of ARIs.

METHODS: By using cluster randomization, classrooms of 744 Mongolian schoolchildren were randomly assigned to different treatments in winter (January–March). This analysis focused on a subset of 247 children who were assigned to daily ingestion of unfortified regular milk (control; \( n = 104 \)) or milk fortified with 300 IU of vitamin D3 (\( n = 143 \)). This comparison was double-blinded. The primary outcome was the number of parent-reported ARIs over the past 3 months.

RESULTS: At baseline, the median serum 25(OH)D level was \( 7 \text{ ng/mL} \) (interquartile range: 5–10 ng/mL). At the end of the trial, follow-up was 99% (\( n = 244 \)), and the median 25(OH)D levels of children in the control versus vitamin D groups was significantly different (7 vs 19 ng/mL; \( P < .001 \)). Compared with controls, children receiving vitamin D reported significantly fewer ARIs during the study period (mean: 0.80 vs 0.45; \( P = .047 \)), with a rate ratio of 0.52 (95% confidence interval: 0.31–0.89). Adjusting for age, gender, and history of wheezing, vitamin D continued to halve the risk of ARI (rate ratio: 0.50 [95% confidence interval: 0.28–0.88]). Similar results were found among children either below or above the median 25(OH)D level at baseline (rate ratio: 0.41 vs 0.57; \( P_{\text{interaction}} = .27 \)).

CONCLUSIONS: Vitamin D supplementation significantly reduced the risk of ARIs in winter among Mongolian children with vitamin D deficiency. Pediatrics 2012;130:e561–e567
Situated in Central Asia, Mongolia is a country where UV-B radiation in winter is inadequate to induce cutaneous vitamin D synthesis. Moreover, cold temperatures throughout much of the year cause Mongolians to limit direct exposure of their skin to sunlight in all but summer. These factors, combined with the absence of vitamin D fortification of food and the limited availability of vitamin D supplements, have led to a high risk for vitamin D deficiency among Mongolians, particularly during winter.

Although vitamin D is known to play a major role in calcium metabolism and bone health, observational studies suggest that low levels may contribute to risk of many different diseases, including acute respiratory infections (ARIs). For example, Camargo et al. recently reported an inverse association between cord blood levels of 25-hydroxyvitamin D (25(OH)D) and risk of ARI in the first 3 months of life. In 2010, Urashima et al. reported the first randomized controlled trial (RCT) on vitamin D and ARIs in children; the authors found use of a vitamin D supplement lowered risk of influenza A (primary outcome) but had no significant effect on influenza B. These seemingly discordant findings contributed to the 2011 conclusion of the Institute of Medicine that the effect of vitamin D status on ARI risk was unclear and additional RCTs are needed.

To further examine the effect of vitamin D status on risk of childhood ARI, we analyzed data from a recently completed RCT involving 744 Mongolian children, a population with low levels of 25(OH)D in winter. Although the trial included 6 arms, we focused on 2 arms with double-blinding: children assigned to daily intake of regular milk versus vitamin D–fortified milk (Fig 1). We hypothesized that vitamin D supplementation of Mongolian children would lower the risk of ARIs.

**METHODS**

**Study Design and Subjects**

The Blue Sky Study was conducted in Ulaanbaatar, Mongolia (latitude 48°N) in association with the Health Sciences University of Mongolia, the Mongolian Ministries of Health and Education, Harvard University, and Boston University. This RCT included 744 Mongolian children from 21 third- and fourth-grade classrooms (96% of 779 invited children). The schools were selected for their size, proximity, and comparable sociodemographic profiles. The primary aims of the trial were to test the effects of vitamin D–fortified beverages or vitamin D pills on serum 25(OH)D levels, somatotropic hormones, and growth and development among prepubertal children; these aims determined the sample size. The primary results of the Blue Sky Study have been published elsewhere. Briefly, delivery of 300 IU daily by using fortified beverages or pill supplements improved 25(OH)D concentrations but failed to raise levels above 20 ng/mL (50 nmol/L) uniformly. There were no significant adverse events.

