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Effect of vitamin D replacement on maternal and neonatal outcomes: a randomised controlled trial in pregnant women with hypovitaminosis D. A protocol

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ABSTRACT

Introduction: The vitamin D recommended doses during pregnancy differ between societies. The WHO guidelines do not recommend routine prenatal supplementation, but they underscore the fact that women with the lowest levels may benefit most. The effects of routine supplementation during pregnancy on maternal and neonatal clinical outcomes have not been investigated in the Middle East, where hypovitaminosis D is prevalent. Our hypothesis is that in Middle Eastern pregnant women, a vitamin D dose of 3000 IU/day is required to reach a desirable maternal 25-hydroxyvitamin D [25(OH)D] level, and to positively impact infant bone mineral content (BMC).

Methods and analysis: This is a multicentre blinded randomised controlled trial. Pregnant women presenting to the Obstetrics and Gynaecology clinics will be approached. Eligible women will be randomised to daily equivalent doses of cholecalciferol, 600 IU or 3000 IU, from 15 to 18 weeks gestation until delivery. Maternal 25(OH)D and chemistries will be assessed at study entry, during the third trimester and at delivery. Neonatal anthropometric variables and 25(OH)D level will be measured at birth, and bone and fat mass assessment by dual-energy X-ray absorptiometry scan at 1 month. A sample size of 280 pregnant women is needed to demonstrate a statistically significant difference in the proportion of women reaching a 25(OH)D level ≥50 nmol/L at delivery, and a difference in infant BMC of 6 (10)g, for a 90% power and a 2.5% level of significance. The proportions of women achieving a target 25(OH)D level will be compared between the two arms, using χ^2. An independent t test will be used to compare mean infant BMC between the two arms. The primary analysis is an intention-to-treat analysis of unadjusted results.

Ethics and dissemination: The protocol has been approved by the Institutional Review Board at the American University of Beirut-Lebanon (IM.GEHF.22). The trial results will be published in peer-reviewed medical journals and presented at scientific conferences.

Trial registration number: NCT02434380.

Strengths and limitations of this study

- This is the first randomised controlled trial (RCT) in the Middle East directly addressing the applicability of the Institute of Medicine recommendations in this specific population, and assessing the effect of vitamin D supplementation on neonatal bone mineral content.
- Multiple maternal and neonatal outcomes that have not been targeted in any previous trial in pregnancy will be assessed as secondary or exploratory outcomes; indeed, the results will guide future research projects in this field.
- Vitamin D and other calciotropic hormones will be measured using the gold standard method, liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- The findings of this trial will help guide the public health policymaker regarding vitamin D supplementation in pregnant women and will allow a step forward in evidence-based recommendations specific to the Middle East.
- Results derived from nutrient RCT, specifically vitamin D supplementation, suffer from several confounding factors related to the baseline status, intake of other nutrients, such as calcium and proteins, and sun exposure, which are difficult to quantify accurately.

INTRODUCTION

Vitamin D physiology during pregnancy

Pregnancy is characterised by physiological changes in mineral metabolism, to allow calcium accretion in the fetal skeleton.[1–3] These changes start in the first trimester, and culminate during the third trimester, a period during which fetal calcium requirements increase exponentially.[2] Indeed, it is in anticipation of such requirements that maternal calcitriol levels increase during

pregnancy. While the total calcitriol levels double in the first trimester, free calcitriol levels do not increase until the third trimester and remain so until lactation.[2 4] Conversely, parathyroid hormone (PTH) levels decrease early on and increase back to mid-normal range by term.[2] The total calcium level decreases during pregnancy, due to haemodilution, while the ionised calcium level remains stable.[2] Vitamin D binding proteins also increase during pregnancy secondary to high oestrogen levels,[4 5] but the 25-hydroxyvitamin D (25(OH)D) level, the single best nutritional indicator of vitamin D status,[6] remains stable.[7] The changes in calcitriol levels led to the description of pregnancy as a state of 'absorptive hypercalciuria'.[1 2] The above adaptive physiology is key to safety considerations when using vitamin D supplementation during pregnancy, as well as to determining key biochemical and hormonal parameters to be monitored.

