



Research paper

Randomized trial of three doses of vitamin D to reduce deficiency in pregnant Mongolian women



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ABSTRACT

Background: In winter in Mongolia, 80% of adults have 25-hydroxyvitamin D (25(OH)D) concentrations <25 nmol/l (<10 ng/ml) and 99% have <50 nmol/l (<20 ng/ml). The vitamin D dose to avert deficiency during pregnancy in this population is unknown.

Methods: We conducted a randomized, controlled, double-blind trial of daily 600, 2000, or 4000 IU vitamin D₃ for pregnant women in Mongolia (ClinicalTrials.gov #NCT02395081). We examined 25(OH)D concentrations at baseline (12–16 weeks' gestation), 36–40 weeks' gestation and in umbilical cord blood, using enzyme linked fluorescent assay. Sample size was determined to detect 0.4 standard deviation differences in 25(OH)D concentrations with 80% power.

Findings: 119 pregnant women were assigned 600 IU, 121 assigned 2000 IU and 120 assigned 4000 IU from February 2015 through December 2016. Eighty-eight percent of participants took ≥80% of assigned supplements. At baseline, 25(OH)D concentrations were similar across arms; overall mean ± standard deviation concentration was 19 ± 22 nmol/l; 91% were < 50 nmol/l. At 36–40 weeks, 25(OH)D concentrations increased to 46 ± 21, 70 ± 23, and 81 ± 29 nmol/l for women assigned 600, 2000, and 4000 IU, respectively (p < 0.0001 across arms; p = 0.002 for 2000 vs. 4000 IU). Mean umbilical cord 25(OH)D concentrations differed by study arm (p < 0.0001 across arms; p < 0.0001 for 2000 vs. 4000 IU) and were proportional to maternal concentrations. There were no adverse events, including hypercalcemia, attributable to vitamin D supplementation.

Interpretation: Daily supplementation of 4000 IU during pregnancy is safe and achieved higher maternal and neonatal 25(OH)D concentrations than 2000 IU. Daily 600 IU supplements are insufficient to prevent vitamin D deficiency in Mongolia.

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1. Introduction

Vitamin D deficiency (25-hydroxyvitamin D (25(OH)D) <50 nmol/l or <20 ng/ml) [1] is widespread in Mongolia, including in the capitol of Ulaanbaatar, the northern steppe, and the southern Gobi desert [2–6]. Despite Mongolia's renowned blue skies, the country sits at a

high latitude (44°–50° N) at which ultraviolet B (UVB) rays are heavily filtered by the atmosphere; UVB exposure is inadequate to induce cutaneous vitamin D production from October through April [3]. Furthermore, Mongolians consume little of the few food sources, such as fish, that naturally contain significant quantities of vitamin D, and there is not yet a national vitamin D food fortification program [2]. The mean daily vitamin D intake from foods is <70 International Units (IU) in any season [2], considerably lower than the 600 IU recommended by the Institute of Medicine [7]. We have conducted a series of surveys among working age men and women across the country which have

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Research in context

Evidence before this study

Most prenatal vitamin formulations contain 400 to 600 IU of vitamin D, which is likely ineffective in preventing vitamin D deficiency (25-hydroxyvitamin D (25(OH)D) <50 nmol/l) in regions with very low vitamin D status. We sought to test in a randomized, double-blind trial the effectiveness of three doses of daily vitamin D supplements to raise 25(OH)D concentrations and avert vitamin D deficiency in a population of pregnant women in Mongolia with baseline 25(OH)D concentrations <25 nmol/l, characteristic of many pregnant populations in the Middle East and Asia. Based on a search of the literature in PubMed in September 2018 with the search terms 'vitamin D, deficiency, pregnancy, 25(OH)D, trial' we found only one study that meets these criteria, by Dawodu and colleagues, in the United Arab Emirates. (Other studies in populations with baseline 25(OH)D concentrations <25 nmol/l or < 30 nmol/l used large doses administered weekly or monthly, which is not relevant to the formulation of daily prenatal supplements, the most common supplementation where clinical screening of vitamin D is not routine.)

Added value of this study

Our study tests the effectiveness of three doses of vitamin D supplements – the minimum (600 IU), an intermediate (2000 IU), and the tolerable upper limit (4000 IU) for daily vitamin D supplementation during pregnancy set by the Institute of Medicine. It is almost twice the size of the previous study among Arab women, which also tested 2000 IU and 4000 IU, but which employed as its lowest dose the now-outdated 400 IU. Clinicians can use the results of this study to inform the supplementation of populations where vitamin D deficiency is prevalent and particularly where vitamin D testing is neither clinically routine or feasible. These data are also critical to researchers designing trials of vitamin D supplementation to prevent pregnancy complications in regions with profound vitamin D deficiency.

Implications of all the available evidence

The two randomized trials of daily vitamin D supplementation in pregnant populations with severe vitamin D deficiency, in the United Arab Emirates and Mongolia, are consistent in their findings. Fewer than half of women randomized to 400 or 600 IU achieved 25(OH)D concentrations above 50 nmol/l. Most women consuming 2000 IU averted vitamin D deficiency. However, the 4000 IU dose was most effective in raising both maternal and neonatal 25(OH)D concentrations above 50 nmol/l. There were no adverse effects of 4000 IU vitamin D during pregnancy, consistent with previous observations and the evaluation of the Institute of Medicine. In regions where vitamin D concentrations are very low, 4000 IU of vitamin D is safe and preferable to standard prenatal vitamin formulations.

shown mean (\pm SD) 25(OH)D concentrations of 19 ± 8 nmol/l (8 ± 3 ng/ml) in winter and 56 ± 20 nmol/l (22 ± 8 ng/ml) in summer [2]. In Ulaanbaatar, 99% of women of reproductive age had serum 25(OH)D concentrations below 50 nmol/l in March and April [3]. Even in summer, 32% of Mongolian women exhibited 25(OH)D concentrations <50 nmol/l [2].

Many, but not all, observational studies of 25(OH)D concentrations and early clinical trials of vitamin D supplements suggest that low

vitamin D status is associated with substantially increased risks of preeclampsia, gestational diabetes, preterm delivery, and fetal growth restriction or low birthweight [8–17]. Inconsistencies in the literature may stem, in part, from variability in population concentrations of 25(OH)D, the doses used in clinical trials, and the concentrations of 25(OH)D achieved [17–19]. Based on these early reports, the Lancet Nutrition Interventions Review Group recommended that maternal vitamin D supplementation warrants further study as an emerging intervention to enhance pregnancy health programs [20]. This call has particular resonance for global health, as large proportions of women in the Middle East and Asia have extremely low vitamin D concentrations [21].

Populations with universally or extremely low 25(OH)D concentrations may serve as ideal sites for proof-of-concept studies to examine the impact of raising vitamin D concentrations on pregnancy outcomes. However, to accomplish this, the dose needed to bring extremely low baseline 25(OH)D concentrations into a range considered adequate or optimal must be established. As the 25(OH)D response of a patient to a given vitamin D dose may depend upon their baseline concentration [22], studies conducted in North America and Europe have limited generalizability to regions with markedly lower vitamin D concentrations.

