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Vitamin D Deficiency in Reproductive Age Mongolian Women: A Cross Sectional Study

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Abstract

Vitamin D production is critical not only for rickets prevention but for its role in several chronic diseases of adulthood. Maternal vitamin D status also has consequences for the developing fetus. This study assessed the prevalence of vitamin D deficiency (serum 25-hydroxyvitamin D [25(OH)D] <20 ng/ml) and insufficiency [25(OH)D = 20–29 ng/ml] in spring, among reproductive age Mongolian women.

Blood was drawn in March and April, 2009 from 420 Mongolian women, 18–44 years of age. Serum 25(OH)D concentrations were measured, anthropometric measurements were performed and information was collected by interview on lifestyle, dietary and reproductive factors. Logarithm-transformed 25(OH)D levels were compared across risk factor categories by analysis of variance. Linear regression analysis was used to assess the independent associations of factors with vitamin D status. Cutaneous vitamin D₃ synthesis was assessed between December and July using a standard 7-dehydrocholesterol ampoule model.

The vast majority of women 415 (98.8%) had serum 25(OH)D <20 ng/ml (50 nmol/l) with an additional 4 women (<1%) in the insufficient range (20–29 ng/ml); only one women (0.2%) had sufficient levels (>30 ng/ml or 75 nmol/l). 25(OH)D concentrations were positively and

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The authors' responsibilities were as follows:

GD, RT, RH, JRE, LF, CJ, MH: designed research study;

DG, DD: conducted research;

RT, DG: analyzed data;

GD, RT: wrote paper;

RH, JRE, LF, CJ, MH, DD, BN: read and contributed to the paper

MH: supervised laboratory analysis.

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independently associated with educational status and use of vitamin D supplements, but not with other demographic, lifestyle, reproductive, or anthropometric factors. 25(OH)D levels were not associated with dietary factors in this population, as there is little access to foods containing vitamin D in Mongolia. No production of previtamin D₃ was observed until March and was maximally effective in April and was sustained through July.

These data suggest that the prevalence of vitamin D deficiency in spring among reproductive age women in Mongolia is high. Given the lack of naturally vitamin D-rich food in the diet and limited use of vitamin D supplements, food fortification and/or supplementation with vitamin D should be considered among these women.

Keywords

25-hydroxyvitamin D; vitamin D; vitamin D deficiency; healthy women; reproductive age

INTRODUCTION

In many countries throughout the world, vitamin D deficiency is a major public health problem that has been linked not only to deficiency diseases such as rickets, but to a range of common chronic diseases in adulthood including diabetes, cancer, infectious diseases, cardiovascular health, and autoimmune diseases (1). In women of reproductive age, vitamin D status is of particular importance, as maternal deficiency has been associated with short- and long-term effects in the offspring (2–4). The Institute of Medicine (IOM) advocates a Recommended Dietary Allowance (RDA) for vitamin D of 600 IU/d for ages 1–70 years, corresponding to a serum 25-hydroxyvitamin D [25(OH)D] level of at least 20 ng/ml (50 nmol/l). This level is thought to meet the health requirements of 97.5% of the United States (<http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx>) (5), and Canadian populations, although others have advised a higher level for optimal health benefits, including the Endocrine Society (6,7).

In summer, the main natural source of vitamin D is the sun as vitamin D is synthesized in the skin after exposure to solar ultraviolet B radiation (wavelength 290–315 nm). Vitamin D is metabolized in the liver to 25-hydroxyvitamin D [25(OH)D] by the enzyme CYP27A1, then further metabolized, primarily in the kidneys, to its biologically active form, 1,25-dihydroxyvitamin D. Serum levels of 25(OH)D, the main circulating metabolite, are accepted as the best indicator of vitamin D status in humans (1,5–7).

In Mongolia, there is almost no vitamin D produced in the skin between October and April because the amount of ultraviolet (UV) B reaching the earth's surface is insufficient at this latitude (the capital, Ulaanbaatar is 48°N) (12). In addition, Mongolia lacks vitamin D-fortified foods, oily fish or other naturally vitamin D-rich foods in the diet (12).

We evaluated the prevalence of vitamin D deficiency and determinants of vitamin D status in early spring in a sample of women of childbearing age living in Mongolia. In addition, we conducted an environmental assessment of the effectiveness of sunlight for producing vitamin D₃ in Ulaanbaatar in ampoules by measuring conversion of 7-dehydrocholesterol to previtamin D₃ (8).

