

Randomized trial of fortified milk and supplements to raise 25-hydroxyvitamin D concentrations in schoolchildren in Mongolia^{1–4}

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ABSTRACT

Background: The optimal public health strategy for maintaining 25-hydroxyvitamin D [25(OH)D] concentrations in schoolchildren in Mongolia is unknown.

Objective: The objective was to compare the effectiveness of different supplement and fortified milk regimens to increase 25(OH)D concentrations in Mongolian schoolchildren.

Design: Twenty-one classrooms of 579 children aged 9–11 y were randomized to interventions with an equivalent content of vitamin D₃: 1) a one-time seasonal supplement of 13,700 IU, 2) 300 IU/d from supplements, 3) 300 IU/d from fortified ultra-high-temperature pasteurized milk from the United States, 4) 300 IU/d from fortified pasteurized Mongolian milk, or 5) unfortified pasteurized Mongolian milk (control).

Results: In January, the mean ($\pm$ SD) serum 25(OH)D concentration was 8 ± 4 ng/mL (20 ± 10 nmol/L), and 98% of the children had a concentration <20 ng/mL (50 nmol/L). In March, concentrations were 8 ± 4 ng/mL after unfortified milk, 20 ± 6 ng/mL after fortified Mongolian milk, 29 ± 10 ng/mL after fortified US milk, 21 ± 6 ng/mL after daily supplements, and 12 ± 4 ng/mL after seasonal supplements (each greater than unfortified milk, $P < 0.01$). Seasonal supplementation was less effective than was daily supplementation ($P < 0.0001$). Despite consuming daily supplements or fortified milk, 41% of the children still had concentrations <20 ng/mL (50 nmol/L). Children with lower baseline 25(OH)D concentrations experienced slightly larger 25(OH)D responses to intervention than did children with higher concentrations ($P = 0.002$).

Conclusions: In this population with extremely low vitamin D concentrations, delivery of 300 IU vitamin D/d via supplements or in fortified milk improved 25(OH)D concentrations but failed to raise concentrations uniformly to >20 ng/mL (50 nmol/L). The daily low-dose intervention was superior to the seasonal larger-dose intervention. Higher doses may be needed to prevent deficiency in schoolchildren in Mongolia and at other northern latitudes. This trial is registered at clinicaltrials.gov as NCT00886379. *Am J Clin Nutr* 2011;94:578–84.

INTRODUCTION

A burgeoning body of research has identified new implications concerning the role of vitamin D in health throughout life (1). For infants and preschoolers, the role of vitamin D deficiency in rickets has been established. In school-age children, vitamin D influences bone mineral deposition (2–4). It may also affect lean

body mass (2), atopic dermatitis (5), asthma (6), and type 1 diabetes (7) and may prevent infection (8). Several chronic diseases associated with low vitamin D may originate in childhood, including diabetes, hypertension, cardiovascular disease, and some cancers (9). The vitamin D status of schoolchildren may be important to short-term and long-term public health.

At least 45% of school-age children have low vitamin D concentrations (<20 ng/mL, 50 nmol/L) in countries as diverse as Lebanon (33° N) (10), northern China (40°N) (11), northern United States (42° N) (12), Spain (43° N) (13), Mongolia (48° N) (14), France (49° N) (15), southern Argentina (55° S) (16), and Finland (60° N) (17). Developed and developing nations need evidence-based strategies to increase vitamin D concentrations in children.

In many populations, children rarely consume the few foods naturally rich in vitamin D, such as fatty fish, fish liver oil, and mushrooms. Alternative dietary sources include fortified foods and supplements. In some countries, fortification of milk, margarine, cereals, or flour is a strategy that relies on passive consumer behavior (18–20). Supplementation regimens—often targeted at pregnant women and infants—have varied from large doses administered as one-time supplements at the outset of winter (“stoss therapie”) to smaller daily, weekly, or monthly doses (21, 22).

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³ Supported by an anonymous foundation. The Tishcon Corporation donated the vitamin D supplements, and the Swanson Foundation contributed the international shipping and storage of supplies.

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Received December 7, 2010. Accepted for publication May 20, 2011.

First published online June 22, 2011; doi: 10.3945/ajcn.110.008771.