To our knowledge, there is only one trial of daily vitamin D supplementation in a pregnant population with mean 25(OH)D concentrations below 25 nmol/l (10 ng/ml) [23], so little is known about the amount of vitamin D supplementation necessary to bring extremely low baseline concentrations of 25(OH)D into the range considered by the Institute of Medicine to be adequate for pregnancy (≥ 50 nmol/l or 20 ng/ml), let alone into higher ranges proposed as optimal for pregnancy health by many scientists (>75 nmol/l or 30 ng/ml; some recommend concentrations >80 nmol/l or 40 ng/ml) [1,9,24]. Therefore, our primary aim was to test the impact of different concentrations of vitamin D supplementation on 25(OH)D concentrations of pregnant women in Mongolia. To generate preliminary data for future studies, we also examined pregnancy characteristics and outcomes as secondary endpoints.

The 2010 Institute of Medicine Report on vitamin D set the recommended daily allowance during pregnancy at 600 IU, and the Tolerable Upper Limit at 4000 IU [7], based on U.S. and western European populations with some dietary sources of vitamin D. Typical prenatal multivitamin formulations sold in Mongolia contain 400 or 500 IU of vitamin D. We tested the impact of 600, 2000, and 4000 IU daily vitamin D₃/day supplements starting at 12–16 weeks' gestation and continued throughout pregnancy on maternal and umbilical cord blood 25(OH)D concentrations in Mongolia.

2. Methods

2.1. Study design

'From Healthy Mother, Healthy Baby' was a randomized, controlled, double-blind study in pregnant women estimating the impact of three doses of daily vitamin D supplements started at 12–16 weeks' gestation on the concentrations of 25(OH)D measured at 36–40 weeks' gestation and in umbilical cord blood (Clinicaltrials.gov #NCT02395081; registration was finalized one month after start. No protocol changes were made in the interim). Serum calcium concentrations were monitored for safety at two months after randomization and at 36–40 weeks of gestation.

The study was approved by the Ethical Review Board of the Mongolian Ministry of Health as well as the Office of Human Research Administration of the Harvard T.H. Chan School of Public Health (Protocol #IRB14–0591). Written informed consent for trial participation and for medical record review was obtained from each participant.

Study conduct was monitored by a Data Safety and Monitoring Board (DSMB) that was comprised of a Mongolian pharmacologist and chair of the Mongolian Ministry of Health Ethical Review Board, a Mongolian obstetrician, two U.S. experts in clinical trials in pregnancy

(a statistician and an obstetrician) and an endocrinologist with expertise in calcium homeostasis. The DSMB met every six months during the study to monitor study progress and safety and was notified immediately of any adverse events potentially attributable to study participation. As 25(OH)D concentrations were not determined until after the end of the trial, stopping rules were based on evidence of benefit or harm regarding adverse clinical events. As these bounds were not met, the trial ended when the last participant delivered.

2.1.1. Study setting

The study was conducted from February 2015 through December 2016 at Mandal Soum Hospital in Selenge Aimag (province) in northern Mongolia, at 50°N latitude. The hospital was selected for its general risk pregnant population, paved roads from Ulaanbaatar, internet access, availability of laboratory facilities (including freezer and generator, in case of electrical outage), and the experience of hospital staff and clinicians in completing research protocols, including umbilical cord blood collection.

2.1.2. Inclusion and exclusion criteria

Women were eligible to participate if they were: ≥ 18 years old; 12–16 weeks pregnant; planning to deliver at Mandal Soum Hospital; and willing to forego vitamin D supplements other than the study capsules. We excluded women with current known seizure disorder, renal failure, parathyroid disease, thyroid disease, sarcoidosis, cancer, or tuberculosis. Women with a history of kidney stones were also excluded. Women with known sensitivity to multivitamin preparations and those already taking vitamin D supplements containing >600 IU/day were ineligible to participate.

Due to the inability to test baseline 25(OH)D concentrations at the provincial study hospital, we did not use baseline 25(OH)D concentrations as a criterion for inclusion or exclusion. Given the 7–8 months of pregnancy after study enrollment, all participants were likely to experience months of low UVB exposure, and, therefore, likely to have low 25(OH)D concentrations at some point in their pregnancies without supplemental vitamin D.

Clinicians referred eligible patients to the onsite Study Coordinator at their first antenatal visit at or after 12 weeks of gestation. Gestational age was based on date of last menstrual period, except in cases where the ultrasound indicated a date >10 days discrepant, in which case ultrasound dating was used. In a private room, the Study Coordinator described the study, reviewed the consent form, and obtained signatures of women who chose to enroll. Women who wanted time to consider were told they could enroll before the 16th week of pregnancy, the date of which was calculated with the Study Coordinator.

2.1.3. Randomization and masking

Stratified block randomization was used to balance the groups by season of enrollment. Dose groups were identified by the six letters A–F (2 letters per dose group) to avoid unintentional unblinding and letters were randomly assigned to each arm. The link to the capsule dose was maintained by the Data Coordinating Center at Brigham and Women's Hospital. Neither participants, clinicians, nor investigators knew the vitamin D content of the capsules, which were identical in size and appearance. Randomization assignments, generated by computer before the start of the study, were provided in consecutively numbered, sealed envelopes to the Study Coordinator, who opened the next sequential envelope to assign each participant to one of the study arms.

2.1.4. Procedures

The Study Coordinator administered a short baseline interview or questionnaire (per participant preference) that collected information on sociodemographic characteristics, medical history, dietary sources of vitamin D, and use of supplements. The Study Coordinator measured height with a stadiometer and weight to determine body mass index

(kg/m^2). A baseline maternal blood sample was drawn, and the study supplements dispensed.

Monthly study visits corresponded with antenatal care visits, from enrollment until delivery. Antenatal care records were abstracted onto a standardized study form to record clinical details, medication use, and adverse events. The delivering clinician completed a checklist to record details and any adverse events of labor and delivery.

Study data were collected and managed using REDCap electronic data capture tools hosted at Partners HealthCare [25].

2.1.5. Source of prenatal vitamins

A standard prenatal multivitamin, which included 300 mg of calcium and varied only in vitamin D₃ content (600, 2000, or 4000 IU), was produced by Tishcon Laboratories (Westbury, NY), a Good-Manufacturing-Practice facility. The full content of the vitamin is provided in Supplement 1. Hoffmann-La Roche Inc. (Basel, Switzerland) supplied the cholecalciferol (vitamin D₃) content, which was validated by Covance Laboratories (Madison, WI) using liquid chromatography-mass spectrometry to ensure the capsules met label claims at the outset of the study. Capsules were shipped by air to Ulaanbaatar and were maintained in the National Center for Maternal and Child Health offices and Mandal Soum Hospital away from direct sunlight until they were dispensed to participants in opaque containers.

2.1.6. Adherence

Adherence to the supplementation regimen was assessed by maternal self-report and capsule counts at each study visit, and was quantified as the proportion of capsules taken relative to the number of capsules dispensed between enrollment and the final prenatal care visit. We defined successful compliance as taking at least 80% of capsules.