Maternal vitamin D status during pregnancy

Vitamin D deficiency during pregnancy is prevalent worldwide, especially in developing countries.[8] In a systematic review of 18 studies conducted in Western countries during the first trimester, white Caucasian pregnant women were found to have a mean 25(OH)D level between 29 and 73 nmol/L.[9] Mean 25(OH)D levels were lower in non-Caucasian pregnant women, ranging between 15.2 and 43 nmol/L.[9] In addition to ethnicity, higher latitude was a significant predisposing factor for hypovitaminosis D.[9] Similarly, in non-Western countries, more than half of the pregnant women who were beyond their first trimester had 25(OH)D levels below 75 nmol/L; these include countries such as India,[10] Kuwait,[11] Pakistan[12] and Turkey.[13] Even lower levels (<25 nmol/L) have been reported at delivery in Saudi Arabia, Iran and the United Arab Emirates.[14] Furthermore, immigrant women were at particular risk.[15 16] An observational study from the Netherlands showed significantly lower 25(OH)D levels during the first trimester in immigrant pregnant women (Turkish, Moroccan and others), compared to western participants.[17]

Association between maternal vitamin D status and maternal adverse outcomes

Vitamin D insufficiency during pregnancy is associated with adverse maternal outcomes such as increased risk of gestational diabetes mellitus (GDM), preeclampsia, caesarean-section delivery and bacterial vaginosis.[18] In a recent meta-analysis of observational studies, the risk of GDM was found to be increased by 40–84% in pregnant women with low 25(OH)D levels, defined as <50 nmol/L or <75 nmol/L, depending on the studies.[19–21] While preeclampsia risk was significantly increased in vitamin D insufficient women,[22] C-section rates were inconsistently affected by vitamin D status.[23] However, these findings remain limited by the inherent biases of observational studies, inconsistent adjustment for confounders, in addition to the wide heterogeneity in vitamin D assays and vitamin D cut-offs definition.

Association between maternal vitamin D level and neonatal adverse outcomes

Low maternal 25(OH)D levels were recently linked to fetal programming, and were found to be associated with adverse events in neonates, resulting in small for gestational age (SGA) at birth,[19 23] and also later on during childhood, leading to reduced muscle and bone mass in offspring at 4 and 9 years.[24 25] This may be explained by the fact that maternal vitamin D is essential for fetal musculoskeletal integrity, as it regulates neonatal bone accrual, possibly through specific proteins that are responsible for placental calcium transport.[26] Recently, data from the Southampton Women's Survey (SWS) showed that maternal 25(OH)D level is significantly correlated with placental amino acid transporters expression, that mediate the transport of various nutrients to the fetus.[27] Furthermore, maternal vitamin D may influence the fetal muscle motor unit size, and consequently muscle mass and strength after birth.[25] It is noteworthy that fetal bone development is one of the predictors of peak bone mass, adult bone mineral content and hip geometry, thus correlating with fracture risk later in life.[26 28]

Vitamin D replacement guidelines during pregnancy

The guidelines regarding vitamin D replacement or supplementation during pregnancy vary substantially.

The 2010 Institute Of Medicine (IOM) Report on Dietary Reference Intakes for Calcium and Vitamin D recommended 600 IU to pregnant women as the recommended daily allowance (RDA), the RDA being the dose that is projected to allow at least 97.5% of the pregnant women population to reach the desirable target 25(OH)D level ≥50 nmol/L.[29] This recommendation was based on observational studies, none of which were conducted in the Middle East.[29] Conversely, the Endocrine Society 2011 guidelines recommended that 1500–2000 IU daily of vitamin D is needed to reach a target 25(OH)D level ≥75 nmol/L (a recommendation that was graded as weak with moderate quality of evidence).[30] The American College of Obstetricians and Gynecologists (ACOG) does not recommend screening for vitamin D level in pregnancy, and abides by the IOM recommendations.[31] Moreover, the WHO 2012 guidelines on vitamin D replacement during pregnancy did not recommend vitamin D supplementation as part of prenatal care.[32] This was based on a meta-analysis of vitamin D trials during pregnancy, which identified a limited number of high-quality studies demonstrating a beneficial effect of supplementation on maternal and neonatal outcomes, and concluded that further randomised controlled trials (RCTs) are needed.[33] In the UK, however, pregnant women are considered at risk of vitamin D deficiency, and supplementation with 400 IU daily is required.[34]

It is not clear whether any of the above recommended doses are applicable to non-western populations, with lower baseline vitamin D levels, such as in Lebanon and other Middle Eastern countries. Indeed, the WHO

pregnancy guidelines clearly stated that "Vitamin D supplementation will probably have the most benefit in populations of poor countries, those with darker skin colour and in populations with a high prevalence of vitamin D deficiency. It is expected that this intervention would be acceptable to women who are not exposed to adequate amounts of sunshine."[32] This is particularly relevant to our population that tends to avoid sunshine, wear concealed clothing or use sunblock, all resulting in the low 25(OH)D levels observed across the life cycle.