Food fortification, daily supplementation, and seasonal supplementation have different costs and distribution challenges for the public health sector. It would be more efficient to administer periodic large-dose supplements than to exhort families or schools to provide daily low-dose supplements or to ensure that children consume fortified foods. However, data are lacking on the comparative efficacy of various strategies. The only study that directly compared equivalent daily, weekly, or monthly regimens reported that a daily administration was more effective than weekly or monthly administration in an elderly population (23). Furthermore, it would be useful to quantify the extent to which the 25(OH)D response to fortified foods or supplements depends on baseline status. Studies have suggested, but not proven, that individuals with a low baseline 25(OH)D concentration have a larger 25(OH)D response to intervention (24, 25).

We therefore sought to test 2 hypotheses: 1) that daily supplementation would be more effective than the same amount of vitamin D delivered once as a seasonal supplement and 2) that children with the lowest baseline 25(OH)D concentrations would benefit the most from intervention. The study was conducted in Ulaanbaatar, Mongolia, where we had previously found mean 25(OH)D concentrations of 17 ± 5 ng/mL (43 ± 12 nmol/L) in early May in a pilot study of 46 schoolchildren (14). At 48°N, Ulaanbaatar is at the same latitude as Montreal, Paris, and Zurich.

SUBJECTS AND METHODS

Study design

The Blue Sky Study is a randomized trial designed to test the effect of vitamin D supplements and fortified cow milk on 25(OH)D and somatotrophic hormone concentrations and on growth and development in children. Twenty-one classrooms of 9–11-y-old Mongolian students from 2 public schools were randomized to one of several intervention arms for 49 d from January through March 2009. Two groups received vitamin D–fortified milk (Mongolian or United States) and 2 groups received vitamin D supplements. Each study arm was designed to deliver a total of 13,700 IU over the course of the intervention, or ≈ 300 IU/d. A fifth group received the typical unfortified Mongolian milk and were given a vitamin D supplement after the study ended. Children in the Mongolian milk arms were blinded to their assignment; other children were not.

Participants

Two public schools were selected for their size, proximity, and comparable sociodemographic profiles. Children were eligible to participate if they had no known allergies to milk. We invited 779 children from 21 third- and fourth-grade classrooms of each school; 744 children (96%) participated and 739 (99%) completed (5 transferred schools and 0 dropped out of the study) the study. We excluded from this analysis an additional 8 children who were absent on the day of a blood draw or refused to have their blood drawn and 152 children participating in an arm not relevant to this analysis. This article reports results for 579 children.

The trial and consent process was approved by the institutional review boards at the Harvard School of Public Health and the

Mongolian Ministry of Health. After an introductory group meeting at each school, parents and children met with trained research assistants to answer questions and give signed parental consent and child assent. There were no significant adverse events. The project funders had no role in the design, implementation, analysis, or interpretation of data.

Classroom randomization

We had adequate statistical power to meet the aims of the study by randomizing ≈ 150 children to each fortified milk arm and 100 children to each supplement arm and unfortified milk arm. The teachers told us that children would prefer milk to supplements, so we decided to individually randomly assign children within each classroom. We instead randomized 21 classrooms of 30–44 children each. To balance the 2 grades and 2 schools across intervention arms and to ensure that intervention supplies would suffice to let every child in a classroom participate, we identified all possible combinations of classroom assignments that satisfied the following criteria: each arm included at least one third- and fourth-grade classroom and at least one classroom from each school, and no classroom combination exceeded our supplies. This yielded 36 unique classroom assignment schemes, each ensuring a modicum of balance across grade and school. We used a random number generator to select one assignment scheme.

Milk study arms

Children in the milk study arms received 710 mL liquid whole cow milk 7 d/wk: ultra-high-temperature (UHT)–pasteurized fortified milk shipped from the United States or pasteurized Mongolian milk that was either unfortified or fortified with the vitamin D₃ premix (Cargill Inc, Minneapolis, MN) used by the US producer. The fortified milks were intended to deliver 100 IU vitamin D₃ per 236 mL (8 oz)—the standard fortification goal in US milk.

Supplement study arms

The other 2 arms each included children who received a vitamin D₃ supplement (Tishcon Corporation, Westbury, NY): one arm received 300 IU 7 d/wk, and the other received a total of 13,700 IU in the first 7 d of the trial as a seasonal supplement (the Institutional Review Board required that ≤ 2000 IU be given on any day).

