

Maternal and neonatal outcomes of vitamin D supplementation in hypovitaminosis D pregnancy: preg-D randomized trial

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Abstract

Context Hypovitaminosis D during pregnancy is common and may have adverse maternal and neonatal effects. While randomized trials show that supplementation improves biochemical vitamin D status, the optimal dose and its effects on neonatal bone outcomes remain uncertain.

Objective To compare the effects of 2 vitamin D supplementation doses during pregnancy on maternal and neonatal serum 25-hydroxyvitamin D (25(OH)D) at delivery, and on neonatal bone mineral content.

Methods In this double-blind trial, pregnant women ≤ 17.5 weeks' gestation with baseline 25(OH)D 10 to 30 ng/mL were randomized to receive either 20 000 IU/week or 10 000 IU every 2 weeks (equivalent to 714 IU/day) vitamin D₃. Primary outcomes were the proportion of women achieving a 25(OH)D level ≥ 20 ng/mL at delivery and neonatal bone mineral content (BMC) at ~ 1 month of age. We used liquid chromatography tandem mass spectrometry (standard LC-MS/MS) at a CDC-certified laboratory.

Results One hundred and ninety-two maternal neonatal pairs had complete biochemical data and 67 neonates had evaluable DXA scans. A significantly higher proportion (100%) of women and neonates (77%) in the higher dose group achieved 25(OH)D levels ≥ 20 ng/mL at delivery, compared with the lower dose group (85% and 40%, respectively, $P = .001$). Mean 25(OH)D in mothers and neonates at delivery followed a similar pattern ($P = .001$). However, neonatal subtotal whole-body BMC did not differ between groups (45.9 ± 8.5 vs 42.2 ± 7.1 g, respectively; $P = .059$), nor did neonatal bone mineral density or bone area. We did not identify any safety concerns related to supplementation.

Conclusion Higher dose vitamin D supplementation safely achieved biochemical sufficiency in mothers and neonates without measurable effect on neonatal bone outcomes.

Keywords vitamin D, pregnancy, neonatal bone mineral content, randomized controlled trial, maternal supplementation, Middle East

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Vitamin D deficiency is highly prevalent during pregnancy worldwide and has been associated, in observational studies, with a range of adverse maternal and neonatal outcomes, including miscarriage, preeclampsia, gestational diabetes, preterm birth, and impaired fetal growth (1-6). However, causality remains uncertain.

Randomized controlled trials of prenatal vitamin D supplementation have yielded heterogeneous results, with limited and inconsistent evidence for effects on major maternal outcomes. This variability is particularly evident in trials from the Middle East and North Africa (MENA) region, where supplementation regimens have ranged from 200 IU/day to 50 000 IU/week, achieved 25-hydroxyvitamin D (25(OH)D) concentrations have varied substantially, and data on clinically meaningful outcomes remain sparse (7).

Neonatal outcomes, including bone outcomes, have been less frequently examined. A meta-analysis of 26 trials reported that antenatal vitamin D supplementation (median dose 2000 IU/day) increased cord blood 25(OH)D concentrations by an average of 11.7 ng/mL (8). However, regional data is limited, with mostly small trials. In a three-arm trial from the United Arab Emirates, 79% of infants in the highest-dose group (4000 IU/day) achieved 25(OH)D ≥ 20 ng/mL, while only 21% to 44% did so in the lower dose arms of 400 and 2000 IU/d, respectively (9). The MAVIDOS trial, which compared 1000 IU/day of vitamin D with placebo, found no differences in neonatal anthropometrics or whole-body Dual-Energy X-ray Absorptiometry (DXA) indices at 2 weeks of age (10). Interpretation of existing trials is further complicated by the widespread use of noncalibrated or nonvalidated vitamin D assays.

In this context, we aimed to compare the efficacy of 2 prenatal vitamin D dosing strategies in a population with a high prevalence of hypovitaminosis D. The primary outcomes were the proportion of women achieving the Institute of Medicine (IOM)/National Academy of Medicine-defined 25(OH)D threshold of ≥ 20 ng/mL at delivery, and neonatal bone mineral content (BMC) at one month of age.

Materials and Methods

This was a phase III double blind randomized controlled superiority trial conducted at the American University of Beirut—Medical Center (AUBMC) and Bahman Hospital in Lebanon. We have previously published a detailed description of the study protocol, and registered the trial at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT 02434380, April 2015) (11). We recruited pregnant women in their first trimester and followed them up from June 30, 2015 to December 6, 2018.

Eligibility criteria

We enrolled pregnant women above 18 years of age, who are at less than 17.5 weeks of gestation, and of Middle Eastern origins. They had baseline 25(OH)D between 10 and 30 ng/mL and were not taking vitamin D supplementation exceeding 200 IU/day. Women taking higher vitamin D doses of 200 to 600 IU/day were included if their prenatal multivitamin D intake was reduced, under physician supervision, to below 200 IU/day.

Exclusion criteria included women with 25(OH)D < 10 ng/mL or > 30 ng/mL, had metabolic bone or chronic diseases associated with mineral or bone abnormalities, were taking medications that could interfere with vitamin D metabolism, or were supplemented with vitamin D exceeding 600 IU/day (11).