Subjects and Methods

Study participants were the mothers of school children attending two primary schools in Ulaanbaatar. Using schools as the recruitment sites provided a central location for data collection. Data were collected in two half-day sessions in March and April, 2009. Eligible were mothers of children attending grades 1 through 4, who were 18–44 years of age, and

who were not pregnant or breastfeeding. Of the 420 invited women, 100% agreed to participate.

Participants completed an interview that ascertained information on demographics, history of recent migration from countryside to Ulaanbaatar, medical and reproductive history, physical activity, diet, smoking status and alcohol consumption. Leisure and work-based physical activity intensity (ascertained as low, moderate and high) was assessed with the Global Physical Activity Questionnaire developed by the World Health Organization, which collects information on both the intensity and total amount of exercise (described previously; 9). To assess dietary intake, participants were presented with a list of foods based on survey data collected by the Nutritional Research Center at the Mongolia Public Health Institute, and asked, “in the past week, how many times did you eat...?” Height was measured using a stadiometer with a centimeter scale; weight in kilograms using a clinical scale with the participant wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kg)/height (m)². Serum samples were stored at -70 ° C until they were shipped to the U.S. biorepository and then to Boston University Medical Center for 25(OH)D assays.

25(OH)D₂ and 25(OH)D₃ were measured by liquid chromatography (LC) - tandem mass spectrometry through a turbulent flow LC system (Cohesive Technologies, Franklin, MA) followed by traditional laminar flow chromatography (10). When assayed, blinded aliquots of pooled sera constituted 10% of each batch of study samples. The blinded coefficient of variation (CV) was 18.4% for total 25(OH)D which is expected when serum 25(OH) concentrations are <10 ng/ml. The interassay CV for the assay is 10% on routine clinical samples.

In addition to the biological component of the study, we conducted an environmental assessment of the effect of season on the sun-induced production of vitamin D₃ exposure in Ulaanbaatar during the same year by placing ampoules of the vitamin D precursor, 7-dehydrocholesterol (7-DHC) at various months for three consecutive days for one hour from 12:30 to 1:30pm outside between December and July. The 7-dehydrocholesterol in ethanol (50 mg/ml) was sealed under argon in borosilicate ampoules, as previously described (8,11). The conversion of 7-dehydrocholesterol to previtamin D₃ was determined by high performance liquid chromatography (HPLC).

Ethics

The study was approved by ethical review boards at Mongolia's Ministry of Health, the National University of Mongolia, the United States National Cancer Institute (National Institutes of Health), and the Harvard School of Public Health. All participants provided written, informed consent.

Statistical Analysis

Total serum 25(OH)D [25(OH)D₂ + 25(OH)D₃] was used as the outcome in this study. Proportions of subjects were calculated who were vitamin D deficient (< 20 ng/ml), or who had levels that were considered “insufficient” for bone and overall health in healthy individuals (>20–<30 ng/ml) (5,6). 25(OH)D concentrations are presented as means and S.D. Analysis of variance (ANOVA), using SAS Proc GLM (general linear models), was used to determine the statistical significance of the differences in mean 25(OH)D (logarithm transformed to improve normality) by categories of the women's characteristics.

Linear regression models were used to further test for independent associations between 25(OH)D and factors that showed some association in the univariate comparisons (based on $p < 0.10$). Exposures that were ordinal (such as age) were modeled with a single exposure

covariate, treated as ordinal, to test for trend using linear regression models (testing overall significance among groups resulted in similar findings). All p-values are two-tailed and statistical significance was defined as $p < 0.05$.

RESULTS

The mean age of the participants was 34.9 years (SD 4.8). The mean weight was 62.0 kg (SD 11.8), mean height was 157.2 cm (SD 5.5), mean waist circumference was 81.0 cm (SD 11.1) and mean BMI was 25.1 (SD 4.5). The mean serum 25(OH)D concentration was 7.6 ± 4.0 ng/ml (18 ± 10 nmol/l), and the median concentration was 6.7 ng/ml. Mean 25(OH)D concentration was higher in women whose blood collection was in April than in March (7.9 vs. 7.0 ng/ml, $p = 0.01$). The vast majority (415, 98.8%) of the participants had serum 25(OH)D levels < 20 ng/ml, while four participants (0.95%) had levels between 20 and 29 ng/ml (Table 1). Only one woman (0.2%) had a 25(OH)D value > 30 ng/ml. Using a cutoff of < 12 ng/ml, the proportion of women with insufficient 25(OH)D values was 88.6% ($n = 372$). Age was uncorrelated with 25(OH)D concentration (Pearson $r = 0.03$, $p = 0.52$). Figure 1 presents serum 25(OH)D levels by age (p -value for linear regression coefficient 0.64). Of the five women with serum 25(OH)D levels above 20 ng/ml, one reported taking vitamin D supplements and the remaining four reported taking multivitamins.