Intervention schedule

We phased in the intervention to minimize digestive upset by the sudden introduction of milk, because 29% of the children were consuming no regular milk at the start of the study. Children in the milk arms received 236 mL/d on days 1–3, 472 mL/d on days 4–5, and 710 mL/d on days 6–49. The daily supplement arm was phased in according to the same schedule. All active study arms were designed to deliver 13,700 IU over 49 d.

Compliance

School staff were trained to distribute the interventions to the classrooms and to record attendance and compliance.

Assessment of diet and other covariates

Trained study assistants helped the children and their parents or guardians to complete questionnaires at the enrollment visit, including a 24-h recall of typical Mongolian dairy products.

Blood samples

Professional phlebotomists drew 8-mL blood samples at baseline and at the conclusion of the intervention. The samples were cooled immediately before transportation to the laboratory, where they were centrifuged, separated into aliquots, and frozen at -20°C .

25(OH)D analysis

Serum samples were transported frozen to the laboratory of MFH (Boston University Medical Center, Boston, MA) for assay of 25(OH)D₂ and 25(OH)D₃ by liquid chromatography–tandem mass spectrometry through a turbulent-low 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) (26). The intraassay CV was 18.7% in this sample with very low 25(OH)D concentrations, whereas it was 8–9% in populations with a wider range of 25(OH)D concentrations in this laboratory. The assay has been validated against the standard of the National Institute of Science and Technology.

Statistical analysis

We conducted an intention-to-treat analyses. To analyze the effect of the interventions on change in serum 25(OH)D concentrations, we fit linear mixed-effect models using the SAS PROC MIXED procedure (version 9.2; SAS Institute Inc, Cary, NC) and tested for a differential change in 25(OH)D concentrations between arms. We adjusted for age, sex, and school and included classroom as a random effect. Similar generalized linear

mixed-effects models were used to assess changes in the odds of having a 25(OH)D concentration <20 ng/mL (50 nmol/L). Secondary sensitivity analyses that further adjusted for compliance yielded nearly identical results (data not shown). We conducted a prespecified test of whether baseline 25(OH)D status differentially affected the effect of each intervention arm. In a model including only the subjects receiving daily supplementation or fortification, the interaction between continuous baseline 25(OH)D and intervention arm in predicting 25(OH)D change was not significant, which suggests a common effect of baseline 25(OH)D across arms. Therefore, we estimated the effect of baseline 25(OH)D concentrations on the response to daily intervention [change in 25(OH)D concentration] by adding it to the model with main effects terms for intervention and covariates, including only the daily intervention arms.

RESULTS

At baseline in January, children were similar in age and body size across intervention arms (Table 1). On average, children consumed 1–2 servings of unfortified dairy products daily.

The distribution of baseline 25(OH)D concentrations in mid-January is shown in Figure 1A. The mean $\pm$ SD 25(OH)D concentration across all arms was 8 ± 4 ng/mL (20 ± 10 nmol/L). Before the intervention, 98% of children had 25(OH)D concentrations <20 ng/mL (<50 nmol/L) and 75% had concentrations <10 ng/mL (25 nmol/L). The baseline concentrations of 25(OH)D were similar across intervention arms (Table 2).

The 25(OH)D concentrations at follow-up for each arm are shown in Table 2. The unchanged distribution of 25(OH)D concentrations in children who were in the unfortified milk arm is shown in Figure 1B. After 49 d, children in the group that received 13,700 IU in the first week as a seasonal supplement had modestly improved 25(OH)D concentrations (Figure 1C); the mean 25(OH)D concentration rose from 8 ± 4 ng/mL (20 ± 10 nmol/L) to 12 ± 4 ng/mL (30 ± 10 nmol/L). The 25(OH)D status improved more in children who received 300 IU/d as fortified milk or supplements (Figure 1D). The mean 25(OH)D