Randomization and blinding

An independent biostatistician prepared the computer-generated randomization sequence, with 1:1 permuted block randomization stratified by center. The senior pharmacist at AUBMC concealed allocation through the use of opaque sequentially numbered boxes, containing the randomly allocated vitamin D dose. All pills were of similar shape, color, size, smell, and taste provided by EURO-PHARM International Canada Inc (Montreal, Canada). EURO-PHARM had no role in the study (protocol development, grant, data collection & analysis, manuscript writing). Study team and all participants were blinded to drug assignment until trial completion.

Study drug

Participants were assigned to receive either a lower dose 10 000 IU every 2 weeks vitamin D3 or a higher 20 000 IU/week vitamin D3 dose. Participants in the higher dose group took 2 active vitamin D pills weekly, whereas women in the lower dose group took 2 placebo tablets, alternating with 1 placebo and 1 active tablet (10 000 IU), weekly. These dosing regimens were selected to examine whether vitamin D recommendations based on the IOM/National Academy of Medicine are sufficient in our regional context (12). The lower dose regimen, equivalent to 714 IU/d, is close to the IOM recommended daily allowance, that is 600 IU/day; whereas the higher dose regimen, equivalent to 2857 IU/day, was selected to test whether a substantially higher yet safe dose (below the tolerable upper intake level, that is 4000 IU/d) would be more effective in achieving vitamin D sufficiency in a population at high risk of hypovitaminosis D. This is particularly relevant to our population, in whom limited sun exposure contributes to low 25(OH)D levels across the life cycle. At each visit, participants received a box containing sufficient tablets (2 per week) until the next visit, with dates at which pills should be administered. Participants returned the study's drug boxes at follow-up visits to assess compliance.

Study visits

Participants attended 4 visits during pregnancy and 1 at 4 to 6 weeks postdelivery. We collected baseline information on demographic and lifestyle factors (smoking, clothing style, exercise, alcohol consumption, and employment), and medical history upon randomization. At each follow-up visit, we measured participants' anthropometrics, and assessed calcium and vitamin D intake from both supplements and diet, using a food frequency questionnaire focusing on items rich in calcium and vitamin D (13). We also recorded the occurrence of any adverse event at each follow-up visit. Detailed description of study visits and data collected at each visit are presented in Appendices 1a and 1b (14). Further details of visits and data collection are described thoroughly in the study protocol (11).

Outcomes and laboratory measurements

The study had 2 primary outcomes: (1) the proportion of women reaching 25(OH)D level ≥ 20 ng/mL at delivery, and (2) neonatal

BMC within 4-6 weeks of delivery. Neonatal 25(OH)D level at delivery was a secondary outcome.

Although we initially measured serum 25(OH)D concentrations using the Roche II total assay (Roche Cat# 07028148, RRID: AB_2909456) in all 330 randomized subjects, we subsequently succeeded in securing additional funding to measure vitamin D using the standardized liquid chromatography–mass spectrometry (LC–MS/MS) assay, in subjects who had remaining sera frozen at -80°C . For LC–MS/MS, samples were frozen at -80°C , shipped on dry ice, and analyzed at the Centre Hospitalier Universitaire (CHU) de Liège (Pr. Etienne Cavalier) using a method certified by the Vitamin D standardization Program (VDSP), and traceable to the CDC and NIST reference standards (15). This assay is known to have a superior performance compared to platform assays, including accuracy of the assay in general (16), and in this population of pregnant women in particular (Manuscript submitted).

The LC–MS/MS procedure included protein precipitation, extraction, chromatographic separation, and tandem mass spectrometry. The assay quantified 25(OH)D₂ and 25(OH)D₃ individually and separated the C3-epimers of 25(OH)D and 25(OH)D₃; total 25(OH)D was calculated as the sum of 25(OH)D₂ and 25(OH)D₃. This method is accredited by the international standard for medicinal laboratories (ISO) 15189 (16).

Other routine biochemical tests were analyzed at the corresponding laboratory of each center. The clinical chemistry lab at AUBMC participates in the quality assurance, evaluation, and accreditation from the College of American Pathologists (www.cap.org) and DEQAS (www.Deqas.org), consistently performing against target values. The clinical chemistry laboratory at Bahman also participates in EQS-Bio Rad (www.bio-rad.com/en-lb/a/qm/proficiency-testing-eqas) as external quality control, and has routine internal quality control from Abbott.

We performed neonatal DXA measurements at 4 to 6 weeks of infant age at the Calcium Metabolism and Osteoporosis Program at AUBMC using the Hologic machine (Horizon A, V.13.5.3.1). Our bone mineral density (BMD) unit facility is certified by the International Society of Clinical Densitometry (ISCD) since 2019. Scans that were successfully done were reviewed by an independent expert (JS) masked to treatment allocation. In scans with motion artifact in one limb, we imputed missing values from the contralateral limb using a regression technique from scans with complete values, as previously described by Shepherd et al (17). Details on DXA scans and their quality assessment can be found in Appendix 1a (14).

Sample size calculation

We calculated the sample size for the 2 primary outcomes and considered the largest estimate for the final sample size.