Most of the demographic, lifestyle, reproductive, anthropometric and dietary factors were not associated with 25(OH)D levels (Tables 2–4). However, there were positive associations of 25(OH)D concentrations with education and longer duration of breastfeeding, with women who had a history of breastfeeding for the shortest total duration having the lowest 25(OH)D levels, although this association did not reach statistical significance. The small number of women who reported taking vitamin D supplements (5.7%) had higher 25(OH)D concentrations. When educational status, average duration of breastfeeding, multivitamin use and vitamin D supplement use were simultaneously included in a linear regression model, higher 25(OH)D levels were observed only with greater educational status ($p = 0.01$) and vitamin D supplementation ($p = 0.01$). Use of vitamin D supplements was not related to level of education (data not shown).

The results of conversion of 7-dehydrocholesterol to previtamin D₃ measured by HPLC suggested that skin production of vitamin D could begin in March and was more effective in producing previtamin D₃ in April, June and July in Mongolia (Figure 2). The effect of heavy clouds during July markedly reduced previtamin D₃ synthesis similar to what was observed in Boston (11).

Discussion

This study revealed that during early spring in Ulaanbaatar, Mongolia, nearly 99% of reproductive age women had serum 25(OH)D levels considered deficient (here defined as < 20 ng/ml), and the remaining 1% with levels considered inadequate (20– < 30 ng/ml) for bone and overall health. Almost 80% of the women had values below 10 ng/ml and almost a third had values below 5 ng/ml. The finding that nearly all women were vitamin D deficient in early spring was observed regardless of the criterion for vitamin D deficiency or insufficiency that was used (< 20 ng/ml vs. < 30 ng/ml) (5,6). Our own estimates of vitamin D production in Ulaanbaatar, suggest that skin production of vitamin D only begins to rise in March (Figure 2) and continued to at least July when the study was completed. Based on observations in Edmonton, Canada it would be expected that synthesis of vitamin D in the skin would cease after October (8). As we measured 25(OH)D concentrations in women slightly after this period (late March, and April), when levels should be rising from their nadir in late winter, these results are not surprising because daily temperatures of 33–63°F

even in May often mean that only the face and hands are uncovered by clothing in these women. Only 5.7% of women reported taking vitamin D supplements.

Vitamin D has long been recognized as an important factor for many chronic conditions in addition to its well-recognized relationship to bone health (12–18). It is of particular relevance to women of reproductive age that maternal 25(OH)D concentrations are critical for the vitamin D status of the offspring (19–22). Additionally, vitamin D deficiency and insufficiency are associated with increased risks of pregnancy complications such as gestational diabetes, preeclampsia, and requirement of a primary Caesarean section (23–24). Though not specifically addressed in our study, 25(OH)D levels may be important in the epidemiology of hepatitis C infection (25–30), a serious problem in Mongolia which leads to an unusually high death rate from liver cancer and cirrhosis. Given the high prevalence of both vitamin D deficiency and hepatitis C infection in Mongolia, exploring the relationship between vitamin D deficiency and the prevalence and severity of non-alcoholic liver disease, as well as the response to antiviral therapy in hepatitis C (31) is warranted. One other study (32) documented vitamin D status among non-pregnant Mongolian women in the summer (July–September) and found that 30% of women had 25(OH)D levels <7 ng/ml and 22% had levels between 7–9 ng/ml.

In a Turkish study, 46% of the mothers had serum 25(OH)D concentrations <10 ng/ml (33) and in a study in Pakistan, 45% of mothers had serum 25OHD concentrations < 10 ng/ml (34). In our study, 80% of the mothers had serum 25OHD levels <10 ng/ml. The problem of vitamin D deficiency in many countries is reflected by the high prevalence of rickets (35). Prentice et al. reported that Mongolia has the highest prevalence of rickets followed by Tibet and Ethiopia (36).