2.1.7. Blood samples

A 10 ml maternal blood sample was drawn at baseline, 2 months after randomization (mean gestational age: 22 weeks), and at the first antenatal visit occurring after 36 weeks' gestation except where, per protocol, the final sample was drawn earlier due to preterm delivery or maternal transfer to another hospital (mean gestational age: 37 weeks; earliest gestational age: 33 weeks). A mixed venous/arterial umbilical cord blood sample was obtained at delivery. Samples, drawn into red top tubes identified only by study ID numbers, were allowed to clot at room temperature and centrifuged. The serum was aliquotted into cryotubes. Except at baseline, one aliquot was tested immediately at the study hospital for serum calcium. The remainder of the aliquots were stored in a freezer at 0° F (−18 °C). At the end of the study, the samples were transported on ice by car to the laboratory in Ulaanbaatar for 25(OH)D assay.

2.1.8. Laboratory measurements

Total serum 25(OH)D concentrations were determined by the VIDAS® enzyme linked fluorescent assay (ELFA) (Biomérieux, Marcy-L'Etoile, France) in the Global Lab LLC of Ulaanbaatar (Altan Sharkhuu, Director). The lower limit of detection (LOD) of the assay was 20 nmol/l (8.1 ng/ml). The intra-assay coefficients of variation (CV) were 3.4, 5.4, and 12.8%, and inter-assay CVs were 2.5, 4.7, and 10.4%, respectively, for quality control samples at mean concentrations of 128, 85, and 35 nmol/l, respectively. The performance of this laboratory with this assay has been validated by the Vitamin D External Quality Assessment Scheme.

As a safety measure, maternal concentrations of serum calcium were measured on unfrozen aliquots drawn two months after randomization and at the 36–40 week visit over the course of the trial, from February 2015 to December 2016. In March 2018, frozen baseline samples were also tested for calcium. All calcium tests were run at Mandal Soum Hospital laboratory on a semi-automated photometer (Humalyzer 3500, Human Diagnostics, Magdeburg, Germany). As we did not test the variability of the clinical calcium assay while the trial was running, we later

(May 2018) ran a set of 40 duplicate samples that had been stored in the freezer and compared them to the original samples. The intra-assay CV on all 40 pairs was 8.0%; the mean difference between the paired samples was -0.05 mmol/l, a 2% difference that was not statistically significant ($p = 0.33$).

2.1.9. Safety monitoring

The DSMB reviewed biannual reports summarizing serum calcium concentrations and other adverse events. These reports were unblinded by the Data Coordinating Center before being provided to the DSMB; at no time were study investigators or staff aware of study assignments. The protocol called for any instances of hypercalcemia (>2.6 mmol/L (10.4 mg/dl) to be reported immediately to the DSMB, and for the participant to discontinue the supplement until the calcium concentrations could be retested; any woman with confirmed hypercalcemia was to be withdrawn from the study. However, no woman had a serum calcium exceeding 2.6 mmol/L at any point in the trial.

2.2. Statistical analysis

Three study arms, each including 120 participants, were needed to detect a 0.4 SD difference between arms in 25(OH)D concentrations with 80% power in a two-sided test at the 0.05 alpha level. Conservatively assuming baseline 25(OH)D concentrations of 50 ± 23 SD nmol/l, this sample size provided adequate power to detect differences in 25(OH)D concentrations between arms as small as 9 nmol/l. The study sample was not sized to detect differences in the secondary endpoints.

The primary aim of the study was to determine the degree to which three doses of vitamin D₃ supplement (600, 2000, and 4000 IU daily) raised 25(OH)D concentrations during pregnancy. We used an intention to treat analysis, including all participants for whom we had a final blood sample (96% of those enrolled), regardless of adherence. We also examined the impact of the supplements on maternal serum calcium concentrations and neonatal 25(OH)D concentrations. Our initial intent was to present only the two month and 36–40 week calcium assays as secondary safety measures; however, surprised by the very low serum calcium apparent in these assays, we later assayed baseline calcium to examine calcium dynamics over pregnancy in the three study arms.

Half (181/360) of the blood samples at baseline and 6% (19/344) of samples at 36 weeks' gestation had 25(OH)D concentrations below the assay LOD of 20 nmol/l (8 ng/ml). Therefore, we employed Tobit regression models to accommodate left-censoring in the dependent variable [26]. The model assumes that values below the LOD continue to follow the normal distribution of those observed above the LOD; this assumption appeared to be borne out by data from previous studies in Mongolian populations with access to a more sensitive DiaSorin Liaison (Stillwater, MN, USA) assay with an LOD of 10 nmol/l. [2] To determine the mean change in serum 25(OH)D from baseline to the end of follow-up for each vitamin D group, we fit a Tobit regression model with an indicator for time (end of follow-up vs. baseline) and interactions between each indicator for group (2000 IU vs. 600 IU and 4000 IU vs. 600 IU) and time [26]. The test of group by time interactions provides a formal statistical assessment of whether the pattern of change in mean serum 25(OH)D differs by vitamin D group. An advantage of the Tobit method over the common practice of assigning to values $<$ LOD the LOD or $\text{LOD}/\sqrt{2}$ is that it more accurately reflects the true uncertainty from having many censored observations $<$ LOD.

Differences in mean serum calcium concentrations across the three vitamin D groups at baseline, two months after randomization and at the end of follow-up were estimated using analyses of variance. Paired *t*-tests were used to evaluate the change in mean serum calcium concentrations from two months after randomization to the end of follow-up in each vitamin D group. The percent of women who had vitamin D deficiency (25(OH)D $<$ 50 nmol/l) at baseline and the end of

pregnancy, as well as proportions with hypocalcemia (<2.1 mmol/L) and hypercalcemia (>2.6 mmol/L) at baseline, 2 months after randomization, and at the end of pregnancy were calculated and compared by chi-square test.

3. Results

Fig. 1 shows the trial profile for the recruitment and randomization of participants from February 2015 to June 2016. Among 512 women approached by clinicians between 12 and 16 weeks' gestation for study participation, 11 women declined to participate. Seventy-six women were excluded because they did not plan to deliver at the hospital; 25 because of threatened miscarriage or plans to abort; 10 due to thyroid disease, seizure disorder, tuberculosis, or kidney stones; 6 who were younger than 18 years of age; and 1 allergic to vitamins. Due to an early protocol misunderstanding, we lacked data on reasons for exclusion for 23 women who were deemed ineligible. Of 360 women enrolled, six had spontaneous or induced abortions, two withdrew, five moved or delivered in other hospitals, and final blood samples were unobtainable for three women who delivered before the 36–40 week antenatal care visit at which the final blood draw was scheduled. Follow-up blood samples were obtained on 344 (96% of enrolled) participants. Umbilical cord blood samples were available for 333 births (93% of enrolled).

Baseline characteristics of participants are presented in Table 1. On average, participants were age 29 years, and most had completed college or university degrees. Twenty percent were nulliparous. The median body mass index (BMI) of the cohort was 25.4 kg/m². Although it appeared that women randomized to the 600 IU group had somewhat higher, and those randomized to the 4000 IU group somewhat lower mean BMI at baseline, these differences were not statistically significant ($p = 0.20$). No women were taking supplements or multivitamins containing >600 IU of vitamin D, either in the month before pregnancy or in the time since they became pregnant. Only 7% of women consumed fish at least once per week. Participants indicated that they utilized milk an average of 4.5 times per day; this milk, which would likely have included the popular 'milk tea,' would not have been a source of vitamin D, as milk is not fortified in Mongolia.