RCTs of vitamin D supplementation during pregnancy

Two landmark RCTs have been conducted in the USA[35] and the UK.[36] Hollis *et al*[35] showed that in pregnant women in the USA, with a baseline 25(OH)D level around 60 nmol/L, a vitamin dose of 4000 IU daily allowed 82% of participants to reach a 25(OH)D level of 80 nmol/L, while only 70% and 50% reached this target in the intermediate (2000 IU daily) and low (400 IU daily) doses, respectively. Cooper *et al*[36] showed that vitamin D supplementation of 1000 IU daily, compared to placebo, in pregnant women in the UK allowed a significant increase in Bone Mineral Content (BMC) of neonates, however, only when they were born in winter. One study from India, comparing non-intervention to vitamin D supplementation groups, with the dose being dependent on 25(OH)D levels at 20 weeks gestation, showed that vitamin D supplementation resulted in a significant difference in the achieved 25(OH)D level at delivery (43.1 (81.3) nmol/L in the former group versus 56.8 (47.5) nmol/L in the latter group).[37] Hollis *et al*[36] and Sablok *et al*[37] showed that vitamin D supplementation decreased the risk of preterm labour, gestational diabetes and hypertensive complications (all combined).

In the Middle East and North Africa region, there are few recent RCTs that attempted to determine the optimal regimen of vitamin D replacement in healthy pregnant women.[38–41] With the exception of Soheilykhah *et al*[40] who assessed the effect of vitamin D supplementation on insulin resistance, the primary outcomes in these studies were mostly maternal and neonatal 25(OH)D levels (see online supplementary appendix 1). None of the other clinically important outcomes, such as neonatal size and other anthropometric measurements, neonatal BMC, GDM and C-section rates, were evaluated as primary outcomes in any of these trials (see online supplementary appendix 1).

We therefore compiled a registry of all ongoing vitamin D trials in pregnancy, as captured by their registration on clinicaltrial.gov[42] (see online supplementary appendix 2). In these trials, different doses of vitamin D, reaching up to 7000 IU daily, are being administered, and the outcomes to be assessed include neonatal weight and length, childhood asthma, maternal bone mineral density, maternal adverse outcomes, including preeclampsia and preterm labour, and neonatal adverse outcomes, such as SGA. Only two of the ongoing trials are being conducted in the Middle East, one in Iran and one in Israel, start supplementation in the third trimester and use vitamin D doses of 7000 IU and 2000 IU daily, respectively. These latter studies aim at assessing the effect of vitamin D supplementation on offspring calcium status, maternal and infant vitamin D status and bone status (by quantitative ultrasound) at 1 year. Neither addresses the applicability of IOM vitamin D dose recommendations in pregnant women in the Middle East. Three completed (unpublished) trials were identified (see online supplementary appendix 2), two from the USA and one from Pakistan. They assessed the effect of various doses of vitamin D on the 25(OH)D level, immune function and periodontal disease.

Hypothesis

The study hypothesis is that a high dose of vitamin D, equivalent to 3000 IU/day, is needed to optimise maternal vitamin D level and neonatal musculoskeletal parameters, compared to a low dose of 600 IU/day.

Objectives

The two primary objectives of this trial, comparing the effect of high-dose versus low-dose vitamin D, are as follows:

- The proportion of women who will reach the IOM defined desirable 25(OH)D level ≥50 nmol/L at delivery.
- Infant BMC at 1 month.

The secondary objectives are to compare the effect of high-dose versus low-dose vitamin D on:

- Maternal outcomes:
  - Mean maternal 25(OH)D level at delivery.
  - Mean maternal PTH level at delivery.
  - Mean change in maternal urine calcium.
- Neonatal outcomes:
  - Mean neonatal 25(OH)D level at delivery.
  - Mean neonatal PTH level at delivery.
  - Mean neonatal fat and lean mass at 1 month.
  - Mean neonatal knee to heel length at birth.

Exploratory outcomes include a composite outcome (incidence of GDM and C-section), maternal weight, blood pressure, ill days, fetal and neonatal anthropometric measures, including neonatal length and weight, rate of small for gestational age, APGAR score, placental weight and 1α-hydroxylase activity, in addition to other placental and genetic studies, that characterise mineral and fuel metabolism.

METHODS AND ANALYSIS

The protocol of this trial was developed on the basis of Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT); see online supplementary appendices 3–6 for further details. This protocol is registered on clinicaltrial.gov (NCT 02434380, April 2015).

Study design

This study is a phase III multicentre blinded randomised controlled superiority trial with two arms, conducted at the American University of Beirut—Medical Center (AUB-MC), Rafic Hariri University Hospital (RHUH) and Bahman Hospital.

Recruitment

Pregnant women in their first trimester will be recruited from the obstetric private clinics and outpatient departments of the three participating centres (AUB-MC, RHUH and Bahman Hospital). Information about the trial will be available as Arabic and English flyers in the obstetrics and gynaecology department, as well as the private clinics and outpatient departments of the three centres. The flow chart of participants and details of study visits are summarised in figure 1.