**Vitamin D Supplementation**

The Blue Sky Study examined 5 approaches to improve the vitamin D status of Mongolian schoolchildren: (1) 300 IU of vitamin D3 daily in Mongolian milk (n = 143); (2) 300 IU of vitamin D3 daily in US milk (n = 143); (3) 300 IU of vitamin D3 daily in a milk substitute (n = 147); (4) 300 IU of vitamin D3 in a daily pill (n = 112); and (5) a total of 13 700 IU of vitamin D3 in pills given over the first 7 days of study (n = 95). The comparison group for these vitamin D interventions was a sixth randomly assigned group given unfortified regular Mongolian milk daily (control; n = 104); all of the children in the control group were given vitamin D supplements after the study ended. Every group, except for the control group, received ~13 700 IU of vitamin D during the 7-week study period (late January to mid-March 2009). Randomization was based on a random number generator, with allocation concealment and off-site assignment of classrooms to a specific intervention. For the current analysis, to ensure that the group assignment was double-blinded and that other differences were minimized, we focused on 2 specific groups: the children receiving unfortified regular Mongolian milk (control group) versus those receiving vitamin D–fortified Mongolian milk (vitamin D group). The added vitamin D was odorless, colorless, and tasteless so children, teachers,
and researchers were unable to tell whether children in any particular classroom were receiving supplementation. This comparison was determined a priori. In a secondary analysis, we examined results in the other vitamin D interventions versus the control group.

**25(OH)D Level**

A blood sample was taken from each child at baseline and at follow-up. This blood was used to determine the serum 25(OH)D concentration by using liquid chromatography–tandem mass spectrometry through a turbulent flow liquid chromatography system (Cohesive Technologies, Franklin, MA) followed by traditional laminar flow chromatography. The analysis was performed by using a TSQ Quantum Ultra triple mass spectrometer (Thermo Finnigan Corporation, San Jose, CA). The intra-assay coefficient of variation was 18.7% in this sample with very low 25(OH)D concentrations; in populations with a wider range of 25(OH)D concentrations, the intra-assay coefficient of variation is 9%. For 8 (3%) of the 247 children in the primary comparison, either the 25(OH)D value at baseline or at follow-up was unavailable.

**Outcomes**

The primary outcome for this analysis, collected by using a survey at completion of the study, was the parent-reported number of ARIs that occurred during the preceding 3 months. ARIs were ascertained by using the following question: “Over the past 3 months, how many chest infections or ‘colds’ has your child had—counting only those infections that lasted for at least 24 hours with symptoms?” The response categories were counts from “none” to “six or more.” Among the 247 children in the primary comparison, outcome data were missing for only 3 children (1 lost to follow-up and 2 discontinued the intervention due to changing schools).

**Assessment of Other Covariates**

Trained study assistants helped the children and their parents/guardians to complete questionnaires at the enrollment and follow-up visits. In addition to sociodemographic characteristics, aspects of respiratory health were assessed. The wheeze question was taken from the International Study of Asthma and Allergies in Childhood.8 “Has your child had wheezing or whistling in the chest at any time in the past?”

**Data Analysis**

All analyses were performed by using Stata version 11.1 (StataCorp LP, College Station, TX). Equality between the 2 groups (regular versus fortified milk) was tested for each of the baseline characteristics by using generalized estimation equations to adjust for intraclass correlation. Depending on the nature of the baseline characteristic, logistic, negative binomial, and linear regression analysis was used.

To test the association between vitamin D supplementation and the number of ARIs in the past 3 months, unadjusted and adjusted random-intercept negative binomial regression were used in an intention-to-treat analysis. We decided a priori to include age, gender, and baseline “ever wheeze” history as covariates in our multivariable analysis. The confounding effect of covariates was assessed by examining the association of vitamin D supplementation with ARI before and after adding the covariates as fixed effects to the model. Moreover, because classrooms (and not individual children) were assigned the different treatment groups, our analyses assume correlation between the children of each class; random effects for each classroom were entered to account for this intraclass correlation. The models were compared by using deviance statistics.

To examine if the effect of vitamin D supplementation on ARI risk varied according to baseline 25(OH)D status, children were stratified according to their median baseline 25(OH)D level, and the multivariable regression analysis was repeated in the 2 subgroups. We formally tested for interaction with a multiplicative term. In a secondary analysis, the primary analysis was repeated but focused on the 2 major vitamin D interventions (vitamin D in beverages or in pills) versus the unfortified milk control. Model results are reported as rate ratios (RRs) with 95% confidence intervals (CIs). A 2-tailed P < .05 was considered statistically significant.