Before starting the study supplements, overall mean (SD) 25(OH)D concentration was 19 ± 22 nmol/l (8 ± 9 ng/ml) and was similar across arms ($p = 0.49$) (Table 1). Ninety-one percent had 25(OH)D concentrations <50 nmol/l (20 ng/ml), and 61% were <25 nmol/l (10 ng/ml).

Eighty-eight percent of women met our criteria for compliance and took at least 80% of their assigned supplements. Mean rates of compliance were 88%, 89%, and 87% among all women randomized to the 600 IU, 2000 IU, and 4000 IU groups, respectively, and did not vary significantly across arms ($p = 0.71$).

Fig. 2 shows maternal baseline and follow-up concentrations of 25(OH)D for the three study arms. At 36–40 weeks, concentrations had risen to 46 ± 21 , 70 ± 23 , and 81 ± 29 nmol/l for women assigned to the 600, 2000, and 4000 IU arms, respectively ($p < 0.0001$ for difference between arms). Compared to their baseline concentrations, maternal 25(OH)D concentrations rose 27 nmol/l (95% confidence interval: 22–32) for women assigned to 600 IU; 52 nmol/l (47–57) for women assigned 2000 IU; and 62 nmol/l (57–68) for women assigned to 4000 IU daily. At 36–40 weeks' gestation, 44% of women assigned to the 600 IU group had 25(OH)D concentrations ≥ 50 nmol/l (20 ng/ml) compared to 84% of women assigned to the 2000 and 4000 IU groups. At the end of pregnancy, 82% of the 600 IU had 25(OH)D concentrations ≥ 25 nmol/l (10 ng/ml), versus 95% in the 2000 and 4000 IU groups (Table 2).

Fig. 3 depicts the mean 25(OH)D concentrations in umbilical cord blood, which differed significantly between arms ($p < 0.001$). The correlation coefficient between maternal 25(OH)D concentrations at the end of pregnancy and umbilical cord blood 25(OH)D was 0.89. Only 17% of newborns in the 600 IU arm had 25(OH)D concentrations ≥ 50 nmol/l (20 ng/ml), compared to 71% of those born to mothers who had taken

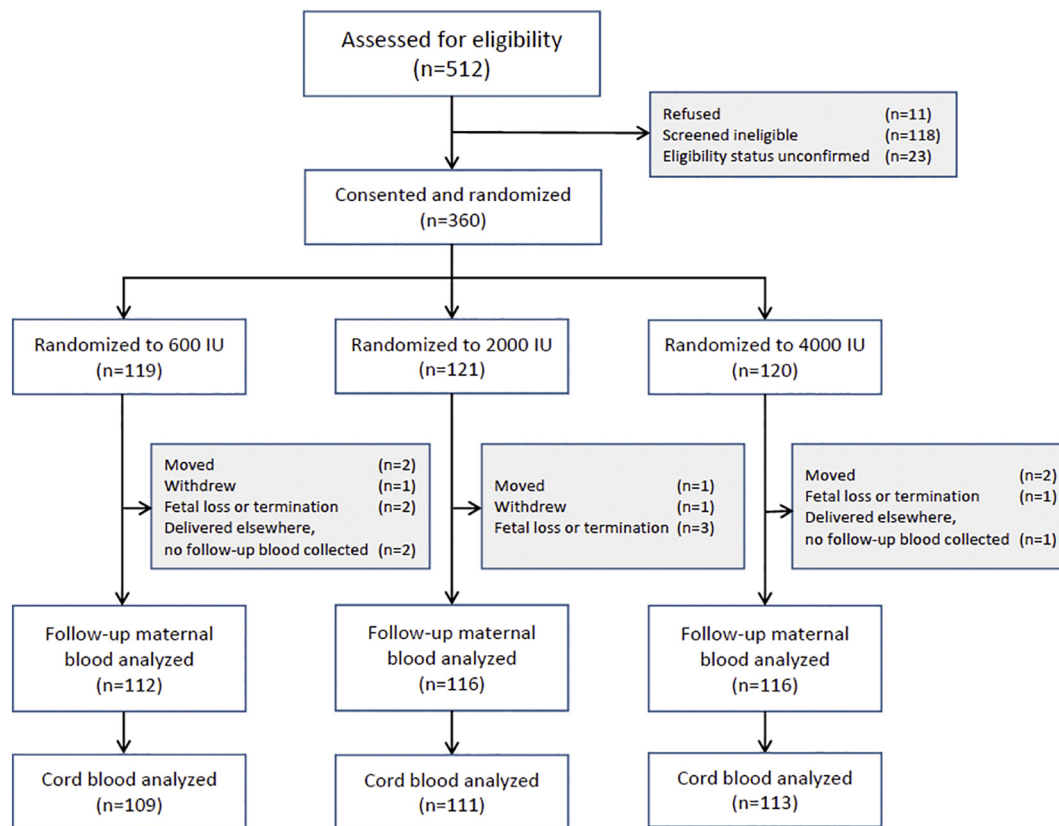


Fig. 1. 'From Healthy Mother, Healthy Baby' Trial profile.

2000 IU daily and 80% of those in the 4000 IU arm. Corresponding proportions of umbilical cord blood 25(OH)D \geq 25 nmol/l (10 ng/ml) were 79%, 91% and 96%.

Maternal serum calcium concentrations were low at baseline (Table 3), at an overall mean of 1.9 ± 0.2 mmol/L. Three quarters (76%) of women were in the hypocalcemic range (<2.1 mmol/L). After two months of supplementation, overall mean serum calcium rose to 2.2 ± 0.2 mmol/L. This increase was statistically significant in every study arm ($p < 0.0001$) and equivalent across arms ($p = 0.24$). After the first two months, mean calcium continued to rise, reaching 2.3 ± 0.2 mmol/L at 36–40 weeks' gestation. These third trimester increases were apparent in every study arm (all $p < 0.005$ for change between two months of supplementation and 36–40 weeks' gestation) and did not differ across study arms ($p = 0.48$). At the end of pregnancy, 12% of participants had hypocalcemia. There were no instances of hypercalcemia (>2.6 mmol/L) in any study arm at any timepoint.

A per protocol analysis restricted to participants who took at least 80% of their assigned supplements yielded nearly identical results to the intention-to-treat analysis, with 43%, 84% and 86% of women with ≥ 50 nmol/l 25(OH)D at the end of pregnancy in the 600, 2000, and 4000 IU groups, respectively. Adjustment for baseline BMI made no material difference to the results (data not shown).

Blood pressures, pregnancy characteristics and outcomes were secondary endpoints for this trial, and are presented in Supplement 2. We observed neither statistically significant differences nor suggestions of trend in any secondary endpoint by vitamin D arm.