Proportion of women reaching a 25(OH)D ≥ 20 ng/mL at delivery

Based on a retrospective review from 2014 at AUBMC, the estimated proportions of pregnant women achieving 25(OH)D level ≥ 20 ng/mL were 75% in the lower dose group, and 98.4% in the higher dose group (11). A total of 50 participants per arm

were required to detect this difference, with 90% power, and a two-sided type I error of 2.5%.

Neonatal BMC

For BMC, sample size estimates were informed by the MAVIDOS trial data demonstrating a 6 g difference in neonatal BMC, with a standard deviation (SD) of 10 g. Detecting this difference with a 90% power and 2.5% type I error required 140 participants per arm. Anticipating a 50% dropout rate for neonatal DXA assessments, the target sample size increased to 280. Due to higher-than-expected attrition (63%), we further expanded the sample to 330 participants with Institutional Review Board (IRB) approval, to preserve statistical power for neonatal bone outcomes.

Statistical analysis

We conducted statistical analyses according to the intention-to-treat principle among participants with 25(OH)D measured by LC–MS/MS. Prior to initiating analysis, we inspected the normality of data across different variables. We summarized baseline characteristics using means $\pm$ SD or frequencies, as appropriate. For comparison between the 2 allocation arms, we used independent *t*-tests for continuous normally distributed variables and the chi-square (χ^2) test for categorical variables. We compared proportions achieving 25(OH)D ≥ 20 ng/mL using a chi-square (χ^2) test, with relative risks, and 95% confidence intervals. We compared neonatal DXA indices using Mann–Whitney *U* tests. A two-sided *P*-value $\leq .025$ was considered statistically significant to account for 2 primary outcomes.

We conducted prespecified subgroup analyses according to baseline 25(OH)D (<20 vs ≥ 20 ng/mL). For adjusted analyses, we used Firth penalized logistic regression to reduce small-sample bias and address separation. Models included treatment allocation arm as the main exposure and were adjusted for clinically relevant covariates, including baseline 25(OH)D (as a continuous variable), prepregnancy body mass index, smoking during pregnancy, and season of enrollment. We also explored effect modification by season of enrollment by including an interaction term between treatment allocation and season (low- vs high-sun) in Firth penalized logistic regression models. We performed analyses using SPSS version 23 (IBM, Armonk, NY, USA) and STATNow18 BE (Texas, USA).

The trial protocol was approved by the IRB at the American University of Beirut (AUB), and we obtained written informed consent from all participants at the prescreening visit. For ethical considerations, we restricted enrollment to women with baseline 25(OH)D levels of 10–30 ng/mL, to avoid exposing deficient women to lower dose supplementation or replete women to higher dose supplementation. An independent Data Safety Monitoring Board (DSMB) oversaw trial conduct, and we reported all serious adverse events (SAEs) to the IRB and DSMB within 48 hours.

Results

Study participants

Of 1600 pregnant women approached between 2015 and 2018, 552 were screened and 330 were randomized (165 per arm; Fig. 1).