TABLE 1

Baseline characteristics of participants by intervention arm: Blue Sky Study¹

	Unfortified Mongolian milk (<i>n</i> = 101)	Fortified Mongolian milk (<i>n</i> = 140)	Fortified UHT milk (<i>n</i> = 37)	Daily vitamin D supplements (<i>n</i> = 109)	Seasonal vitamin D supplements (<i>n</i> = 92)	<i>P</i> value
Age (y)	10 ± 1^2	10 ± 1	10 ± 1	10 ± 1	10 ± 1	0.99
Height (cm)	133 ± 6	135 ± 7	133 ± 7	133 ± 7	133 ± 7	0.96
Weight (kg)	30 ± 6	30 ± 5	30 ± 5	29 ± 5	30 ± 5	0.98
BMI (kg/m ²)	17 ± 2	17 ± 2	17 ± 2	17 ± 2	17 ± 2	0.45
School no. 62 (%)	33	52	74	64	29	0.83
School no. 65 (%)	67	48	26	36	71	
Grade 3 (%)	63	51	46	62	59	0.99
Grade 4 (%)	37	51	54	38	41	
Male (%)	50	54	50	59	55	0.60
Female (%)	50	46	50	41	45	
Consumed dairy products yesterday (%) ³	57	65	68	64	71	0.81
Consumed no dairy products yesterday (%) ³	43	35	32	36	29	

¹ The differences in means were derived from mixed models, and the differences in proportions were derived from generalized linear models adjusted for classroom clustering. UHT, ultra-high temperature.

² Mean $\pm$ SD (all such values).

³ Dairy products included milk, milk tea, yogurt, cheese, or *aaruul* (dried curdled milk).

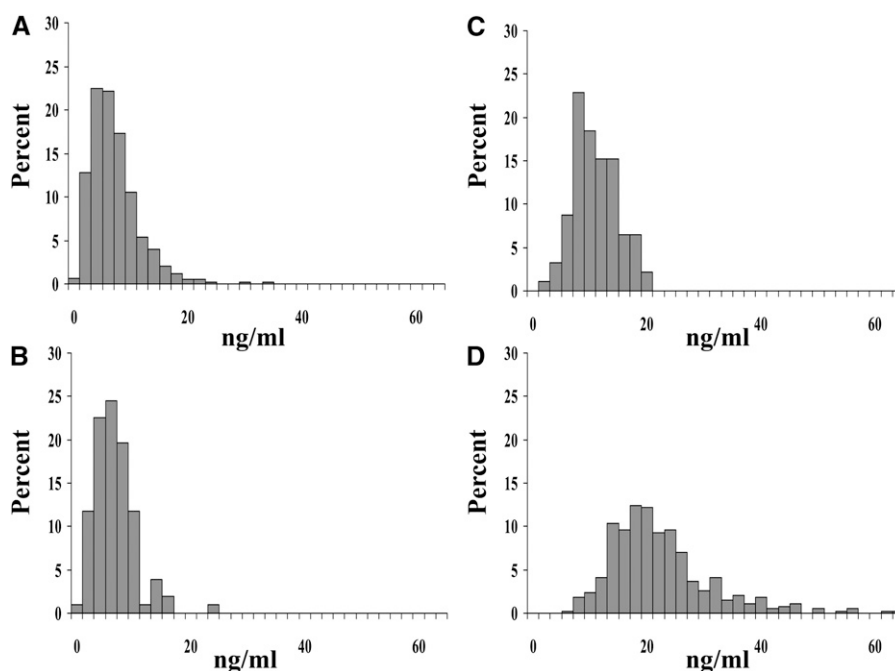


FIGURE 1. Distribution of serum 25-hydroxyvitamin D concentrations in schoolchildren aged 9–11 y in the Blue Sky Study, Ulaanbaatar, Mongolia. A: all 579 children at baseline, January 2009; B: after 7 wk of drinking unfortified milk ($n = 101$); C: 7 wk after a one-time seasonal supplement of 13,700 IU ($n = 92$); and D: after 7 wk of consuming vitamin D (300 IU/d) as a supplement or as fortified milk ($n = 386$).

concentration of children receiving ≈ 300 IU as a daily supplement or as fortified milk rose from 8 ± 4 ng/mL (20 ± 10 nmol/L) to 23 ± 9 ng/mL (57 ± 22 nmol/L).

The mean 25(OH)D changes after adjustment for age (in d), sex, school, and classroom are shown in **Figure 2**. The increases in 25(OH)D concentrations ranged from 9 ng/mL (22 nmol/L) with the seasonal supplement to 24 ng/mL (60 nmol/L) with the fortified UHT milk; each arm was significantly different from unfortified milk (all $P < 0.003$). The UHT milk increased 25(OH)D concentrations more than did the fortified Mongolian milk ($P < 0.0001$) or daily supplements ($P = 0.0004$). The fortified Mongolian milk and the daily supplements raised 25(OH)D concentrations to a similar degree ($P = 0.22$). After adjustment, children who consumed a daily supplement had twice the improvement (18 ng/mL) in 25(OH)D concentrations than did

those who had consumed the same amount of vitamin D as a seasonal supplement at the outset of the trial (9 ng/mL) (Figure 2; $P < 0.0001$).