4. Interpretation

The mean baseline concentration of 25(OH)D documented among the pregnant Mongolian women in this trial, at 19 nmol/l (8 ng/ml), is among the lowest recorded globally [21], and is well below the

minimum concentration considered adequate (50 nmol/l (20 ng/ml)) during pregnancy. Serum calcium concentrations were very low at enrolment, with approximately three-quarters of women in the hypocalcemic range (<2.1 mmol/L).

At enrolment, roughly half of participants reported taking some variety of nutritional supplement; however, only 6% consumed supplements containing vitamin D. The lowest dose administered in this trial, 600 IU, is higher than the 400 or 500 IU typically contained in prenatal vitamins consumed in Mongolia. This daily 600 IU supplement failed to adequately raise mean maternal 25(OH)D concentrations, with slightly more than half of mothers remaining <50 nmol/l (20 ng/ml) at the end of follow-up. Only the daily 4000 IU supplement raised mean maternal concentrations above 75 nmol/l (30 ng/ml) considered by many to be a more appropriate range during pregnancy [1,9,17,24]. Nevertheless, even with 4000 IU, 16% of mothers had concentrations <50 nmol/l (20 ng/ml). This does not appear to reflect compliance issues, as the per protocol analysis restricted to women whose capsule counts were at least 80% compliant yielded nearly identical proportions with low vitamin D status. This suggests either error in the compliance measure or that even 4000 IU daily was insufficient to raise some women's 25(OH)D concentrations above a deficiency threshold. Others have reported large variability in individual serum 25(OH)D responses, both to vitamin D supplements and ultraviolet light [22].

The newborns' umbilical cord blood concentrations reflected those of their mothers. The 600 IU dose was clearly inadequate to prevent newborn vitamin D deficiency, with the vast majority with 25(OH)D < 50 nmol/l (20 ng/ml). This is consistent with the high prevalence of rickets in Mongolia [27]. Only in the 4000 IU group were 80% of newborns above the 50 nmol/l (20 ng/ml) threshold for deficiency.

To our knowledge, only one daily vitamin D dosing study has been conducted among pregnant women with mean 25(OH)D

Table 1
Baseline characteristics of study participants by Vitamin D dose trial arm.

	600 IU n = 119	2000 IU n = 121	4000 IU n = 120	Total n = 360
Age at enrollment, mean (SD)	28.3 (5.6)	28.5 (5.7)	28.5 (5.4)	28.5 (5.5)
25(OH)D, mean nmol/l (SD) ^a	18 (21)	20 (24)	20 (22)	19 (22)
Education, n (%)				
None	0 (0)	1 (1)	1 (1)	2 (1)
Less than high school graduation	5 (4)	2 (2)	5 (4)	12 (3)
Graduated high school	56 (47)	49 (41)	46 (39)	151 (42)
Graduated college or university	58 (49)	69 (57)	68 (58)	195 (54)
Housing type, n (%)				
Ger (traditional felted yurt)	23 (19)	26 (21)	18 (15)	67 (19)
Apartment	22 (18)	19 (16)	27 (23)	68 (19)
House	74 (62)	76 (63)	75 (63)	225 (63)
Parity, n (%)				
Nulliparous	25 (21)	23 (19)	22 (19)	70 (20)
1 birth	41 (35)	49 (41)	47 (40)	137 (39)
2 births	34 (29)	29 (24)	32 (27)	95 (29)
≥3 births	17 (15)	18 (15)	18 (15)	53 (15)
Missing	2	2	1	5
Body Habitus, mean (SD)				
Weight, kilograms	66.6 (12.0)	65.2 (13.4)	64.3 (12.4)	65.4 (12.6)
Height, meters	1.58 (0.05)	1.58 (0.06)	1.59 (0.06)	1.58 (0.06)
Body mass index, kg/m ²	26.6 (4.4)	26.2 (4.7)	25.5 (4.6)	26.1 (4.6)
Weight status, n (%)				
Underweight (<18.5), n (%)	0 (0)	1 (1)	0 (0)	1 (0)
Normal weight (18.5–24.9), n (%)	48 (40)	51 (42)	65 (54)	164 (46)
Overweight (25–29.9), n (%)	47 (40)	51 (42)	35 (29)	133 (37)
Obese (≥30), n (%)	24 (20)	18 (15)	20 (17)	62 (17)
Smoking, n (%)				
Never	112 (94)	113 (93)	116 (97)	341 (95)
Past	7 (6)	6 (5)	4 (3)	17 (5)
Current	0 (0)	2 (2)	0 (0)	2 (1)
Vitamin use in month before pregnancy, n (%)				
None	112 (94)	113 (93)	114 (95)	339 (94)
Multivitamin or prenatal vitamin	2 (2)	2 (2)	3 (3)	7 (2)
Vitamin D	0 (0)	0 (0)	0 (0)	0 (0)
Iron ^b	2 (2)	1 (1)	1 (1)	4 (1)
Folic acid ^b	1 (1)	4 (3)	0 (0)	5 (1)
Herbal or other	3 (3)	1 (1)	2 (2)	6 (2)
Vitamin use at study enrollment (12–16 weeks; gestation), n (%)				
None	60 (50)	69 (57)	61 (51)	190 (53)
Multivitamin or prenatal vitamin	8 (7)	5 (4)	10 (8)	23 (6)
Vitamin D	0 (0)	0 (0)	0 (0)	0 (0)
Iron ^c	11 (9)	13 (11)	9 (8)	33 (9)
Folic acid	44 (37)	39 (32)	44 (37)	130 (36)
Herbal or other	1 (1)	0 (0)	2 (2)	3 (1)
Diet				
Eat fish ≥1 time per week, n (%)	8 (7)	7 (6)	10 (8)	25 (7)
Utilized milk, ^d times/day, mean (SD)	4.3 (2)	4.7 (2)	4.4 (2)	4.5 (2)
Season, ^e n (%)				
Winter	29 (24)	26 (22)	27 (23)	82 (23)
Spring	42 (35)	44 (36)	44 (37)	130 (36)
Summer	23 (19)	26 (22)	25 (21)	74 (21)
Fall	25 (21)	25 (21)	24 (20)	74 (21)

^a Estimated from Tobit regression for left censored data since n = 181 women had baseline 25(OH)D concentrations below the limit of detection of 8 ng/ml.

^b N = 1 woman in the 600 IU arm took both iron and folic acid and is counted in both the iron and folic acid groups.

^c N = 19 women took both iron and folic acid and are counted in both the iron and folic acid groups. There were N = 5 in the 600 IU arm, N = 8 in the 2000 IU arm, and N = 6 in the 4000 IU arm.

^d Milk sold in Mongolia was not fortified with vitamin D at the time of the trial; we queried 'In a typical day, how many times do you drink milk?' Participants' responses may have included the popular 'milk tea.'