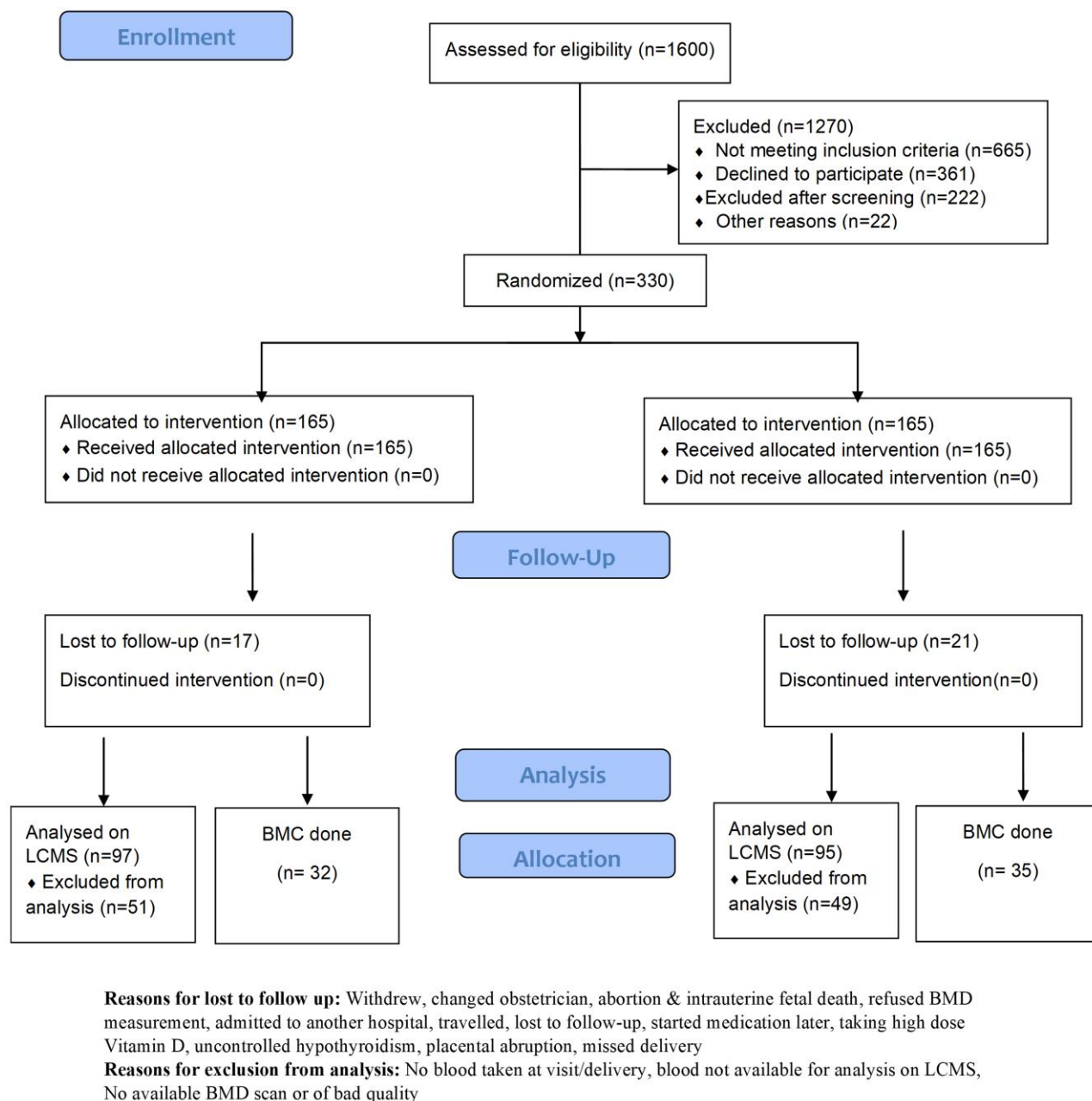


Figure 1 CONSORT 2010 flow diagram. Flow diagram of participants from screening and eligibility assessment to allocation, follow-up, and analysis, according to the CONSORT 2010 guidelines. The figure details exclusions, losses to follow-up, and reasons for exclusion from biochemical and neonatal bone mineral content (BMC) analyses. Final analyses included 192 women with complete LC-MS/MS 25(OH)D data and a subset of mother-neonate pairs with evaluable neonatal BMC measurements.

During follow-up (until December 2018), 38 participants withdrew, leaving 292 with outcome data. Of the 330 randomized women, 192 had sufficient stored serum for LC-MS/MS measurement of 25 (OH)D at all time points and were included in the primary analyses; 138 were excluded due to unavailable serum. The LC-MS/MS sample was therefore smaller than the original randomized sample because standardized testing was performed when additional funding became available, and only stored sera could be assayed. Excluded participants had similar baseline characteristics to those included, although they were slightly younger, less educated, and more likely to be veiled within the lower dose group (Appendix 1c)

(14). Participants continued to receive their prenatal care and supplements (with vitamin D not exceeding 200 IU/day) as advised by their primary physicians.

Serum 25(OH)D results were available for 192 maternal and 170 cord blood samples. Neonatal DXA scans of acceptable quality were obtained in 67 infants; the remainder were excluded primarily due to motion artifacts, as only one scan attempt was permitted by the IRB. Neonatal anthropometric measures did not differ between infants with and without DXA scans (data not shown).

Baseline characteristics were well balanced between treatment groups (Table 1). Mean maternal age was 30 ± 5 years, gestational

Table 1 Baseline characteristics of the enrolled pregnant women with complete data for 25(OH)D by LC-MS/MS, from both centers, by treatment arm

		Overall (N = 192)	20 000 IU D3/week (N = 97)	10 000 IU D3 biweekly (N = 95)
		Mean ± SD	Mean ± SD	Mean ± SD
Gestational age (weeks)		16.6 ± 1.2	16.5 ± 1.2	16.7 ± 1.2
Maternal age (years)		30.0 ± 4.7	29.4 ± 4.6	30.7 ± 4.8
Systolic blood pressure (mmHg)		112.5 ± 12.4	112.5 ± 11.6	112.5 ± 13.3
Diastolic blood pressure (mmHg)		68.2 ± 9	67.4 ± 9.7	69.1 ± 9.3
Maternal BMI (kg/m ²)		24.9 ± 3.9	24.7 ± 4.1	25.2 ± 3.8
Years of veiling (years)		15.6 ± 5.7	15.2 ± 5.2	16.1 ± 6.3
		N (%)	N (%)	N (%)
Parity	Nulliparous	70 (37)	40 (41)	30 (32)
	Primiparous	67 (35)	32 (33)	35 (37)
	Multiparous	55 (29)	25 (26)	52 (32)
Gravida	Primigravid	63 (33)	36 (37)	27 (28)
	Multigravid	129 (67)	61 (63)	68 (72)
Previous abortions		58 (30)	24 (25)	34 (36)
Education	Below high school	16 (8)	9 (9)	7 (7)
	High school	21 (11)	13 (13)	8 (8)
	College	155 (81)	75 (77)	80 (84)
Currently employed		96 (50)	47 (49)	49 (52)
Current smoking		23 (12)	10 (10)	13 (14)
Previous smoking		71 (37)	32 (33)	39 (41)
Secondhand smoking exposure		96 (50)	47 (49)	49 (52)
Current alcohol intake		1 (0.5)	0 (0)	1 (1)
Previous alcohol intake		29 (15)	14 (14)	15 (16)
Current exercise		25 (13)	11 (11)	14 (15)
Previous exercise		79 (41)	41 (42)	38 (40)
Veiling		103 (54)	58 (60)	45 (47)
Presence of any health condition ^a		58 (30)	30 (31)	28 (30)

Abbreviation: BMI, body mass index.