Randomisation

The allocation sequence will be a computer-generated, permuted block randomisation, stratified by study centre, with a 1:1 allocation. The statistician will be responsible for sequence generation and treatment assignment. The senior pharmacist at AUB-MC will be responsible for treatment allocation.

Concealment and blinding

Vitamin D and placebo pills are manufactured to have a similar shape, colour, size, smell and taste. The study medications will be stored at the AUB-MC pharmacy, and placebo and/or vitamin D pills will be dispensed in boxes. Boxes will be sequentially numbered as per the random allocation list by the pharmacist. The pharmacist keeps the list linking the randomisation code to the participant identity/trial number and to the delivered box number. At each visit, the pharmacist allocates a box to every participant, containing enough pills until the next visit, with dates at which pills should be administered. The research assistant collects the boxes in sealed envelopes prior to each participant's visit and delivers them at the end of the visit.

The research assistants, the healthcare providers, the principal investigator, the co-investigators and the biostatistician do not have access to code break, and are all blinded to the treatment allocation. The only personnel who will not be blinded will be the pharmacist.

Investigational medicinal product

All participants receive once per week two tablets that are similar in shape, colour, size, smell and taste. Each tablet can be either a placebo or a 10 000 IU vitamin D (cholecalciferol), provided by Europharm.

The high-dose group receives two tablets of 10 000 IU weekly (equivalent daily dose 2857 IU).

The low-dose group receives one tablet of 10 000 IU and one tablet of placebo alternating with two tablets of placebo on a weekly basis (equivalent daily dose 714 IU).

Vitamin D supplementation present in prenatal multivitamins will be permitted up to 200 IU daily, which will raise the aforementioned treatment doses to approximately 3050 IU and 900 IU daily, respectively.

The manufacturers had no role in the study design or implementation.

Study visits

A. Prescreening visit

Trained research assistants will approach pregnant women who are in their first trimester during their routine prenatal visits to study sites. Eligible pregnant women willing to participate and to be compliant with the study protocol, and who provide written informed consent, will be invited to a screening visit.

B. Screening visit

The screening visit will be scheduled to coincide with the nuchal translucency appointment date (between 11 and 13 weeks of gestation). During this visit, eligibility criteria will be verified and blood tests for 25(OH)D level, calcium, phosphate, magnesium, creatinine and thyroid stimulating hormone (TSH) will be withdrawn. Urine calcium will be assessed in a fasting urine spot or 24 h urine collection (table 1). The level of 25(OH)D will be measured using the electrochemiluminescence immunoassay (ECLIA) at the AUB-MC Clinical Chemistry laboratory. Reference ranges using this assay are defined as follows: Deficiency <25 nmol/L, insufficiency 25–62.4 nmol/L, desirable >62 nmol/L, toxic >374 nmol/L. AUB-MC clinical chemistry laboratory partakes in the quality assurance, evaluation and accreditation by the College of American Pathologists[43] and is a participant in the Vitamin D External Quality Assurance Surveillance (DEQAS) programme.[44]

Eligibility criteria

Inclusion criteria:

- Pregnant women gestational age (GA) <14 weeks at screening visit.[i]
- Middle Eastern origin; Middle East countries as defined by the World Bank (Bahrain, Egypt, Iran, Iraq, Palestine, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Syria, the United Arab Emirates, Yemen).[45]
- 25(OH)D level between 25 and 75 nmol/L.
- Age >18 years.

Vitamin D supplementation ≤200 IU daily.[ii]

Exclusion criteria:

[i]In the case where the pregnant woman presents after 13 weeks GA, she is still eligible for the screening visit provided that screening blood tests are carried out before 16.5 weeks of gestation and the first visit in the trial occurs before 18 weeks GA.

[ii]If daily vitamin D supplementation is between 200 and 600 IU daily, at enrollment, the pregnant women will be advised to adjust prenatal multivitamin doses, in consultation with her primary obstetrician, to ensure that total vitamin D supplementation during the study does not exceed 1400 IU/week, in consultation with the primary obstetrician.