^e Seasons are based on the Northern hemisphere equinoxes and solstices. Winter is December 21 to March 19; Spring is March 20 to June 20; Summer is June 21 to September 21; Fall is September 22 to December 20.

concentrations <25 nmol/l (10 ng/ml). In the United Arab Emirates, pregnant women had mean baseline 25(OH)D concentrations of 21 nmol/l (8 ng/ml), nearly identical to the baseline concentrations we observed in Mongolia [23]. In that group of 192 pregnant women randomized to receive 400, 2000, or 4000 IU daily from 12 to 16 weeks' gestation, 48%, 76% and 91% achieved concentrations ≥50 nmol/l (20 ng/ml), comparable to the 44%, 84% and 85% we observed for the 600, 2000, and 4000 IU groups [23]. That study noted that the increase of 27 nmol/l 25(OH)D over the course of pregnancy in their 400 IU group was several times greater than the expected

4–8 nmol/l; we also observed a 27 nmol/l increment in our 600 IU arm. Perhaps, as has been observed elsewhere [6], the 25(OH)D response is greater among individuals with extremely low baseline concentrations.

A recent trial conducted by Rostami and colleagues in Iran tested a screening and treatment program to improve 25(OH)D concentrations [16]. Women in the screening arm with moderate ((25(OH)D 25–50 nmol/l (10–20 ng/ml)) or severe (25(OH)D < 25 nmol/l (<10 ng/ml)) vitamin D deficiency were randomized to one of eight intervention arms in which doses of 50,000 IU or 300,000 IU were

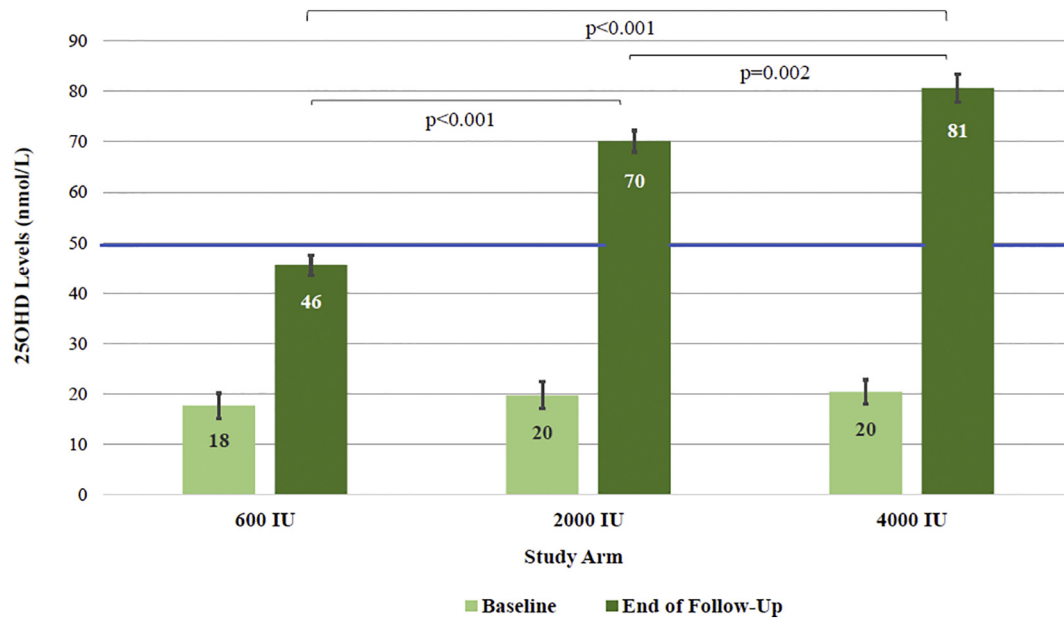


Fig. 2. Mean (standard error) 25(OH)D concentrations at baseline and end of follow-up by trial arm. The blue line at 50 nmol/l (20 ng/ml) indicates the threshold for Vitamin D deficiency [1]. Means are estimated using a Tobit model to account for left censoring of samples below the assay limit of detection (20 nmol/l). Sample sizes were 119, 121, and 120 at baseline, and 112, 116, and 116 at end of follow-up, respectively, for the 600 IU, 2000 IU and 4000 IU arms.

administered according to various weekly or monthly schedules. Among the 800 women randomized to the screening and treatment program, 53% achieved 25(OH)D concentrations >50 nmol/l (mean concentration 52 nmol/l); women in the screening and treatment program had 60% less preeclampsia and 40% less preterm delivery. Despite equal or greater success in raising 25(OH)D concentrations at the time of delivery, we did not observe similar clinical benefits with regard to pregnancy outcomes. This might be the result of low statistical power, differences in outcome measurement, or perhaps differences in the doses and modes of administration. The participants in the Iranian study, some of whom were randomized to initial high dose therapy that was not continued throughout pregnancy, may have had higher peak 25(OH)D concentrations, but lower sustained concentrations, than women taking the daily supplements we employed. Future studies should compare daily versus weekly or monthly methods of repleting low vitamin D.

In the United States, Hollis and colleagues examined the impact of 400, 2000 and 4000 IU on maternal 25(OH)D concentrations at delivery [28]. The mean baseline 25(OH)D concentration of the 350 women who completed the study was 51 nmol/l (20 ng/ml), double the mean concentration in our study. Concentrations of 25(OH)D at a month before delivery were 79, 105 and 119 nmol/l, some 50% to 60% higher than final concentrations in our study, but with proportional differences across study arms. Like ours, the study was underpowered to test the impact on clinical outcomes. However, as in the present study and the study in the United Arab Emirates [23], there were no adverse events

attributable to vitamin D, and the authors concluded that 4000 IU daily was safe.

In our study, maternal serum calcium concentrations rose in all three trial arms to a similar degree. The baseline samples were assayed from frozen sera that had been stored for up to two years, while the calcium assays at two months after randomization and at 36–40 weeks' gestation were run on fresh samples as part of safety monitoring. The rise in calcium between baseline and two months may be, in part, an artifact of degradation of calcium in baseline samples assayed after frozen storage. However, this would not explain the continuing increase between two months and the end of gestation, based only on assay of fresh samples.

Serum calcium does not typically increase during the course of normal pregnancy [29,30]. For example, in the daily vitamin D trial in the United Arab Emirates, which reported normal baseline serum calcium concentrations, concentrations tended to fall over the course of pregnancy in all vitamin D groups (participants were provided only with daily vitamin D supplements, not a prenatal multivitamin supplement containing both vitamin D and calcium, as in the current study) [23]. However, in a recent trial of weekly vitamin D supplements (plus 500 mg daily calcium) in Bangladesh, where the mean serum calcium concentration in women receiving no supplemental vitamin D was 2.22 mmol/l, although women receiving only 4200 IU of vitamin D per week had no increase in serum calcium, those receiving 16,800 or 28,000 IU weekly (equivalent to 4000 IU daily) had increases in serum calcium of 0.02–0.03 mmol/l at 30 weeks' gestation; in some higher dose groups, serum calcium continued to rise to delivery to

Table 2
Concentrations of maternal serum 25(OH)D (nmol/l) at end of follow-up by trial arm.