Children who had the lowest 25(OH)D concentrations at baseline had a slightly greater serum 25(OH)D response to daily interventions ($P = 0.002$). For every 10-ng/mL (25 nmol/L) higher baseline 25(OH)D concentration, there was a 2.5-ng/mL (95% CI: 1.0, 4.1; 6.2 nmol/L, 95% CI: 2.5, 10.2) smaller improvement in serum 25(OH)D in response to the 300-IU/d interventions. For example, a child who started the trial with a 25(OH)D concentration of 10 ng/mL (25 nmol/L) would be predicted to have a 12-ng/mL (30-nmol/L) increase from the 300-IU daily supplement, whereas a child whose baseline concentration was 20 ng/mL (50 nmol/L) would be expected to have only a 10-ng/mL (25-nmol/L) increase from the same daily supplement regimen.

TABLE 2

Concentrations of serum 25-hydroxyvitamin D [25(OH)D] and compliance by intervention arm: Blue Sky Study¹

	Unfortified Mongolian milk ($n = 101$)	Fortified Mongolian milk ($n = 140$)	Fortified UHT milk ($n = 137$)	Daily vitamin D supplements ($n = 109$)	Seasonal vitamin D supplements ² ($n = 92$)	<i>P</i> value
25(OH)D at baseline (ng/mL)	8 ± 4^3	8 ± 4	10 ± 5	7 ± 3	8 ± 4	0.16
25(OH)D at follow-up (ng/mL)	8 ± 4	20 ± 6	29 ± 10	21 ± 6	12 ± 4	<0.0001
Proportion of intended dose received (%)	87 ± 8	83 ± 11	89 ± 6	87 ± 8	98 ± 6	<0.0001
25(OH)D <20 ng/mL (50 nmol/L)						
At baseline (%)	99	99	96	99	100	0.54
At follow-up (%)	99	59	15	51	98	<0.0001

¹ The differences in means were derived from mixed models, and the differences in proportions were derived from generalized linear models adjusted for classroom clustering. UHT, ultra-high temperature.

² Children in the seasonal vitamin D supplement arm received vitamin D supplements (13,000 IU) in the first week of the trial.

³ Mean $\pm$ SD (all such values).

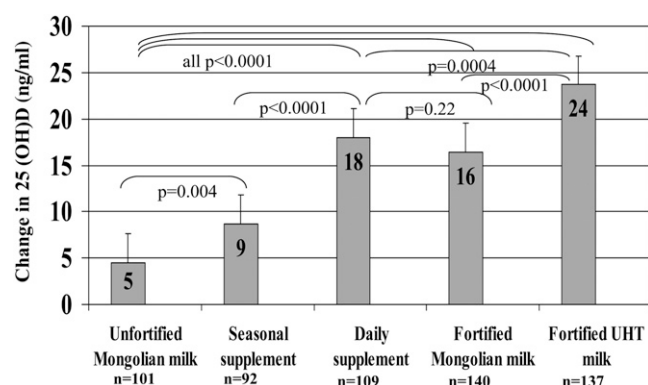


FIGURE 2. Change in baseline serum 25-hydroxyvitamin D [25(OH)D] concentrations, by intervention arm, in the Blue Sky Study, Ulaanbaatar, Mongolia. Values were adjusted for sex [β coefficient predicting change in 25(OH)D concentrations for males = 0.32 ng/mL; $P = 0.43$], age ($\beta = 0.44$ ng/mL per year of age; $P = 0.12$), school (β for school no. 65 = -0.2 ng/mL; $P = 0.82$), and classroom clustering in generalized linear mixed-effects models. UHT, ultra-high temperature.

DISCUSSION

Very low 25(OH)D concentrations (mean: 8 ng/mL, or 20 nmol/L) were endemic among Mongolian schoolchildren during the winter. Low vitamin D concentrations are to be expected in a population as far north as 48° that eats little fish, rarely uses supplements, and lacks fortified foods. This profoundly low mean vitamin D concentration is consistent with the high incidence of rickets in Mongolia, estimated at 24–70% (27–29). As expected, children who consumed unfortified milk had no improvement in 25(OH)D concentrations, because cow milk contains only trace amounts of vitamin D unless fortified.