In our study, serum 25(OH)D levels were not associated independently with any of the factors studied, including several lifestyle, reproductive, anthropometric and dietary variables apart from education level and vitamin D supplements. Whereas parity was unrelated to 25(OH)D levels, average months of breastfeeding each child was marginally associated in univariate analyses, an association that was further attenuated with adjustment for vitamin D supplement use and education. Lower education-economic level was found to predict lower vitamin D status of mothers in Turkey, respectively (34). We also found that women with higher education had higher 25(OH)D levels. This may have been due to generally better health or access to imported, vitamin D fortified foods. In addition, BMI has been demonstrated to be inversely correlated with serum 25(OH)D levels (37, 38), possibly owing to the sequestration of vitamin D in adipose tissue resulting in reduced bioavailability (39), or to less outdoor physical activity. We did not observe an association between BMI and 25(OH)D levels in the present data, possibly because all of the 25(OH)D values were low. These data suggest that vitamin D status is not influenced by factors other than supplementation and education status in the Mongolian population.

There are some limitations to our study. One of them is that the study could not reflect seasonal differences because the study was only conducted in the spring. Also the study represents sample of the population of reproductive age women in capital city only. We were not able to measure levels of parathyroid hormone (PTH) and calcium in blood. The first one is indirect biomarker for vitamin D deficiency and rise when levels of 25(OH)D fall below 30 ng/ml (1). The other, as proposed in earlier studies, it was suggests that active calcium absorption is optimal when the level of 25(OH)D is 30 ng/ml (40). When calcium intake is insufficient, production of 1,25-dihydroxyvitamin D increases enhancing 25-hydroxyvitamin D-24-hydroxylase activity and increasing the catabolism of 25(OH)D (1,41).

The recommended intake of vitamin D in Mongolia for children <5 years is one dose of 1250 µg (50,000IU) each month during the cold season of the year to prevent rickets (<http://moh.mn/moh%20db/HealthReports.nsf/homepage?OpenFrameSet>) but there is no recommendation for the adult population. The Canadian Cancer Society (42) recommends 1000 IU of vitamin D daily during the fall and winter for Canadians who are located at the same high northern latitude as Mongols. Moreover, 1000 IU of vitamin D daily all year is recommended for those who are unlikely to produce enough vitamin D during summer, such as the elderly, people with dark skin, and those who avoid sunlight exposure.

In Mongolia, the majority of milk consumed is provided directly to local processing plants by traditional herding households, and sometimes directly to consumers without processing. Therefore, fortification of wheat might be a better vehicle for providing vitamin D for the Mongolian population, and ideally this would be combined with provision of supplements. Given the limits on the amount of vitamin D that can be added to the food supply to avoid toxicity among individuals who might consume large amounts of the fortified staple, a combined strategy of fortification and supplement use may be necessary to raise 25(OH)D levels above 20 ng/ml in the winter in Mongolia. Much work is required to increase public awareness about the importance of reaching and maintaining, optimal serum 25(OH)D levels throughout the year in Mongolia.

In conclusion, our data indicate that a high prevalence of profound vitamin D deficiency in the spring is common in reproductive age women living in Ulaanbaatar, Mongolia. Food fortification with vitamin D warrants consideration.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
BMI	body mass index
RDA	recommended dietary allowance

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Highlights

- We reported vitamin D levels in two populations of healthy Mongolian women: urban born and rural born
- We documented lowest vitamin D levels in the world
- We also showed severe prevalence of vitamin D deficiency
- No vitamin D fortified and/or vitamin D rich food available in the country
- Actions to improve vitamin D levels are required in this specific population

Figure 1.
Plot of serum 25-hydroxyvitamin D [25(OH)D] values by age, March–April 2009

Figure 2.

Percent conversion of 7-dehydroxycholesterol (7-DHC) to previtamin D₃ (preD₃) in ampoules containing 7-DHC. They were placed outside in direct sunlight in Ulaanbaatar in at various months for one hour (from 12:30 p.m. to 1:30 p.m) at noon. The samples were stored in the dark and evaluated by high-performance liquid chromatography (HPLC).

*The data for May is not stated because the ampoule was damaged

Table 1

Distribution of serum 25-hydroxyvitamin D [25(OH)D] levels

25(OH)D Level (ng/ml)	N	%
<5	119	28.3
5–<10	214	51.0
10–<15	64	15.2
15–<20	18	4.3
20–<30	4	0.95
30+	1	0.24

Table 2

Mean (SD) serum 25-hydroxyvitamin D [25(OH)D] (ng/ml) levels by demographic and lifestyle variables

