

## Original Article

# Effects of vitamin D fortified milk on vitamin D status in Mongolian school age children

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Mongolians are at high risk for vitamin D deficiency because of their residence at northern latitude, reduced exposure to UV-B rays during the winter months, and a low availability of vitamin-D fortified foods. We performed a pilot study in May 2005 to estimate the prevalence of hypovitaminosis D in Mongolian school age children and to determine the feasibility of conducting a longer and larger trial with fortified milk and vitamin D supplements. In a group of 46 Mongolian children (22 girls and 24 boys) aged 9-11 years, 76% (35) had levels of 25-hydroxyvitamin D (25(OH)D) below 50nmol/L (20ng/mL) and 32% had levels below 37nmol/L (15ng/mL). After a month of consuming 710 ml of vitamin D-fortified (total 300IU or 7.5µg) milk daily, only 3 of the children were below 50nmol/L (20ng/mL) and none below 37nmol/L (15ng/mL). These results reveal prevalent and serious 25(OH)D deficiency among Mongolian prepubertal school age children that appears to be ameliorated by a month of consuming approximately 7.5µg of vitamin D3 in fortified milk.

**Key Words:** milk, vitamin D deficiency, growth, rickets, Mongolia

## INTRODUCTION

Mongolia is a landlocked country in Central Asia surrounded on the east, south and west by China and to the north by Russia. At latitudes above 37°N and below 37°S, ultraviolet light is too weak to induce sufficient cutaneous vitamin D synthesis during winter.<sup>1</sup> Mongolians are at high risk for vitamin D deficiency because of their residence at northern latitude (45°) and reduced exposure to UV-B rays during the winter months. Further, daytime winter temperatures of -20 to -40°C cause Mongols to stay more covered up. During summer months, however, ultraviolet light from the sun may allow the skin of both adults and children, with active outdoor lifestyles, to produce enough vitamin D.<sup>2</sup> Vitamin-D fortified foods are not widely available.

In a survey conducted by the United Nations Children's Fund and the Ministry of Health in 1992, 44% of children less than age 5 had clinical signs of rickets.<sup>3</sup> The highest prevalence rates were in the capital city of Ulaanbaatar. The city has experienced rapid growth over the last decade since the Soviet withdrawal, as harsh economic conditions in the countryside forced migration to the city. Data on the serum 25(OH)D levels in school children are scarce, particularly during the prepubertal growth spurt, a critical time for bone development.

Vitamin D deficiency not only causes rickets among children, but is also associated with an increased risk of cardiovascular disease,<sup>4,5</sup> multiple sclerosis,<sup>6</sup> rheumatoid

arthritis,<sup>7</sup> and type I diabetes mellitus, later in life.<sup>8,9</sup> Epidemiological studies show that higher serum 25(OH)D status, and/or environmental ultraviolet exposure is associated with lower rates of breast,<sup>10</sup> ovarian,<sup>11</sup> prostate<sup>12,13</sup> and colorectal cancers.<sup>14,15</sup> New evidence has accumulated that vitamin D can have important functions in the immune system, specifically the innate immune system.<sup>16</sup>

## MATERIALS and METHODS

The study was conducted over one month in May in a public school from the Songino-Khairhan district, located in the eastern part of Ulaanbaatar. Participants included a third grade classroom of 46 girls and boys. A letter was sent home to parents explaining the study and inviting parents and children to an informational meeting about the study. After the question and answer period, parents who wished for their children to participate signed an informed consent, and children signed an assent. All participants and their parents completed an enrollment survey that queried food allergies, age, gender, dairy food

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preferences, and recent consumption of milk, yogurt and cheese. Dietary data was also collected at two time points, by 24 hour recall.

The study was approved by the Mongolian Ministry of Education and Ministry of Health Ethical Review Board and the Human Subjects Committee of the Harvard School of Public Health.

The local school doctor reviewed each child's medical history. A registered nurse weighed the children, using standard double beam scales available at the clinic. Heights were measured with children standing shoeless with backs against a vertical surface and a right-angle level brought to the crown of the head.