	600 IU (n = 112)	2000 IU (n = 116)	4000 IU (n = 120)	P-Value
25(OH)D, mean nmol/l (SD) ^a	46 (21)	70 (23)	81 (29)	<0.0001
Range of 25(OH)D	<20–104	<20–136	<20–154	
Increase in 25(OH)D (95% confidence interval) from baseline to follow-up	27 (22, 32)	52 (47, 57)	62 (57, 68)	
Number (%) ≥50 nmol/l or 20 ng/ml	49 (44)	97 (84)	102 (85)	<0.0001
Number (%) ≥25 nmol/l or 10 ng/ml	92 (82)	110 (95)	114 (95)	<0.001

^a Values are from Tobit model to accommodate the 12, 4, and 3 women with 25(OH)D concentrations below the assay limit of detection (20 nmol/l or 8 ng/ml), respectively, in the 600, 2000, and 4000 IU arms.

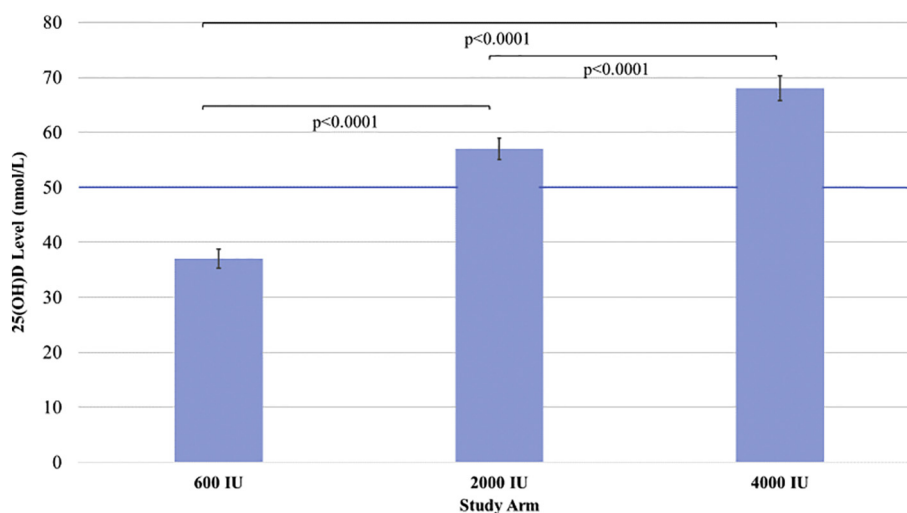


Fig. 3. Mean (standard error) 25(OH)D concentrations in umbilical cord blood at end of follow-up by trial arm. The blue line at 50 nmol/l (20 ng/ml) indicates the threshold for Vitamin D deficiency [1]. Sample sizes were 109, 111, and 113 at baseline, for the 600 IU, 2000 IU and 4000 IU arms. Means are estimated using a Tobit model to account for left censoring of samples below the assay limit of detection (20 nmol/l).

0.04–0.05 mmol/l above unsupplemented concentrations [31]. The rise in serum calcium that we observed over the course of pregnancy may have resulted from the 300 mg of calcium/day and/or the vitamin D content of the supplements, which presumably improved absorption of calcium. Although we did not measure calcium intake from foods in this study, participants indicated that they utilized milk, on average, 4.5 times per day, an imprecise indicator of dairy consumption. Data are inconsistent regarding the calcium content of the Mongolian diet [27,32]. If, in fact, the increases in serum calcium in this trial are attributable to the repletion of vitamin D, then the similar increase across arms suggests a threshold effect, with 600 IU/day sufficient to improve calcium absorption. Given the high dairy content of the Mongolian diet, further studies need to determine the cause of the prevalent hypocalcemia we observed among pregnant women.

There were no instances of hypercalcemia and no adverse events attributable to the vitamin D regimen, suggesting that up to 4000 IU daily is safe for pregnant Mongolian women. This is consistent with previous

reports of no adverse events in trials involving >2000 pregnant women consuming daily 4000 IU supplements [9] and with the Institute of Medicine determination that 4000 IU is safe during pregnancy [19].

A limitation of the current study is our reliance on a 25(OH)D assay whose lower limit of detection was higher than many of the samples, especially at baseline. More sensitive assays were not available in Mongolia at the time we assayed the samples. Rather than rely on the practice of using the LOD or $\text{LOD}/\sqrt{2}$, we instead utilized an established model to accommodate left censored data with appropriate uncertainty. Our umbilical cord 25(OH)D concentrations may have been overestimated by our use of an ELFA assay that does not eliminate artifacts specific to umbilical cord blood. However, the high correlation between maternal and newborn 25(OH)D concentrations ($r = 0.89$) and the similar ratio of newborn to maternal concentrations (ranging from 0.80 to 0.84 across arms) suggests that any inflation of umbilical cord 25(OH)D concentrations was similar across arms and unlikely to jeopardize relative comparisons between study arms. The literature on

Table 3

Maternal serum calcium concentrations at baseline, at two months after randomization, and at 36–40 weeks by trial arm^a.

	600 IU	2000 IU	4000 IU	P-Value across arms ^b
Serum calcium, mmol/L, mean (SD)				
Baseline	1.9 (0.3)	1.9 (0.2)	2.0 (0.2)	0.82
Two months after randomization	2.2 (0.2)	2.2 (0.2)	2.2 (0.2)	0.24
36–40 weeks' gestation	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)	0.48
P-value for change within arm				
Baseline to two months ^c	<0.0001	<0.0001	<0.0001	
Two months to 36–40 weeks ^d	0.0004	0.008	0.005	
Hypocalcemia (<2.1 mmol/L), n (%)				
Baseline	88 (74)	91 (85)	95 (79)	0.61
Two months after randomization	25 (22)	20 (17)	27 (23)	0.51
36–40 weeks' gestation	12 (11)	10 (9)	19 (17)	0.16
P-value for change within arm				
Baseline to two months ^e	<0.0001	<0.0001	<0.0001	
Two months to 36–40 weeks ^f	0.07	0.06	0.50	
Hypercalcemia (>2.6 mmol/L), n (%)				
Baseline	0 (0)	0 (0)	0 (0)	
Two months after randomization	0 (0)	0 (0)	0 (0)	
36–40 weeks' gestation	0 (0)	0 (0)	0 (0)	

^a There were 119, 121 and 120 samples tested at baseline; 116, 118, and 119 tested at two months; and 112, 113, and 113 tested at 36–40 weeks, respectively, for the 600 IU, 2000 IU and 4000 IU groups. Baseline samples were tested on frozen aliquots; later samples were tested in fresh sera.

^b p-value from analysis of variance (for means) or chi-square (for proportions) comparing study arms at each time point.

^c p-value is from a paired t-test of within-arm means at baseline and at 2 months.

^d p-value is from a paired t-test of within-arm means at 2 months and at 36–40 weeks.

^e p-value is from McNemar's test for paired data comparing baseline to 2 months within each study arm.

^f p-value is from McNemar's test for paired data comparing 2 months to 36–40 weeks within each study arm.

umbilical cord concentrations employs a variety of assays; both the ratio of (0.82) and correlation between (0.89) umbilical cord and maternal 25(OH)D concentrations in our study is similar to the thirty studies reviewed by Saraf and colleagues [33]. We were not able to measure albumin or ionized calcium to determine whether the change in total calcium reflected a change in free calcium. We relied on capsule counts, which may overestimate compliance compared to more objective measures. The study's strengths include the high compliance and nearly complete (96% of women enrolled) data collection. The almost universal vitamin D deficiency allowed us to examine the impact of three vitamin D doses among women with extremely low 25(OH)D concentrations.