^aRespiratory, cardiovascular, gastrointestinal, genitourinary, musculoskeletal, neurological, endocrine-metabolic, blood/lymphatic, dermatologic, psychiatric disorder, allergy, others.

age at enrollment 16.6 ± 1.2 weeks, and baseline 25(OH)D was 16.8 ± 8.3 ng/mL (Table 1). Mean baseline BMI was 25 ± 4 kg/m², 54% of participants were veiled, one-third were nulliparous, 81% had a college degree, and 30% reported at least one comorbidity (Table 1). Baseline biochemical parameters, including serum calcium, phosphate, creatinine, and thyroid function, were within the normal range (Table 2). Mean dietary calcium and vitamin D intakes were low (556 ± 242 mg/day and 99 ± 71 IU/day, respectively), with minimal supplemental intake. No significant differences were observed between treatment arms.

Maternal and neonatal vitamin D status

At baseline, 35% of the participants in the higher dose group and 34% of participants in the lower dose group had serum levels ≥ 20 ng/mL. At delivery, all women in the higher dose group achieved 25(OH)D ≥ 20 ng/mL compared with 85% in the lower dose group (absolute difference 15%; 95% CI 8-22%; $P = .001$; Fig. 2). Mean maternal 25(OH)D concentrations were higher in the higher dose group (42 ± 11 vs 30 ± 11 ng/mL, respectively; $P = .001$; Fig. 3). Maternal serum calcium levels were similar between groups.

Cord blood findings mirrored maternal results: 77% of neonates in the higher dose group achieved 25(OH)D ≥ 20 ng/mL vs 39% in the lower dose group (absolute difference 38%; 95% CI 25-52%; $P = .001$). Cord blood 25(OH)D concentrations were likewise higher in the higher dose group; 25 ± 7 vs 18 ± 7 ng/mL in the lower dose; $P = .001$. The relative risk of achieving 25(OH)D ≥ 20 ng/mL with higher dose supplementation was 1.17 (95% CI 1.08-1.28) in mothers and 1.99 (95% CI 1.50-2.65) in cord blood, compared to the lower dose; the number needed to treat was 7 for maternal vitamin D sufficiency.

Among the 124 participants with baseline 25(OH)D < 20 ng/mL, 100% in the higher dose vs 81% in the lower dose group achieved ≥ 20 ng/mL ($P < .001$). In the multivariable analysis, the treatment arm was associated with vitamin D deficiency ($P = .01$) while the other covariates (baseline 25(OH)D, prepregnancy BMI, smoking during pregnancy, and season of enrollment) were not significantly associated with vitamin D deficiency (data not shown). However, we were unable to perform interaction between season and arm, as prespecified in protocol, because all vitamin D deficiency events occurred in one treatment arm; the data exhibited complete separation, rendering interaction estimates unstable and statistically

Table 2 Baseline intake of calcium and vitamin D and biochemical baseline characteristics of the enrolled pregnant women from both centers, by treatment arm

	Overall (N = 192)	20 000 IU D3/week (N = 97)	10 000 IU D3 biweekly (N = 95)
	Mean ± SD	Mean ± SD	Mean ± SD
Total dietary calcium intake (mg/day)	556.2 ± 242.1	559.5 ± 247.8	552.9 ± 237.4
Total dietary vitamin D intake (IU/day)	98.9 ± 70.6	99.9 ± 63.8	97.8 ± 77.4
Total supplemental calcium (mg/day)	119.6 ± 174.5	134.8 ± 210.1	104.2 ± 127.7
Total supplemental vitamin D (IU/day)	67.9 ± 124.8	69.3 ± 149.1	66.6 ± 94.7
Baseline (first trimester) 25OHD (ng/mL)	16.8 ± 8.3	16.6 ± 8.4	17.0 ± 8.2
Calcium (mg/dL)	9.3 ± 0.4	9.3 ± 0.5	9.3 ± 0.3
Creatinine (mg/dL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Phosphate (mg/dL)	3.6 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
Magnesium (mg/dL)	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2
Total protein (g/L)	68.3 ± 4.7	68.6 ± 4.9	67.9 ± 4.6
Albumin (g/L)	41.3 ± 2.9	41.4 ± 3.2	41.2 ± 2.8
Globulins (g/L)	26.9 ± 3.7	27.1 ± 3.9	26.7 ± 3.4
TSH (mIU/mL)	1.3 ± 0.8	1.2 ± 0.7	1.4 ± 1.8

Abbreviations: 25OHD, 25 hydroxyvitamin D; TSH, thyroid stimulating hormone.