	N (%)	Mean (SD)	p ^I
Total sample	420 (100)	7.6 (4.0)	
Birthplace			0.27
Ulaanbaatar	178 (42)	7.8 (4.5)	
All other provinces	242 (58)	7.4 (3.7)	
Age (years)			0.68*
<30	67 (16)	7.6 (3.4)	
30–34	158 (37)	7.4 (4.3)	
35–39	116 (28)	7.5 (3.9)	
40+	79 (19)	7.8 (4.3)	
Marital Status			0.94
Single	8 (2)	7.6 (2.7)	
Married	359 (85)	7.5 (3.8)	
Divorced or separated	41 (10)	7.8 (6.1)	
Widowed	12 (3)	7.4 (4.9)	
Education			0.01
High school or less	153 (36)	7.3 (3.5)	
Some college	75 (18)	6.9 (3.5)	
Graduated college	152 (36)	7.6 (3.8)	
Graduate school	40 (10)	9.6 (6.6)	
House type			0.37
Indoor with plumbing	271 (65)	7.4 (3.6)	
Outdoor (yurt)	149 (35)	7.8 (4.8)	
Current Smoking Status			0.12
Yes	55 (13)	6.8 (2.9)	
No	365 (87)	7.7 (4.2)	
Current alcohol status			0.90
Yes	95 (23)	7.5 (5.0)	
No	325 (77)	7.6 (3.7)	
Work physical activity level			0.90*
Low	96 (23)	7.7 (3.9)	
Moderate	111 (26)	7.3 (3.5)	
Vigorous	213 (51)	7.6 (4.3)	
Leisure physical activity level			0.17*
Low	240 (57)	7.2 (3.6)	
Moderate	62 (15)	8.5 (4.2)	
Vigorous	116 (28)	7.7 (4.7)	

¹ p-value from analysis of variance models.

* p-value is from trend in level of 25(OH)D across categories tested using linear regression models.

Table 3

Mean (SD) serum 25-hydroxyvitamin D [25(OH)D] (ng/ml) levels by reproductive factors and anthropometric measurements

	N (%)	Mean (SD)	p ¹
Parity			0.44
1	110 (26)	7.3 (4.7)	
2	183 (44)	7.7 (4.0)	
3	88 (21)	7.3 (3.3)	
4+	39 (9)	8.2 (4.1)	
Current use of contraceptives			0.31
Yes	106 (25)	7.9 (4.7)	
No	313 (75)	7.4 (3.8)	
Average months breastfed per child			0.10 [*]
<12	119 (29)	7.1 (3.2)	
12–24	145 (35)	7.6 (3.6)	
>24 ^{**}	146 (36)	7.9 (5.4)	
Weight (quartiles; kg)			0.58 [*]
35.8 – 53.2	104 (25)	7.7 (3.8)	
53.3 – 60.3	106 (25)	7.6 (5.0)	
60.4 – 68.9	104 (25)	7.5 (3.6)	
69.0 – 119.1	106 (25)	7.4 (3.5)	
Height (quartiles; cm)			0.88 [*]
138.6 – 153.3	105 (25)	7.5 (4.0)	
153.4 – 156.9	102 (25)	7.5 (3.3)	
157.0 – 160.6	107 (25)	7.9 (4.2)	
160.7 – 172.7	106 (25)	7.3 (4.6)	
Body Mass Index (kg/m²)			0.85 [*]
≤ 20	36 (9)	7.3 (3.8)	
21–24	194 (46)	7.7 (4.5)	
25–<30	134 (32)	7.5 (3.4)	
30+	56 (13)	7.4 (3.9)	

¹ p-value from analysis of variance models.

^{*} p-value is from trend in level of 25(OH)D across categories tested using linear regression models.

^{**} information on breastfeeding was missing for 10 participants.

Table 4

Mean (SD) serum 25-hydroxyvitamin D [25(OH)D] (ng/ml) levels by dietary intake

	N (%)	Mean (SD)	p ^I
Meat, meat product servings/day			0.53 [*]
0	12 (3)	8.1 (3.7)	
1	127 (30)	7.3 (4.7)	
2	153 (36)	7.6 (4.0)	
3+	128 (31)	7.7 (3.5)	
Dairy servings/day			0.11 [*]
<2	83 (20)	7.0 (3.1)	
2–3	166 (39)	7.5 (4.1)	
4+	171 (41)	7.9 (4.4)	
Vitamin D supplement use			0.03
Yes	23 (5)	9.3 (3.8)	
No	397 (95)	7.4 (4.0)	
Multivitamin use			0.07
Yes	221 (53)	7.9 (4.2)	
No	199 (47)	7.2 (3.9)	

^I p-value from analysis of variance models.

^{*} p-value is from trend in level of 25(OH)D across categories tested using linear regression models.