For one month each child drank three 236 mL tetrapack boxes daily of conventional UHT-processed fortified whole milk (100 IU vitamin or 2.5 µg D3 per serving) from a large U.S. milk producer (Borden). During the school-week, participants drank their portion of milk under the supervision of a homeroom teacher and trained personnel. During holidays and weekends, the children drank the milk at home under their parents' supervision. A physician examined each child for bone deformities consistent with rickets, ranking each symptom as mild or moderate/severe: angular deformities of the knees, Harrison's groove, pigeon chest, and widened wrists or "symptom bracelet".

Approximately 8 mL of blood was obtained by vein puncture from each child into standard red top tubes pre- and post-intervention. After blood was centrifuged, 3 ml of serum was drawn into 3 x 2 ml cyrotubes, frozen, and shipped to the United States (biochemical determination of serum 25(OH)D levels is not available in Mongolia at present). Vitamin D analysis was performed at Boston Children's Hospital by enzyme-linked immunoabsorbent assay (ELISA) with reagents from ALPCO Diagnostics (Windham, NH). The average intra-assay coefficients of variation were below 10%. Samples were identifiable only by study number.

Descriptive statistics (mean, standard deviation and range) were calculated for all variables. A paired Student's t-test was used to examine change in serum 25(OH)D levels before and after the intervention. Statistical analyses were performed using SPSS 8.0 for Windows and SAS 9.1 software.

## RESULTS

After one month of drinking milk, all children had an increase in height and weight (Table 1). Over the course of one month, girls grew a mean 1.1 (1.0 sd) centimeters, 0.7 (0.8) kg, and had a mean drop in BMI of -0.3 (0.3) kg/m<sup>2</sup>. Boys grew a mean 1.0 (1.1) centimeters, 0.2 (0.7) kg, and had a mean decrease in BMI of -0.26 (0.3) kg/m<sup>2</sup>.

Dairy intake was very low among Mongolian children in the study (Table 1). The most common source of any dairy products was milk tea. Sixty eight percent of children reported no milk intake in the week prior to the start of the intervention. Fifteen percent of children had no dairy intake at all, confirming anecdotal reports of low dairy intake among Mongolian urban children.

We observed mild bone deformities consistent with possible rickets in 40% of the children, and more severe deformities indicative of probable rickets in 36% of chil-

**Table 1.** Mean (SD) characteristics of participants before and after one month of drinking 710 mL of vitamin D fortified whole milk daily

	Girls	Boys
Number	22	24
Age	10.6 (0.6)	10.4 (0.7)
Weight before (kg)	28.8 (4.7)	29.1 (3.6)
Weight after (kg)	29.5 (4.7)	29.1 (3.6)
Weight change (kg)	+0.7 (0.8)	+0.2 (0.7)
Height before (cm)	130.8 (6.2)	132.5 (6.7)
Height after (cm)	131.9 (6.1)	133.6 (6.7)
Height change (cm)	+1.1 (1.0)	+1.0 (1.1)
BMI before (kg /m <sup>2</sup> )	16.8 (1.6)	16.5 (1.2)
BMI after (kg /m <sup>2</sup> )	16.5 (1.5)	16.3 (1.2)
BMI change (kg /m <sup>2</sup> )	-0.3 (0.3)	-0.3 (0.3)
Tanner stage >1	4% (1/22)	12% (3/24)
In past week:		
Servings of milk	0.5 (1.0)	0.8 (1.3)
Servings of other dairy <sup>†</sup>	4.1 (4.0)	6.5 (6.0)

<sup>†</sup> "Other dairy" for the Mongolia children consisted of cheese, yoghurt, or milk added to tea.