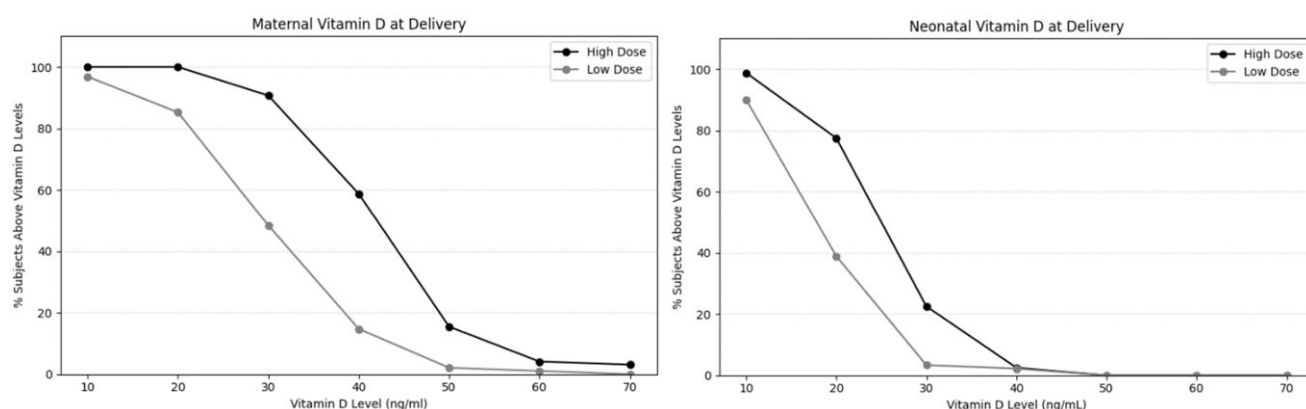


Figure 2 Sigmoidal curves showing the percentage of mothers and neonates reaching a spectrum of desirable serum 25(OH)D levels at delivery. Sigmoidal dose–response curves illustrating the cumulative percentage of mothers (left) and neonates (right) achieving increasing 25(OH)D thresholds at delivery, stratified by vitamin D supplementation dose (20 000 IU/week vs 10 000 IU every 2 weeks). Maternal and neonatal 25(OH)D concentrations were measured by standardized LC–MS/MS.

noninformative. Medication adherence was high and comparable between groups (>97% at all visits).

Neonatal bone outcomes

Among infants with interpretable DXA scans, maternal 25(OH)D at delivery was higher in the higher dose group than in the lower dose group (39.9 ± 7.2 vs 30.4 ± 9.9 ng/mL; $P < .001$; Table 3). Neonatal 25(OH)D concentrations showed a similar pattern (23.9 ± 5.4 vs 18.2 ± 7.5 ng/mL; $P = .012$). Despite these biochemical differences, subtotal whole-body BMC at a mean age of 5.1 weeks did not differ between groups (45.97 ± 8.5 vs 42.17 ± 7.1 g; $P = .059$; Table 4), nor did subtotal BMD or bone area. Neonatal anthropometric measures, including birth weight, length, and head circumference, were also comparable between groups (data not shown). There was no correlation between

umbilical cord 25(OH)D concentrations and neonatal BMC at 1 month; similar results for maternal 25(OH)D at delivery and neonatal Bone Mineral Content (BMC) at one month.

Safety and adverse events

Despite >95% adherence, we did not observe potential vitamin D toxicity, defined as serum 25(OH)D >150 ng/mL and/or hypercalcemia attributable to vitamin D supplementation, in either treatment arm (18). Serum 25(OH)D levels >60 ng/mL occurred in one participant in the lower dose group and 4 in the higher dose group, none of whom developed hypercalcemia (Fig. 3). One mother in the lower dose group had an elevated serum calcium level at delivery (11.1 mg/dL; upper limit of normal 10.5 mg/dL), with a 25(OH)D level of 15.4 ng/mL. The corresponding cord blood calcium level was 9.6 mg/dL, and