A 13,700-IU seasonal supplement at the outset of the trial increased mean 25(OH)D concentrations by 4 ng/mL (10 nmol/L) compared with unfortified milk, but was inferior to daily supplements at improving serum 25(OH)D status over 49 d. Children who received the seasonal supplement, delivered in the first week of the trial, had a mean postintervention 25(OH)D concentration roughly half that of children who received the same total dose meted out as daily supplements. After 7 wk, nearly all (98%) the children who received the seasonal supplement again had a 25(OH)D concentration <20 ng/mL (50 nmol/L), compared with 41% of children who received daily supplements or fortified milk. The half-life of 25(OH)D is 2–3 wk (30, 31); 13,700 IU in January did not last until March. It is likely that the seasonal-dose arm achieved higher 25(OH)D concentrations than did the other arms in the first weeks of the intervention; we were not able to draw multiple blood samples to test this. Other studies, using seasonal doses >100,000 IU, have generally found that 25(OH)D concentrations have an initial peak that declines toward baseline concentrations within months (22, 32–34). Although effective as a treatment of vitamin D deficiency, these high doses are unlikely to be accepted for widespread prophylactic use in children. Additional work is needed to optimize the effectiveness, safety, and acceptability of a seasonal dosing regimen for children. In the meantime, these findings concur with Chel et al's (23) results in the elderly, ie, that daily or weekly schedules result in higher long-term 25(OH)D concentrations than do monthly or periodic schedules.

The effect of the intervention varied by baseline 25(OH)D concentration: the lower the baseline 25(OH)D concentration, the

greater the effect of the intervention. This finding is consistent with comparisons across study populations. Most studies have reported increases in 25(OH)D of 1 to 2 ng/mL per 100-IU/d intake for school-age populations whose baseline concentrations were 10–30 ng/mL (25–74 nmol/L) (2, 3, 13, 23, 35). We observed increases of 5 ng/mL (15 nmol/L) per 100 IU/d for this group of schoolchildren, with a mean baseline concentration of 8 ng/mL (20 nmol/L), similar to increases observed in Chinese newborns whose mean concentrations at birth were 5–6 ng/mL (12–15 nmol/L) (36). This suggestion of a greater treatment response at lower baseline 25(OH)D concentrations has been directly tested only twice and never quantified. Holick and Chen (37) reported that elderly nursing home residents whose baseline 25(OH)D concentrations were <20 ng/mL (50 nmol/L) had a greater response to vitamin D₂ supplements than did residents with higher 25(OH)D concentrations. In contrast, a study of 212 adolescents with a mean baseline 25(OH)D concentration of 19 ng/mL (47 nmol/L) reported no statistically significant interaction between baseline 25(OH)D status and intervention (4). However, neither the 25(OH)D range nor the test of interaction was reported, and the study may have been underpowered to detect an interaction between treatment and baseline status. Our larger study detected a statistically significant, if clinically modest, gradient in the 25(OH)D response to the intervention across baseline 25(OH)D status: for every 10-ng/mL (25-nmol/L) higher baseline 25(OH)D concentration, the children had a 2.5-ng/mL (6-nmol/L) lower response to the 300-IU/d intervention.

Our study had several limitations. Chief among them was the inability to monitor the exact vitamin D₃ content of the intervention arms during the course of the study—a shortcoming imposed by location and budget, because no Mongolian laboratories were able to measure vitamin D₃ in milk or supplements. This is important, because the vitamin D content of commercially available milk varies widely (25). One source of variability is the degradation of vitamin D over time. We worked with the US and Mongolian producers to estimate and compensate for the expected loss of vitamin D₃ due to various pasteurization methods and during the months of storage and shipment. We had planned to estimate the vitamin D₃ content of each arm during the trial by assaying samples in the United States after the trial and interpolating vitamin D₃ concentrations between pretrial and posttrial measurements. However, the commercial laboratory assay variability proved to be so great that it precluded precise estimation of the vitamin D₃. As a result, although we are confident in the comparison of daily to seasonal supplements (which used the same pills) and of each arm to unfortified milk (which contained no vitamin D₃), the comparisons of fortified milks with each other and with daily supplements were difficult because of the uncertainty regarding their exact vitamin D₃ content. We planned the active interventions arms to have comparable vitamin D₃ contents, but we were unable to pinpoint how successful we were at achieving parity between fortified milks and supplements.