**RESULTS**

Table 1 displays the characteristics of children in the 2 randomly assigned groups: those assigned to receive regular Mongolian milk (control) versus those assigned to receive vitamin D–fortified Mongolian milk. As expected, there were no significant differences between the randomly assigned groups at baseline. Specifically, the median serum 25(OH)D concentrations of children in the control and intervention groups were nearly identical (6.8 vs 7.0 ng/mL; P = .58). In January, 99% of these apparently healthy Mongolian schoolchildren were vitamin D deficient (using a definition of <20 ng/mL). At follow-up ~7 weeks later, the 2 groups differed significantly in their median serum 25(OH)D level (7.2 vs 18.9 ng/mL; P < .001). Even in the intervention group, however, most children continued to have serum 25(OH)D levels <20 ng/mL.

Figure 2 shows the parent-reported number of ARIs at follow-up in both groups. Compared with children in the control group, children receiving vitamin D–fortified milk were less likely to report 1 or more ARIs over the winter. These frequencies suggest an inverse association between vitamin D supplementation and risk of ARI. Indeed, the unadjusted regression model in...
Table 1 found that vitamin D supplementation approximately halved the risk of ARI (RR: 0.52). Addition of the child’s baseline wheeze history as a covariate in the model did not materially change the results (RR: 0.50). A positive wheeze history seemed to increase risk of ARI, although the finding was not statistically significant (RR: 1.44 [95% CI: 0.91–2.29]). Adjusting additionally for age and gender (both P > .30) did not materially affect results for either vitamin D (RR: 0.50) or wheeze history (RR: 1.48 [95% CI: 0.92–2.37]).

After stratifying on the baseline median level of 25(OH)D (6.97 ng/mL) and adjusting for age, gender, wheeze history, and correlation within school classes, the addition of vitamin D to milk lowered the child’s risk of ARI among children with both lower (RR: 0.41 [95% CI: 0.20–0.82]) and higher (RR: 0.57 [95% CI: 0.33–0.96]) levels of serum 25(OH)D. The interaction term (vitamin D supplementation × baseline 25(OH)D below/above median) was not significant (P = .27).

In a secondary analysis, children were analyzed from all arms with follow-up data for the parent-reported number of ARIs over the past 3 months (n = 713). The same statistical analyses were performed as done for our primary analysis, and consistent results were found. For example, in the random-intercept negative binomial model adjusted for age, gender, and wheezing history, the RR for ARI was lower for children receiving the different types of vitamin D supplementation compared with those receiving unfortified milk (control): for vitamin D supplement in beverages, RR was 0.66 (95% CI: 0.41–1.03); for vitamin D supplement in pills, RR was 0.69 (95% CI: 0.40–1.18). In this larger sample, a positive baseline wheezing history was associated with a significantly higher risk of ARI (RR: 1.43 [95% CI: 1.08–1.87]).

**DISCUSSION**

In this randomized, double-blind, placebo-controlled trial of Mongolian schoolchildren in winter, vitamin D₃ supplementation of milk with only 300 IU daily produced a clinically and statistically significant decrease in risk of ARI (adjusted RR: 0.50 [95% CI: 0.29–0.88]). The ARI decrease was seen in children both above and below the median baseline 25(OH)D level of 7 ng/mL; there was no evidence for a difference in benefit in this vitamin D–deficient population (with vitamin D deficiency defined as <20 ng/mL).

Although vitamin D is widely recognized for its importance in calcium metabolism and bone health, researchers worldwide have focused for several years on a growing number of possible, noncalcemic health effects. One of the more promising areas is the relation between vitamin D status and respiratory infections. In addition to both historical and contemporary work on the potential benefits of sunlight and vitamin D supplementation on tuberculosis, there is growing appreciation that low, but nonrachitic, levels of vitamin D may also affect risk of other respiratory infections. Indeed, several observational studies suggest that better vitamin D status is associated with lower risk of infection-related phenomena such as childhood wheezing. Recent studies in *Pediatrics* have shown that cord blood levels of 25(OH)D have a strong inverse