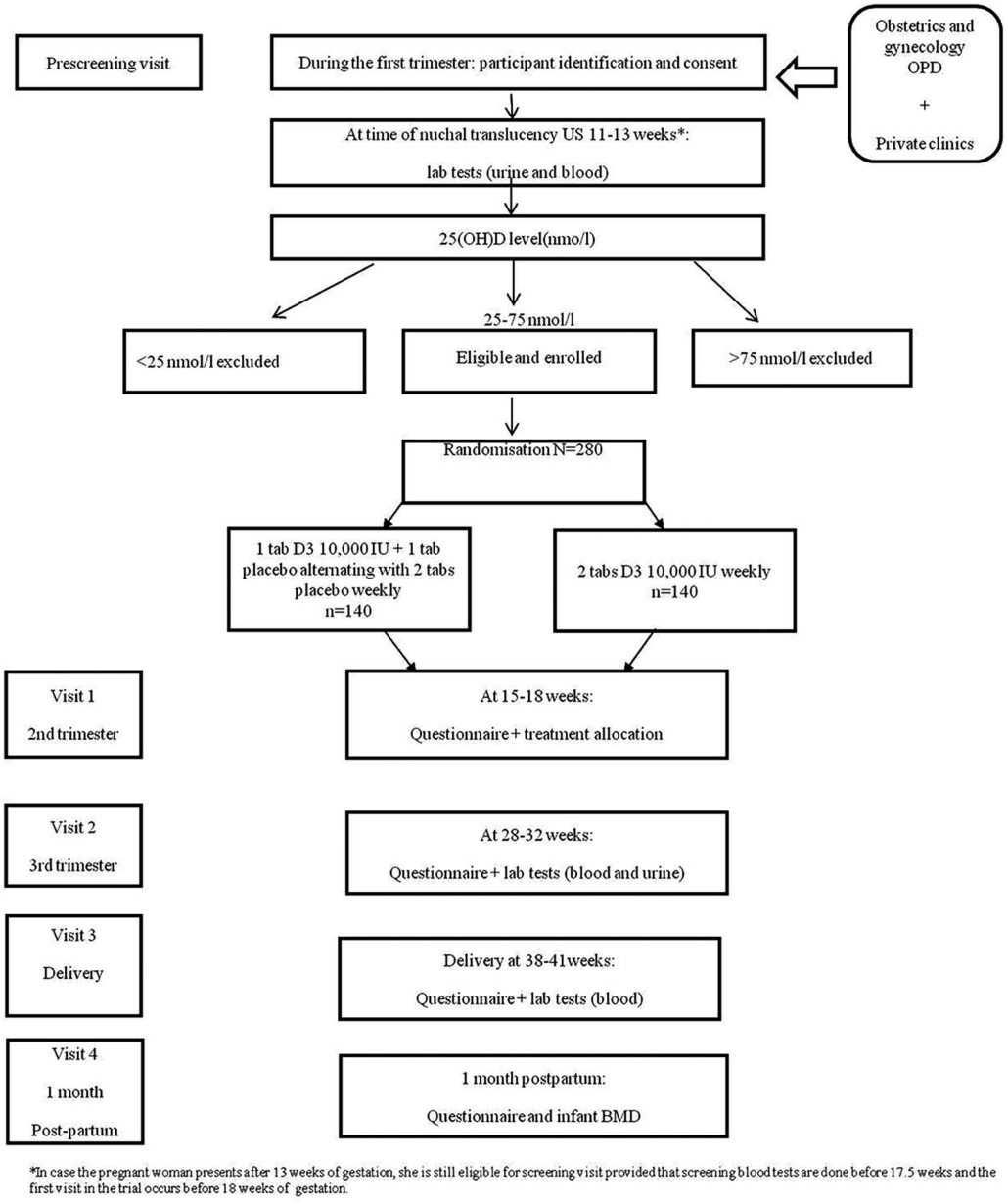

Figure 1 Trial flow chart. BMD, bone mineral density.

- 25(OH)D level <25 nmol/L, as it would be unethical to randomise pregnant women to the low dose of vitamin D, and 25(OH)D level >75 nmol/L (30 ng/mL), as vitamin D supplementation with routine prenatal multivitamins would be sufficient.
- Known metabolic bone disease or chronic diseases associated with bone abnormalities (renal or liver diseases.
- Current medications likely to interfere with vitamin D metabolism (enzyme inducing anticonvulsants, antituberculosis).
- Vitamin D supplementation >600 IU daily.[iii]

[iii]*If a pregnant woman is on a high dose of vitamin D supplementation, > 600 IU daily, vitamin D should be stopped at least 1 month prior to study entry, at the discretion of her primary physician.

- Fetal physical anomalies on the initial ultrasound.
- Renal stones.
- Hyperparathyroidism.
- Uncontrolled thyroid dysfunction.
- Diagnosis of cancer in the past 10 years (other than basal cell carcinoma).
- Serum calcium >10 mg/dL.
- Diabetes mellitus type 1 or type 2.
- Previous GDM.
- Allergy to any component of vitamin D formulation.