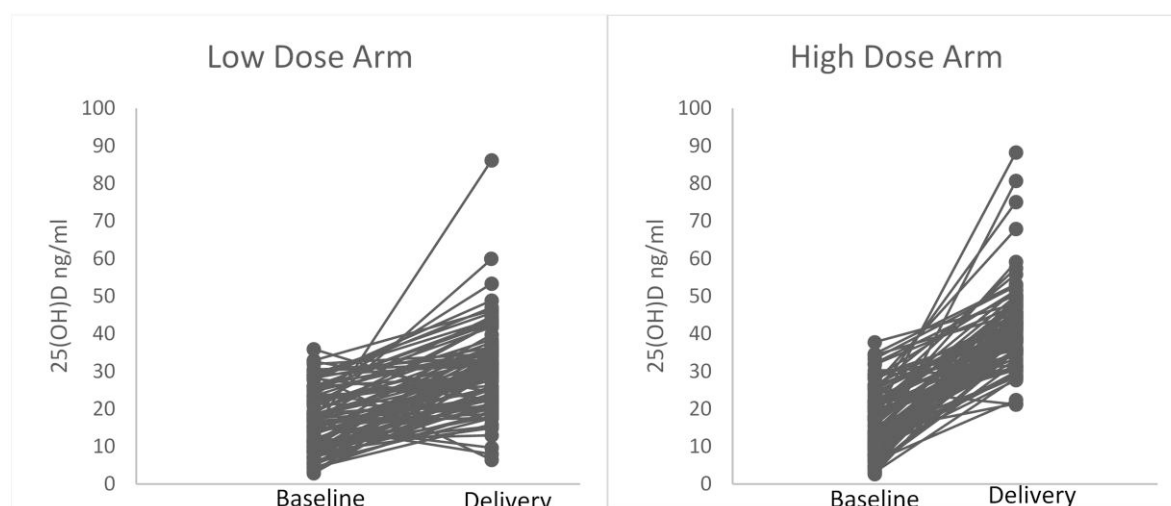


Figure 3 Individual change in maternal 25(OH)D level during pregnancy, by treatment arm. Plot showing individual changes in maternal serum 25(OH)D concentrations from the first trimester to delivery in women randomized to 20 000 IU/week (higher dose) or 10 000 IU every 2 weeks (lower dose) of vitamin D₃. Each line represents one participant.

Table 3 Characteristics of maternal–neonatal pairs with BMC scans and neonatal outcomes

		20 000 IU D3/week (N = 32)	10 000 IU D3 biweekly (N = 35)
Neonate gender	Female	N (%) 16 (50)	N (%) 20 (57)
	Male	16 (50)	15 (43)
		Mean ± SD	Mean ± SD
Gestational age at birth (weeks)		38.8 ± 0.9	38.8 ± 1.2
Maternal 25(OH)D at delivery (ng/mL)		39.9 ± 7.2	30.4 ± 9.9
		(N = 43) ^a	
Neonatal (25OHD) at birth (ng/mL)		23.9 ± 5.4	18.2 ± 7.5
		(N = 38) ^a	
Apgar score at 5 minutes after birth		10.0 ± 0.8	10.0 ± 0.5
Neonatal age at BMD scan (weeks)		5.1 ± 1.4	5.1 ± 1.1

Abbreviation: BMD, bone mineral density.

^aSignificant difference between arms (Mann–Whitney test).

Table 4 BMC scan results

Neonatal BMC results			
	20 000 IU D3/week (n = 31)	10 000 IU D3 biweekly (n = 34)	Effect size
	Mean ± SD	Mean ± SD	
Sub total BMC (g)	45.97 ± 8.5	42.17 ± 7.06	0.49
Sub total BMD (g/cm²)	0.159 ± 0.016	0.154 ± 0.016	0.31
Sub total bone area (cm²)	284.9 ± 35.3	276.7 ± 32	0.24

Abbreviations: BMC, bone mineral content; BMD, bone mineral density.

25(OH)D was 24.7 ng/mL. None of the study subjects reported symptoms consistent with the occurrence of kidney stones. The proportion of neonates with a cord blood calcium >11 mg/dL was 8.6%; their corresponding median 25(OH)D was 17.2 ng/mL [12.0–26.8] (19).

There was no significant difference in SAEs between the 2 doses, throughout the whole study, or between visits. A total of 13 serious adverse events were reported including preterm contractions, congenital anomaly and neonatal intensive care unit (NICU) admission during the study period; 2 between

visits 1 and 2 (1 in each arm), and 11 between visits 2 and 3 (7 in higher dose and 4 in lower dose arm), for a total of 8 in the higher dose group and 5 in the lower dose group. There were also no differences in the adverse events between the 2 arms: 38 were noted in the higher dose arm ($N = 97$) and 44 in the lower dose arm ($N = 95$). The same pattern for SAEs and AEs was observed in the total randomized study population of 330 maternal neonate pairs between the 2 arms (data not shown).

Discussion

In this double-blind randomized controlled trial of pregnant women with hypovitaminosis D, supplementation with 20 000 IU/week vitamin D3 was significantly more effective than 10 000 IU every 2 weeks in achieving maternal and neonatal vitamin D sufficiency at delivery. Using a validated LC-MS/MS assay in a CDC-certified laboratory, all women and 77% of neonates in the higher dose group achieved serum 25(OH)D ≥ 20 ng/mL, compared with 85% of mothers and only 39% of neonates in the lower dose arm. The primary neonatal outcome of subtotal whole-body BMC was slightly higher in the higher dose group (45.9 g vs 42.2 g), compared to the lower dose group, but not statistically significant ($P = .059$). Higher dose supplementation was safe and well-tolerated, with no increase in the rate of serious adverse events.

Maternal and neonatal 25(OH)D response

Our study group and others have demonstrated that baseline 25(OH) levels, type, and dose of vitamin D predict achieved levels (7, 20). The observed dose-response in our trial, 0.9 ng/mL per 100 IU/day in the higher dose group and 1.8 ng/mL per 100 IU/day in the lower dose group, mirrors findings from other pregnancy trials using comparable dosing ranges and LC-MS/MS assays (21, 22). Notably, diminishing incremental increases at higher doses have been consistently reported.