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**TABLE 1** Characteristics of 244 Children According to Their Randomly Assigned Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Milk (n = 103)</th>
<th>Milk + Vitamin D (n = 141)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>9.8 ± 1.0</td>
<td>10.1 ± 0.9</td>
<td>.51</td>
</tr>
<tr>
<td>Male, %</td>
<td>50</td>
<td>54</td>
<td>.25</td>
</tr>
<tr>
<td>BMI</td>
<td>16.5 ± 2.1</td>
<td>16.5 ± 1.9</td>
<td>.85</td>
</tr>
<tr>
<td>Ever wheeze, %</td>
<td>13</td>
<td>14</td>
<td>.87</td>
</tr>
<tr>
<td>Wheeze last 12 mo, %</td>
<td>10</td>
<td>10</td>
<td>.74</td>
</tr>
<tr>
<td>Respiratory medication at baseline, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>9</td>
<td>.83</td>
</tr>
<tr>
<td>Vitamin D status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median 25(OH)D at baseline (IQR), ng/mL</td>
<td>6.8 (4.3–9.5)</td>
<td>7.0 (5.0–9.9)</td>
<td>.58</td>
</tr>
<tr>
<td>Median 25(OH)D at follow-up (IQR), ng/mL</td>
<td>7.2 (5.5–9.7)</td>
<td>18.9 (15.5–22.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ARIs over past 3 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.52 ± 1.10</td>
<td>0.41 ± 0.87</td>
<td>.16</td>
</tr>
<tr>
<td>At follow-up</td>
<td>0.80 ± 0.95</td>
<td>0.45 ± 0.86</td>
<td>.047</td>
</tr>
</tbody>
</table>

Unless otherwise indicated, data are presented as mean ± SD. IQR, interquartile range.

* P is based on generalized estimating equations used to fit the logistic, negative binomial, and linear regression models of the different characteristics (binary, count, and continuous variables, respectively) on vitamin D supplementation.

<sup>a</sup> Respiratory medication includes any medicines (oral, inhaled, or injected) that the child had taken during the past week for breathing problems, including asthma.

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**FIGURE 2**

Effect of vitamin D supplementation of milk on number of ARIs.
association with ARIs during the first 3 months of life\(^4\) and with ARIs due to respiratory syncytial virus during the first year.\(^6\)

Association is not causation, however, and many have called for RCTs to formally test this promising association. In addition to post hoc analyses of data from bone health RCTs in older women,\(^17\) a few recent RCTs have specifically focused on the relationship between vitamin D status and ARI risk. The adult trials are either null\(^18\) or provide moderate support for a protective effect of vitamin D supplements on risk of viral ARIs during winter.\(^19\) In children, there is 1 comparable trial by Urashima et al\(^5\) in Japan. In a sample of 334 schoolchildren, the investigators found that 1200 IU of vitamin D\(_3\) supplement daily lowered the risk of influenza A (RR: 0.58 [95% CI: 0.34–0.99]) but, for unclear reasons, did not affect the risk of influenza B (RR: 1.39 [95% CI: 0.90–2.15]) and therefore yielded an overall null result for influenza risk. Our Mongolian findings suggest that the association between vitamin D status and ARI risk is indeed causal, at least among school-aged children with very low vitamin D status in early winter.

The 2011 Institute of Medicine report suggested that most Americans have adequate vitamin D status and do not require supplements or serum 25(OH)D testing.\(^6\) For children, however, Mansbach et al\(^20\) demonstrated that ~20% of US children ages 1 to 11 years have vitamin D levels <20 ng/mL. Moreover, the percentage climbs to ~50% among specific demographic groups, such as African-American children. Others have reported similar 25(OH)D values in a variety of populations, including infants, health care workers, and the elderly.\(^13\) Specific patient populations, such as individuals with HIV or chronic respiratory disorders, also are at higher risk of having undiagnosed vitamin D deficiency. Thus, although the Mongolian 25(OH)D results may, at first glance, seem like a finding applicable to only a small segment of the US population, the observed 25(OH)D levels actually are not uncommon in several segments of the US population.