Other limitations included randomization by classrooms instead of by individuals; however, adjustment for classroom clustering and the potential effects of school, classroom, age, and sex had little effect on the estimates. It would have been ideal to collect serial blood samples to describe 25(OH)D concentrations throughout the study, which would have enabled us to record peak 25(OH)D concentrations, which were probably higher for the



seasonal supplement group. Because 25(OH)D concentrations plateaued 1–2 mo after the start of daily supplementation, we likely captured the full effect of the daily interventions (22). The strengths of the study included the first direct comparison of seasonal compared with daily supplements in children and its large size, which enabled us to detect an interaction between baseline 25(OH)D status and intervention.

In this randomized trial of nutritional vitamin D interventions in a “real life” school setting, daily supplementation with 300 IU was more effective than was seasonal supplementation, but was insufficient to redress vitamin D deficiency for every child. Seasonal supplements may be more effective at higher doses, but further work to balance effectiveness with appropriateness for children is needed. Vitamin D recommendations for children are currently in flux, with ongoing questions regarding the minimal effective dose and the maximally safe dose for children. Our data suggest that either daily supplementation or food fortification with concentrations >300 IU are needed to maintain healthy vitamin D concentrations in children resident in regions where vitamin D concentrations are very low.

We thank the medical and public health students from the Health Sciences University of Mongolia for assistance with the research. We are grateful for the assistance from the teachers and staff of Public Schools no. 62 and no. 65 and the doctors affiliated with the schools. KH Giimaa and the staff of Guum Milk provided invaluable assistance with the production and delivery of the fortified and unfortified Mongolian milk. We also thank Former Ambassador R Bold, Walter Willett, Allyson Abrams, Alice Kungu, and Sarah Hagan for their assistance and Gossner Foods and Gyals Laboratory for their critical help with the production, supplies, and logistics.

The authors' responsibilities were as follows—JWR-E, DG, and ALF: conceived the study and obtained funding; JWR-E, DG, KK, NS, MFH, AB, and ALF: participated in the study design; JWR-E and KK: performed the data analysis; JR: wrote the manuscript; DG and BD: recruited the participants and acquired the data; and MFH: supervised the laboratory analysis. All authors assisted with the editing of the manuscript and approved the final version of the manuscript. Neither of the sponsors had any role in the design, implementation, analysis, or interpretation of the study. None of the authors had any personal or financial conflicts of interest.