C. First visit

During the first visit at 15–18 weeks GA, a questionnaire will be administered to collect maternal information on parity, demographics, smoking and alcohol history, exercise, previous medical problems, medications, dietary calcium and vitamin D intake, in addition to relevant paternal information. Pregnant women will

Table 1 Trial events and outcomes measures

Trial event and outcomes measures	11–13 weeks (screening visit)	15–18 weeks (visit 1)	20 weeks	24–28 weeks	28–32 weeks (visit 2)	37–42 weeks Delivery (visit 3)	1 month post partum (visit 4)
Maternal height		✓					
Maternal weight, blood pressure		✓			✓	✓	
Maternal health and diet assessment		✓			✓	✓	
Maternal 25(OH)D, Crea, Ca	×				×	×	
Maternal cell count, ph, mg, alb, TSH, $1,25(OH)_2D$	×						
PTH	×					×	
Maternal glucose (1 h)				✓			
Maternal 24 h (or spot) urine collection for calcium and creatinine		×			×		
Maternal vitamin D binding protein	×					×	
Maternal genetic pathways of vitamin D metabolism						×	
Fetal US:	✓						
▶ crown -rump							
Fetal US:			✓				
▶ Femur length							
▶ Abdominal circumference							
▶ Head circumference							
▶ Biparietal diameter							
Newborn weight, length, knee to heel length APGAR score						✓	
Placental weight						×	
Placental studies						×	
Newborn 25(OH)D, Ca, PTH (cord blood)						×	
Newborn genetic pathways of vitamin D metabolism						×	
Infant bone/fat mass							×
Infant weight and length							✓
Infant health and diet assessment							✓

✓Test performed for clinical purpose.
×Test performed for research purpose.
PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; US, ultrasound

be randomised early in their second trimester to one of two vitamin D doses as discussed above.

D. Second visit

This visit will take place at 28–32 weeks GA, during which maternal weight and blood pressure will be recorded, maternal health and diet assessed, in addition to assessment of adverse events, if any. We will check compliance to trial medication by pill counting. Blood and urine tests will be carried out (see table 1).

E. Third visit

The third visit will coincide with the participant's delivery. When entering labour, the research team will be informed about each participant by the obstetrician, or by the participant, or her partner. The research assistant will visit the participant on the first day post partum, and will record information on delivery mode, delivery course and complications, if any. In addition, neonatal measurements at birth such as length, weight and knee—heel length will be recorded in triplicate. Knee-heel length will be measured using handheld vernier calipers.[46] Knee—heel length measurement is operator dependent; hence, measurements will be carried out in triplicate, and only by paediatricians/neonatologists who are trained on how to use such instruments.

The neonatal 25(OH)D level will be obtained from cord blood, whereas maternal blood tests will be withdrawn when the pregnant woman presents in labour. In addition, blood tests at delivery include genetic studies such as vitamin D genes polymorphism and RNA expression of vitamin D polymorphisms. After delivery, placental sampling will be performed by a trained medical doctor and samples will be preserved and stored at −80°C.

F. Fourth visit

This visit will occur when the infant is 1 month of age. He will undergo bone mineral density (BMD) assessment by dual-energy X-ray absorptiometry (DXA) scan, Hologic machine, Horizon A, V.13.5.3.1, at AUBMC. Infant DXA assessment is performed by technicians certified by the International Society for Clinical Densitometry (ISCD). The technician positions the laser light so that it is centred about 2 cm below the iliac crest (or umbilicus/belly button) on the child, and observes the emerging image to ensure that the spine is centrally positioned and straight, and that the top of the iliac crests and all of L5 are visible.

In addition, during this visit, information about the infant's health and feeding will be recorded using an interviewer-led questionnaire.

Sample size calculation and justification

Sample size was calculated for the two primary outcomes: the proportion of pregnant women who will reach a 25(OH)D ≥50 nmol/L at delivery, and the infant BMC at 1 month; the largest number was considered the final sample size. Given that we have two primary outcomes, type I error was considered 2.5%.[47] Sample size calculation was performed online.[48]

Sample size calculation for the proportion of women who will reach a 25(OH)D ≥50 nmol/L at delivery

On the basis of a retrospective lab study conducted at AUB-MC in 2014, the median 25(OH)D level in the Lebanese population was found to be 52 nmol/L (20.9 ng/mL). The low-dose group will receive 10 000 IU vitamin D weekly, equivalent to 700 IU daily. The high-dose group will receive 20 000 IU vitamin D weekly, equivalent to 2850 IU daily. Considering that each 100 IU vitamin D supplementation increases the level by 1.7 nmol/L,[49] the expected levels reached in the low-dose and high-dose arms would be 67 nmol/L and 106 nmol/L, respectively. This computation takes into consideration that all groups will be taking additional 200 IU vitamin D daily from their prenatal vitamin pills, thus increasing the final vitamin D intake approximately to 900 IU/day in the low-dose group and 3050 IU/day in the high-dose group. The expected proportions of pregnant women who would reach a 25(OH)D level ≥50 nmol/L, using a SD of 24.9 nmol/L, and assuming normality, would be 75% and 98.4%, in the low-dose and high-dose arms, respectively. To detect statistical significance between the two groups, for a 90% power and a type I error of 2.5%, 50 participants per arm are needed.