Inadequate 25(OH)D concentrations may impact the health of pregnant women and their offspring. A large proportion of the world's women of reproductive age have very low vitamin D status; a recent review documented that 38% to 60% of pregnant women surveyed in Kuwait, Pakistan, Turkey, and India have 25(OH)D concentrations <30 nmol/l (<12 ng/ml) [21]. In these regions, as demonstrated by this study in Mongolia, pregnant women may require vitamin D supplementation of at least 4000 IU daily to avoid vitamin D deficiency and reach 25(OH)D concentrations that may be optimal for pregnancy health.

Declaration of interests

No authors have a conflict of interest

Author contributions

D. Enkhmaa: study design, literature search, data collection, data interpretation, writing.

L. Tanz: study design, data analysis, figures, data interpretation, writing.

D. Ganmaa: Study design, data collection, data interpretation, editing

Sh. Enkhtur: Study design, data collection, editing

B. Oyun-Erdene: study design, data collection, data management, data interpretation, editing.

J. Stuart: study design, data collection, data analysis, data interpretation, editing.

G. Chen: study design, data collection, data analysis, data interpretation, editing.

A. Carr: study design, literature search, data collection, data management, editing.

E.W. Seely: study design, literature search, data interpretation, editing.

G. Fitzmaurice: Study design, data analysis, data interpretation, editing

Yo. Buyandelger: Study design, data collection, editing

B. Sarantsetseg: Study design, data collection, editing

G. Gantsetseg: Study design, data collection, data management, editing

J. Rich-Edwards: study design, literature search, data collection, data analysis, data interpretation, writing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2018.11.060>.

References

- [1] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911–30.
- [2] Bromage S, Rich-Edwards JW, Tselmen D, et al. Seasonal epidemiology of serum 25-hydroxyvitamin D concentrations among healthy adults living in rural and urban areas in Mongolia. *Nutrients* 2016;8(10).
- [3] Ganmaa D, Holick MF, Rich-Edwards JW, et al. Vitamin D deficiency in reproductive age Mongolian women: A cross sectional study. *J Steroid Biochem Mol Biol* 2014; 139:1–6.
- [4] 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(1):68–71.
- [5] Ganmaa D, Giovannucci E, Bloom BR, et al. Vitamin D, tuberculin skin test conversion, and latent tuberculosis in Mongolian school-age children: a randomized, double-blind, placebo-controlled feasibility trial. *Am J Clin Nutr* 2012;96(2): 391–6.
- [6] Rich-Edwards JW, Ganmaa D, Kleinman K, et al. Randomized trial of fortified milk and supplements to raise 25-hydroxyvitamin D concentrations in schoolchildren in Mongolia. *Am J Clin Nutr* 2011;94(2):578–84.
- [7] Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D, Calcium. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. The national academies collection: Reports funded by national institutes of health. Dietary reference intakes for calcium and vitamin D. Washington (DC): National Academies Press (US). National Academy of Sciences; 2011.
- [8] De-Regil LM, Palacios C, Lombardo LK, Pena-Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev* 2016;1 [Cd008873].
- [9] Wagner CL, Hollis BW, Kotsa K, Fakhoury H, Karras SN. Vitamin D administration during pregnancy as prevention for pregnancy, neonatal and postnatal complications. *Rev Endocr Metab Disord* 2017;18(3):307–22.
- [10] Wei SQ. Vitamin D and pregnancy outcomes. *Curr Opin Obstet Gynecol* 2014;26(6): 438–47.
- [11] Qin LL, Lu FG, Yang SH, Xu HL, Luo BA. Does maternal vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. *Nutrients* 2016;8 (5).
- [12] Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ* 2013;346:f1169.
- [13] Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014;348:g2035.
- [14] Harvey NC, Holroyd C, Ntani G, et al. Vitamin D supplementation in pregnancy: a systematic review. *Health Technol Assess* 2014;18(45):1–190.
- [15] McDonnell SL, Baggerly KA, Baggerly CA, et al. Maternal 25(OH)D concentrations \geq 40 ng/ml associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. *PLoS One* 2017;12(7):e0180483.
- [16] Rostami M, Tehrani FR, Simbar M, et al. Effectiveness of prenatal vitamin D deficiency screening and treatment program: A stratified randomized field trial. *J Clin Endocrinol Metab* 2018;103(8):2936–48.
- [17] Hollis BW, Wagner CL. Vitamin D and pregnancy: Skeletal effects, nonskeletal effects, and birth outcomes. *Calcif Tissue Int* 2013;92(2):128–39.
- [18] Pludowski P, Holick MF, Grant WB, et al. Vitamin D supplementation guidelines. *J Steroid Biochem Mol Biol* 2018;175:125–35.
- [19] Wagner CL, Hollis BW. The Implications of vitamin D status during pregnancy on mother and her developing child. *Front Endocrinol* 2018;9:500.
- [20] Bhatta ZA, Das JK, Rizvi A, et al. Evidence-based interventions for improvement of maternal and child nutrition: What can be done and at what cost? *Lancet* 2013; 382(9890):452–77.
- [21] Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol* 2014;144(Pt A):138–45.
- [22] Aloia JF, Patel M, Dimaano R, et al. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr* 2008;87(6):1952–8.
- [23] Dawodu A, Saadi HF, Bekdache G, Javed Y, Altaye M, Hollis BW. Randomized controlled trial (RCT) of vitamin D supplementation in pregnancy in a population with endemic vitamin D deficiency. *J Clin Endocrinol Metab* 2013;98(6):2337–46.
- [24] Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005;135(2):317–22.
- [25] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42(2): 377–81.
- [26] Tobin J. Estimation of relations for limited dependent variables. *Econometrica* 1958; 26:24–36.
- [27] Uush T. Prevalence of classic signs and symptoms of rickets and vitamin D deficiency in Mongolian children and women. *J Steroid Biochem Mol Biol* 2013;136:207–10.

- [28] Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* 2011;26(10):2341–57.
- [29] Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Calcium metabolism in normal pregnancy: A longitudinal study. *Am J Obstet Gynecol* 1979;133(7):781–90.
- [30] Seely EW, Brown EM, Demaggio DM, Weldon DK, Graves SW. A prospective study of calciotropic hormones in pregnancy and post partum: reciprocal changes in serum intact parathyroid hormone and 1,25-dihydroxyvitamin D. *Am J Obstet Gynecol* 1997;176(1):214–7 Pt 1.
- [31] Roth DE, Morris SK, Zlotkin S, et al. Vitamin D supplementation in pregnancy and lactation and infant growth. *New England J Med* 2018;379(6):535–46.
- [32] Troisi R, Ganmaa D, dos Santos Silva I, et al. The role of hormones in the differences in the incidence of breast cancer between Mongolia and the United Kingdom. *PLoS One* 2014;9(12):e114455.
- [33] Saraf R, Morton SM, Camargo Jr CA, Grant CC. Global summary of maternal and newborn vitamin D status - a systematic review. *Matern Child Nutr* 2016;12(4):647–68.