**Table 2.** Change in serum 25(OH)D levels before and after one month of drinking 710 mL of vitamin D fortified whole milk daily

	25(OH)D		
	mean ± SD (range)	<50 nmol/L below normal	<37 nmol/L below critical level
Before	43.1 ± 12.0 (25.2-86.4)	76%	32%
After	66.1 ± 11.2 (45.9-101)	7%	0%

dren. At the start of the intervention, 76% of the children had 25(OH)D levels <50 nmol/L (20ng/mL) (lower limit of normal) and 32% of the children had 25(OH)D levels <37 nmol/L (15ng/mL). After one month of drinking vitamin D fortified milk, only 7% had 25(OH)D levels <50 nmol/L (20ng/mL) and none <37 nmol/L (15ng/mL) (Table 2). Recent data using various biomarkers such as intact parathyroid hormone (PTH), intestinal calcium absorption, and skeletal density measurements suggest that levels of 25(OH)D should be maintained at or above 80 nmol/L (32ng/mL) for optimal health.<sup>17</sup> Only one child had 25(OH)D level >80 nmol/L (32ng/mL) at start of the intervention and only 5 children had levels >80 nmol/L (32ng/mL) after the intervention.

## DISCUSSION

Without historical data on these children, it is difficult to say whether the signs of rickets reflected resolved or active cases. Also some of the improvement in 25(OH)D status in the schoolchildren in May, may have been the result of increasing sunlight exposure. As this feasibility study was not designed to include a control group, we cannot determine the extent to which the increase in 25(OH)D levels was due to the milk intervention or to the increasing sunlight. The study was not budgeted to allow the determination of the PTH levels as an additional surrogate for the degree of vitamin D deficiency. The main objective for our pilot study was not the response of 25(OH)D to milk (or sunlight) but rather the extent of the deficiency in this population. These data are among the

first to evaluate 25(OH)D status and clinical signs of rickets among school-age children in Mongolia.

Clinical sign of rickets depends on the age of onset and the severity of deficiency. Rickets will be more severe if the deficiency is coincident with a period of rapid growth. Rickets thus usually occurs in the first two years of life but de novo emergence of the disease during adolescence has also been described.<sup>18,20</sup> During these two critical periods of life, rapid growth occurs and peak bone mass is achieved.<sup>19</sup> The symptoms observed in the study may represent either prevalent or incident cases; we cannot tell due to the short period of observation. Not only is this population vitamin D deficient, but in all likelihood there is widespread calcium deficiency as well. Fraser reported that the dietary calcium intake of children under 5 years of age was only 46% of the Mongolian recommended daily intake (265mg per day).<sup>2</sup> In our study, we documented that dairy intake, a major source of dietary calcium, was also very low in this population. (Table 1)

Presently, there is a limited source of vitamin D fortified milk in Mongolia. Mass Mongolian milk production is currently quite low; one Mongolian manufacturer sells vitamin D fortified milk; most commercially available milk is imported unfortified from Russia, China, and New Zealand. This study provides important information for Mongolian health sector planning with regard to vitamin D deficiency in childhood and the potential of increased risk of adult diseases. It would be advisable for Mongolia to test alternative vitamin D repletion strategies, including supplementation and milk and/or other food fortification.

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#### AUTHOR DISCLOSURES

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### 維生素 D 強化牛奶對蒙古學齡兒童維生素 D 狀態之效應

居處在北方高緯度而冬季鮮能接受到 UV-B 紫外線的照射，以及難以獲得有維生素 D 強化的食物，使得蒙古人成為缺乏維生素 D 的高危險群。我們在 2005 年 5 月進行一個先驅研究來估計蒙古學齡兒童中低維生素 D 的盛行率，並且評估實施一個較長期也較具規模以強化牛奶和維生素 D 補充劑試驗的可行性。46 位 9-11 歲的蒙古兒童(22 位女孩，24 位男孩)，76%(35 位)其 25-羥基維生素 D 低於 50nmol/L (20ng/mL)，32%低於 37nmol/L (15ng/mL)。在每日食用添加維生素 D 的牛奶 710mL(共 300IU 或 7.5μg)一個月後，只有 3 個兒童低於 50nmol/L (20ng/mL)、沒有人低於 37nmol/L (15ng/mL)。這些結果顯示蒙古青春前期學齡兒童有嚴重的 25(OH)D 缺乏，可以飲用添加約 7.5μg 維生素 D3 的牛奶一個月來改善。

關鍵字：牛奶、維生素 D 缺乏、生長、軟骨症、蒙古。