REFERENCES

- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.
- El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *J Clin Endocrinol Metab* 2006;91:405–12.
- Viljakainen HT, Palssa A, Karkkainen M, et al. A seasonal variation of calcitropic hormones, bone turnover and bone mineral density in early and mid-puberty girls—a cross-sectional study. *Br J Nutr* 2006;96:124–30.
- Viljakainen HT, Natri AM, Karkkainen M, et al. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *J Bone Miner Res* 2006;21:836–44.
- Sidbury R, Sullivan AF, Thadhani RI, Camargo CA Jr. Randomized controlled trial of vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. *Br J Dermatol* 2008;159:245–7.
- Freishtat RJ, Iqbal SF, Pillai DK, et al. High prevalence of vitamin D deficiency among inner-city African-American youth with asthma in Washington, DC. *J Pediatr* 2010;156:948–52.
- Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* 2008;93:512–7.
- Walker VP, Modlin RL. The vitamin D connection to pediatric infections and immune function. *Pediatr Res* 2009;65:106R–13R.
- Holick M. The Vitamin D deficiency pandemic: a forgotten hormone important for health. *Public Health Rev* 2010;17.
- El-Hajj Fuleihan G, Nabulsi M, Choucair M, et al. Hypovitaminosis D in healthy schoolchildren. *Pediatrics* 2001;107:E53.
- Du X, Zhu K, Trube A, et al. School-milk intervention trial enhances growth and bone mineral accretion in Chinese girls aged 10–12 years in Beijing. *Br J Nutr* 2004;92:159–68.
- Gordon CM, DePeters KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* 2004;158:531–7.
- Docio S, Riancho JA, Perez A, Olmos JM, Amado JA, Gonzalez-Macias J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies? *J Bone Miner Res* 1998;13:544–8.
- Ganmaa D, Tserendolgor U, Frazier L, Nakamoto E, Jargalsaikhan N, Rich-Edwards J. Effects of vitamin D fortified milk on vitamin D status in Mongolian school age children. *Asia Pac J Clin Nutr* 2008;17:68–71.
- Guillemant J, Le HT, Maria A, Allemandou A, Peres G, Guillemant S. Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D3 supplements. *Osteoporos Int* 2001;12:875–9.
- Oliveri MB, Ladizesky M, Mautalen CA, Alonso A, Martinez L. Seasonal variations of 25 hydroxyvitamin D and parathyroid hormone in Ushuaia (Argentina), the southernmost city of the world. *Bone Miner* 1993;20:99–108.
- Lehtonen-Veromaa M, Mottonen T, Irjala K, et al. Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *Eur J Clin Nutr* 1999;53:746–51.
- Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr* 2004;80(Suppl):1710S–6S.
- Laaksi IT, Ruohola JP, Ylikomi TJ, et al. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. *Eur J Clin Nutr* 2006;60:1035–8.
- MOST/USAID. Summary of mandatory and voluntary staple food fortification in developing countries. 2000 (updated 2000; cited 2010 August 9). Available from: <http://www.mostproject.org/PDF/fortificationritu.PDF> (cited 9 August 2010).
- Markestad T, Ulstein M, Aksnes L, Aarskog D. Serum concentrations of vitamin D metabolites in vitamin D supplemented pregnant women. A longitudinal study. *Acta Obstet Gynecol Scand* 1986;65:63–7.
- Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999;69:842–56.
- Chel V, Wijnhoven HA, Smit JH, Ooms M, Lips P. Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int* 2008;19:663–71 (PMCID: 2277446).
- Ellis G, Cooke WT. Serum concentrations of 25-hydroxy vitamin D in Europeans and Asians after oral vitamin D3. *BMJ* 1978;1:685–6.
- Chen TC, Shao A, Heath H III, Holick MF. An update on the vitamin D content of fortified milk from the United States and Canada. *N Engl J Med* 1993;329:1507.
- Holick MF, Siris ES, Binkley N, et al. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* 2005;90:3215–24.
- Tserendolgor U, Mawson JT, Macdonald AC, Oyunbileg M. Prevalence of rickets in Mongolia. *Asia Pac J Clin Nutr* 1998;7:352–8.
- Ministry of Health U. Nutrition status of population of Mongolia 2000: Second National Nutrition Survey. Ulaanbaatar, Mongolia: Nutritional Research Center of Public Health Institute, Mongolia, 2002.
- Fraser DR. Vitamin D deficiency in Asia. *J Steroid Biochem Mol Biol* 2001;89–90:491–5.
- Holick M. Vitamin D: biosynthesis, metabolism, and mode of action. *Endocrinology*. New York, NY: Grune and Stratton, 1989:902–26.
- Haddad JG Jr, Rojanasathit S. Acute administration of 25-hydroxycholecalciferol in man. *J Clin Endocrinol Metab* 1976;42:284–90.
- Oliveri B, Cassinelli H, Mautalen C, Ayala M. Vitamin D prophylaxis in children with a single dose of 150000 IU of vitamin D. *Eur J Clin Nutr* 1996;50:807–10.

33. Markestad T, Hesse V, Siebenhuner M, et al. Intermittent high-dose vitamin D prophylaxis during infancy: effect on vitamin D metabolites, calcium, and phosphorus. *Am J Clin Nutr* 1987;46:652–8.
34. Zeghoud F, Ben-Mekhibi H, Djeghri N, Garabedian M. Vitamin D prophylaxis during infancy: comparison of the long-term effects of three intermittent doses (15, 5, or 2.5 mg) on 25-hydroxyvitamin D concentrations. *Am J Clin Nutr* 1994;60:393–6.
35. Maalouf J, Nabulsi M, Vieth R, et al. Short- and long-term safety of weekly high-dose vitamin D3 supplementation in school children. *J Clin Endocrinol Metab* 2008;93:2693–701.
36. Specker BL, Ho ML, Oestreich A, et al. Prospective study of vitamin D supplementation and rickets in China. *J Pediatr* 1992;120:733–9.
37. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008;87(suppl):1080S–6S.