Large randomized trials further support these observations. In a trial from Bangladesh, Roth et al demonstrated that daily equivalent doses of 600 to 4000 IU achieved maternal 25(OH)D concentrations of 27.9 and 45.4 ng/mL, respectively, with $\geq 88.6\%$ reaching sufficiency, and a delta change of 0.87 ng/mL per 100 IU at the highest dose (23). Grant et al similarly reported diminishing dose-response beyond 1000 IU/day (21). The MAVIDOS trial showed that 1000 IU/day increased maternal 25(OH)D to a mean of 27.1 ng/mL, equivalent to a 0.84 ng/mL rise per 100 IU/day, albeit using a platform immunoassay (10). An exception is an Irish trial, with a baseline 25(OH)D of 21.9 ng/mL, reported a 2.1 ng/mL increase in serum 25(OH)D per 100 IU/day in the lower dose of supplementation (400 IU/d from the study and 876 IU/day as total D intake) (24). Across trials, higher doses consistently resulted in higher proportions (81-100%) of women achieving serum 25(OH)D ≥ 20 ng/mL (10, 21, 23, 24).

Importantly, despite exceeding the IOM recommendation, the 10 000 IU dose every 2 weeks in our trial was insufficient to ensure vitamin D sufficiency, leaving 15% of mothers and 61% of neonates below the target threshold. These findings

align with a regional meta-analysis from the MENA region, which demonstrated substantially lower rates of sufficiency with doses < 800 IU/day compared with doses > 2000 IU/day (7). Current guidelines do not adequately account for such high-risk populations (25, 26).

Neonatal vitamin D status closely reflected maternal concentrations. Higher maternal supplementation resulted in significantly higher cord blood 25(OH)D levels, consistent with prior trials demonstrating proportional but attenuated fetal transfer. Across studies, only higher maternal doses reliably achieved neonatal sufficiency (21-24).

Neonatal bone outcomes

Although maternal vitamin D status plays a key role in fetal skeletal development (27), we did not observe significant differences in neonatal BMC or other bone indices between dosing groups. This finding is consistent with the MAVIDOS trial, which showed no overall effect of maternal supplementation with 1000 IU D3/day on neonatal whole-body BMC at birth, despite a season-specific benefit among winter births (10). Four-year follow-up of MAVIDOS children demonstrated higher offspring BMD and lean mass in the supplemented group compared with placebo (28). Similarly, long-term follow-up from the Copenhagen cohort demonstrated improved childhood bone mineralization with high-dose prenatal vitamin D3 supplementation with 2800 IU/d, compared to 400 IU/day, particularly among vitamin D-deficient mothers and winter births (29). Given the limited number of DXA scans in our study, the absence of a statistically significant neonatal BMC difference should be interpreted cautiously and may reflect insufficient power to detect modest skeletal effects in early infancy. Thus, longer follow-up may be required to determine whether biochemical improvements translate into later skeletal benefits.

Limitations and strengths

Limitations of our study include reduced power for neonatal bone outcomes due to parental refusal and motion artifacts affecting DXA scans, the smaller LC-MS/MS sample relative to the randomized cohort, the lack of direct assessment of sunlight exposure, and limited generalizability beyond similar populations. Motion artifact is a recognized limitation of neonatal DXA because scans must be obtained while infants remain still; the IRB at our institution permitted only one scan attempt, which increased the number of unanalyzable scans. To limit bias, scans were reviewed by an independent expert masked to treatment allocation. Scans with high motion artifact were considered inadequate and those missing limb values were imputed from the contralateral limb using a regression-based method when appropriate. Nevertheless, the reduced number of interpretable scans limited the precision and prevented the study from achieving the originally planned analytic sample for neonatal BMC. Importantly, its strengths include the randomized, double-blind design; evaluation of both biochemical and skeletal endpoints; use of gold-standard LC-MS/MS assays traceable to CDC and NIST pregnancy reference standards; and blinded expert review of neonatal DXA scans. To the best of our knowledge, this is the first randomized trial in the region to integrate accurate vitamin D measurement with neonatal bone outcomes.

Clinical and public health implications

Our findings challenge clinical practice and public health guidelines on vitamin D supplementation in pregnancy. Indeed, the dose of 600 IU/day recommended by the IOM (12) and that of 200 IU/day by the WHO (25) fall short of achieving biochemical sufficiency in many pregnant women and their neonates. However, the Endocrine Society recommends empiric vitamin D supplementation through fortified foods, prenatal vitamins, or supplements based on evidence from clinical trials in healthy pregnant women in which vitamin D equivalent doses ranged from 600 to 5000 IU/day, with an estimated weighted average intake of approximately 2500 IU/day (26).

Conclusion

This trial provides robust, region-specific evidence that prenatal vitamin D doses near current guideline recommendations are insufficient to achieve maternal and neonatal sufficiency. A daily dose equivalent to 2857 IU safely and effectively corrected hypovitaminosis D, supporting reconsideration of prenatal vitamin D dosing recommendations in the MENA and globally. These findings support further evaluation of prenatal vitamin D dosing strategies in high-risk populations, with further follow-up of infant and childhood skeletal outcomes.

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Disclosures

The authors have nothing to disclose.

Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical trial information

[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02434380) NCT02434380.

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