Calculation was also carried out on the basis of the results of a recently completed systematic review and meta-analysis of RCTs from the Middle East and North Africa (MENA), conducted by Chakhtoura *et al*, as part of a Master of Sciences in Health Research thesis project (available online from the Jafet Library at the American University of Beirut—Lebanon). This meta-analysis showed that, in pregnant women from the MENA region, a vitamin D dose of 800–2000 IU daily results in an increase in the 25(OH)D level by 2.5 nmol/L, and a high dose of >2000 IU daily results in an increase in the 25(OH)D level by 1.67 nmol/L. Starting from a baseline 25(OH)D level of 52 nmol/L, the 25(OH)D levels achieved would be 74.5 nmol/L and 103 nmol/L in the low-dose and high-dose groups, respectively. Accordingly, 83.6% and 98.3% would reach the target 25(OH)D level of 50 nmol/L, and 72 participants per arm are needed for an 80% power and a type I error of 2.5%. It is noteworthy that the studies included in the aforementioned meta-analysis had a baseline 25(OH)D level of 20–27 nmol/L, lower than the expected levels in our participants.

Sample size calculation for infant BMC

Estimations were based on the preliminary results of the MAVIDOS trial conducted by our collaborators at Southampton University, UK. They showed a significant difference of 6 g (SD 10 g) in neonatal mean BMC in the vitamin D supplemented group, compared to placebo, in the winter season.[36] For a 90% power and a type I error of 2.5%, considering an SD of BMC of 10 g, to detect a 6 g difference in BMC between high-dose and low-dose groups, 69 participants per arm are needed. Taking into consideration that 25(OH)D levels in RHUH and Bahman hospital are lower compared to

pregnant women presenting to AUB-MC and to pregnant women in the UK, a significant improvement in BMC is expected throughout the year in the high-dose group compared to the low-dose group.

The largest sample size of 69 participants per arm is our target. If we consider a 50% dropout rate, to be conservative, approximately 140 participants per arm should be recruited for a total of 280 pregnant women for the whole study. If we consider that 50% of pregnant women presenting to clinics are eligible, approximately a total of 560 pregnant women should be screened initially. If 30% of pregnant women accept to participate in clinical trials, approximately 1870 pregnant women should be approached initially.

Statistical analysis

Baseline demographic characteristics will be summarised using frequencies and percentages for categorical characteristics, and mean ±SD (or median and range) for continuous variables. Normality of all variables will be checked. Comparisons between dose groups will be performed using χ^2 tests for categorical variables, and t test for continuous variables, as appropriate.

Unadjusted analysis

Two primary outcomes are considered:

A. The proportion of women who reach a 25(OH)D ≥50 nmol/L: binary outcome; the percent of women achieving 25(OH)D ≥50 nmol/L in the low dose will be compared to those in the high dose using χ^2, by constructing a 95% CI for the difference and computing an unadjusted RR and its 95% CI, along with the p value. A number needed to treat (NNT) will also be computed.
B. The mean infant BMC at 1 month: continuous outcome; an independent t test will be used to compare mean BMC between the two arms. 95% CI for the difference will be calculated.

Secondary and exploratory outcomes:

For secondary and exploratory outcomes, a t test will be used for continuous outcomes and χ^2 will be used for binary outcomes to compare means and proportions, respectively. Non-parametric tests including the Wilcoxon sign rank test and Fisher's exact test will be used, respectively, instead of t test and χ^2, when needed.

Relative Risk (RR) with corresponding CIs will be calculated for dichotomous variables, and difference in means with their 95% CIs will be used for additional analysis of continuous variables.

The primary analysis is an intention-to-treat analysis (ITT) of unadjusted results, ITT being defined as the analysis of all participants as randomised, regardless of whether they respected the study protocol or not (effectiveness). The p values will be reported to four decimal places.

For the primary outcomes, p values will be considered statistically significant if ≤0.025.

SPSS V.23 will be used to conduct statistical analysis.

In case of missing data, analysis restricted to results of individuals with complete data will be carried out (with retrospective power calculation) and compared to analysis resulting from multiple imputations to try to test the robustness of results.[50]

Additional analysis

Subgroup analysis

As discussed earlier, the IOM targets a 25(OH)D level of ≥50 nmol/L[29] and the Endocrine Society targets a level of ≥75 nmol/L.[30] Subgroup analysis based on a 25(OH)D level at study entry (<50 nmol/L vs ≥50 nmol/L) will be carried out to explore whether the treatment effects persist across all 25(OH)D categories, whether below or above 50 nmol/L.