The primary publication from the Blue Sky Study\(^7\) emphasized that all of the vitamin D interventions failed to raise 25(OH)D levels >20 ng/mL uniformly. To reach this 20-ng/mL threshold requires supplement doses >300 IU daily. At the time the trial was designed, the Institute of Medicine recommended 200 IU daily for all individuals from birth to age 50 years.\(^21\) The 2011 Institute of Medicine report has since increased the recommendation to 400 IU daily for children.\(^6\) Although this higher dose is certainly an improvement, we suspect that even 400 IU daily will fall short for many children with vitamin D deficiency. Other groups, such as the Canadian Paediatric Society,\(^22\) recommend 1000 IU daily for children, and we believe that this higher dose is more likely to achieve adequate 25(OH)D levels for almost all children without pushing them to a potentially toxic level. In addition to traditional concerns about hypercalcemia, which begin at 25(OH)D levels ≥100 ng/mL,\(^23\) there are emerging concerns about increased atopy risk with less dramatically elevated levels, such as cord blood levels >40 ng/mL (>100 nmol/L).\(^24\) Should the latter concern be affirmed in ongoing pregnancy/infancy RCTs, it would support the importance of occasional 25(OH)D testing in pregnant women and children and the creation of tailored vitamin D regimens to optimize the achieved serum level of 25(OH)D.

The protective effect of vitamin D on ARI risk is consistent with the growing literature on vitamin D and innate immunity.\(^25,26\) For example, laboratory studies have shown that activated vitamin D induces cathelicidin (an endogenous antimicrobial peptide) in bronchial epithelial cells.\(^27\) The in vivo effects of UV-B exposure or oral vitamin D supplementation on airway levels of cathelicidin are not established, but recent experimental studies indicate that these approaches raise skin\(^28,29\) and circulating\(^30\) levels of cathelicidin. In adults, low levels of circulating cathelicidin have been associated with higher rates of infectious disease mortality.\(^31\) Similar studies in children are not yet available.

The current RCT has some potential limitations. Serum 25(OH)D levels are generally thought to stabilize after 2 months of treatment,\(^3\) which suggests that 25(OH)D levels were rising over the course of this winter trial. Extending the RCT would have provided more follow-up time with the groups at their most different levels of serum 25(OH)D. However, the arrival of spring in Mongolia, and a progressive rise in ambient UV-B, argued against continuation of our trial beyond March. Future studies of vitamin D and ARI may wish to start their vitamin D intervention in the fall, or even summer, to assure a maximal 25(OH)D difference before the typical fall/winter burst of ARIs. Another limitation is the imprecision of the primary outcome. Because number of ARIs was captured from a single follow-up interview with the child’s parent, rather than several frequent medical evaluations, it is likely that there is

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**TABLE 2** Effect of Vitamin D Supplementation of Milk on Number of ARIs

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-intercept negative binomial model(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>244</td>
<td>0.52</td>
<td>0.31–0.89</td>
</tr>
<tr>
<td>Adjusted for baseline wheeze history</td>
<td>231</td>
<td>0.50</td>
<td>0.28–0.92</td>
</tr>
<tr>
<td>Adjusted for baseline wheeze history, age, and gender</td>
<td>231</td>
<td>0.50</td>
<td>0.28–0.92</td>
</tr>
</tbody>
</table>

\(^a\) Adjusts for correlation within randomly assigned classrooms.
misclassification. However, this misclassification should not differ between the unfortified versus fortified milk arms, for which children, parents, and interviewers were blinded. If anything, this nondifferential misclassification would tend to bias results toward the null. This acknowledged imprecision may have contributed to the results seen in our secondary analysis, in which multiple unblinded study arms were combined for analysis. Future trials with better outcome definitions (eg, real-time physician evaluations, identification of viral etiology) would further advance our understanding of how vitamin D status affects risk of ARI.

CONCLUSIONS

This double-blinded RCT of Mongolian children in winter found that vitamin D₃ supplementation with only 300 IU daily led to a clinically and statistically significant reduction in risk of parent-reported ARIs. The halving of ARIs has important public health implications for Mongolian children and perhaps other populations with low levels of serum 25(OH)D. Although the latitude of Ulaanbaatar (48° N) may seem unusually high, it actually is similar to that of Montreal and Paris; many major North American and European cities are at even higher latitudes. Although our RCT data support a causal link, additional RCTs are needed to examine the association in other populations at increased risk of both vitamin D deficiency and ARI. Populations fitting that profile include infants, health care workers, and the elderly; individuals with HIV or other immunodeficiency conditions; and individuals with asthma or other chronic respiratory disorders. Moreover, future trials can examine the efficacy of different vitamin D supplementation regimens on ARI risk in general, as well as risk of ARIs caused by specific pathogens, such as respiratory syncytial virus and human rhinovirus.

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