Subgroup analysis based on the season will be also performed to check for interaction between the vitamin D dose and the season of pregnant women enrolment.

Sensitivity analysis

Sensitivity analysis will be performed, including Per Protocol analysis and as treated analysis. In addition, an adjusted analysis will be performed, including adjustment for variables that are not evenly distributed between the two arms, if any, and adjustment for variables that are clinically important (even if there is no imbalance in the baseline characteristics of the 2 groups); this includes the baseline 25(OH)D level, pre-pregnancy body mass index, season at enrolment and smoking status.

Ethical considerations

We will restrict enrolment to pregnant women whose 25(OH)D levels range between 25 and 75 nmol/L. This is because it will be unethical to include women with levels <25 nmol/L in the trial, as there is a risk to randomly allocate them to the low-dose arm. In addition, women with a 25(OH)D level >75 nmol/L will be excluded in order to prevent reaching supranormal levels of 25(OH)D should they be allocated to the high-vitamin D dose. It is noteworthy that high doses of vitamin D (up to 4000 IU daily) have been used in previous trials conducted during pregnancy with no reported adverse events (see online supplementary appendix 1).

The infant radiation exposure resulting from the study procedure, BMD testing by DXA, is minimal. The radiation dose is estimated at 0.007 mSv for whole body DXA. This dose is equivalent to 20 h of exposure to background radiation, based on the Duke Radiation Safety online assessment and statement.[51]

Safety considerations

Information on adverse events will be regularly collected soon after starting the trial intervention and during each trial visit. In between visits, all participants will be called by the research team every 2 weeks to emphasise compliance with treatment regimens and to enquire about adverse events. All information will be documented in case report forms and discussed with the Trial

Monitoring Committee (TMC) (see online supplementary appendix 3 for further details). The TMC will report any serious adverse event to IRB and the Data Safety and Monitoring Board (DSMB) within 48 h.

Dissemination

Trial results will be communicated to participants, to the public, and to healthcare professionals at AUB-MC and in Lebanon. Results will be presented in scientific meetings and conferences and published in peer-reviewed medical journals, whether the results are in the expected direction or not.

DISCUSSION

Hypovitaminosis D is a well-recognised common public health problem in Lebanon and in most countries of the Middle East. Many observational studies suggest that maternal hypovitaminosis D is associated with adverse maternal and neonatal outcomes. Vitamin D RCTs in pregnancy are scarce, with small sample sizes, and their primary outcomes are mostly limited to measuring 25 (OH)D levels in mothers and neonates. Furthermore, given the lack of evidence-based guidelines that define the optimal RDA for vitamin D supplementation during pregnancy in our population, and the limited number of randomised clinical trials completed so far in our region, this trial will fill an important knowledge gap. We will conduct this RCT to test the impact of two different doses of vitamin D replacement on clinically relevant maternal and neonatal outcomes in Middle Eastern women. The Lebanese and other Middle Eastern women in the reproductive age are ideally suited for such trials, in view of the fact that the median 25(OH)D levels in this age group is relatively low. These levels are reflective of the median low levels registered in most countries from the Middle East, as well as those from Northern Africa.[14] The doses used will allow us to directly address the applicability of the IOM in our region. The findings of this trial will help guide the public health policymaker regarding vitamin D supplementation in pregnant women and will allow a step forward in evidence-based recommendations specific to the Middle East. Multiple outcomes that have never been targeted in any previous trial in pregnancy will be assessed as secondary or exploratory outcomes; indeed, the results will guide future research projects in this field.

Findings from our trial, and similar to results derived from nutrient RCTs, are prone to the confounding effect of several factors.[52] Indeed, the baseline 25(OH)D level, the dietary intake of vitamin D and other nutrients, such as calcium and proteins, sun exposure and others, remain important predictors affecting the response to vitamin D supplementation, but are very difficult to quantify accurately.

Trial status

The study was launched on 27 July 2015.

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Contributors GEHF conceived the idea of the trial. MC, GEHF and AN designed and developed the study protocol. ZM provided advice on the sample size, study design and randomisation. CC and NH provided advice on the trial design, end points, conduct and operating procedures. AA and MN provided advice on the logistic planning of the study. All authors made significant contributions to the protocol development. They all reviewed the draft versions and approved the final version of this manuscript.

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Competing interests Professor Cyrus Cooper reports having received consultancy and honoraria from Alliance for Better Bone Health, Amgen, Eli Lilly, GSK, Medtronic, Merck, Novartis, Pfizer, Roche, Servier, Takeda and UCB, outside the submitted work. All other authors report no conflicts of interest.

Ethics approval This protocol has been approved by the Institutional review Board (IRB) at AUB-MC and RHUH and by the medical committee at Bahman Hospital (Protocol number IM.GEHF 22).

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