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

M Chakhtoura,1 A Nassar,2 A Arabi,1 C Cooper,3 N Harvey,3 Z Mahfoud,4 M Nabulsi,5 G El-Hajj Fuleihan1

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 χ². 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 calcitropic 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
Association between maternal vitamin D status 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, and also later on during childhood, leading to reduced muscle and bone mass in offspring at 4 and 9 years.

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. 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. Furthermore, maternal vitamin D may influence the fetal muscle motor unit size, and consequently muscle mass and strength after birth.

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.

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. This recommendation was based on observational studies, none of which were conducted in the Middle East.

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). The American College of Obstetricians and Gynecologists (ACOG) does not recommend screening for vitamin D level in pregnancy, and abides by the IOM recommendations.

Moreover, the WHO 2012 guidelines on vitamin D replacement during pregnancy did not recommend vitamin D supplementation as part of prenatal care. 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.

In the UK, however, pregnant women are considered at risk of vitamin D deficiency, and supplementation with 400 IU daily is required.

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 sup-
plementation 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.” 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 USA35
and the UK.36 Hollis et al35 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 al36 showed that
vitamin D supplementation of 1000 IU daily, compared
to placebo, in pregnant women in the UK allowed a sig-
nificant 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 sig-
nificant 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 al36
and Sablok et al37 showed that vitamin D supplementation
decreased the risk of preterm labour, gestational dia-
betes 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 Soheilikhah et al40
who assessed the effect of vitamin D supplementation on insulin resistance, the primary out-
comes in these studies were mostly maternal and neo-
natal 25(OH)D levels (see online supplementary
appendix 1). None of the other clinically important out-
comes, 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 regis-
tration on clinicaltrial.gov42 (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 tri-
quarter 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 mater-
nal vitamin D level and neonatal musculoskeletal para-
eters, 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 anthropomet-
ric 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 supplemen-
tary 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 random allocation list by the pharmacist. The pharmacist will not be 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).

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 participates in the quality assurance, evaluation and accreditation by the College of American Pathologists and is a participant in the Vitamin D External Quality Assurance Surveillance (DEQAS) programme.

Eligibility criteria
Inclusion criteria:
▸ Pregnant women gestational age (GA) <14 weeks at screening visit.
▸ 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).
▸ 25(OH)D level between 25 and 75 nmol/L.
▸ Age >18 years.
▸ Vitamin D supplementation ≤200 IU daily.

Exclusion criteria:

1In 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.
2If 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.
▸ 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

▸ 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

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

*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.

Chakhtoura M, et al. BMJ Open 2016;6:e010818. doi:10.1136/bmjopen-2015-010818
| Trial event and outcomes measures                                      | 11–13 weeks (screening visit) | 15–18 weeks (visit 1) | 20 weeks | 24–28 weeks (visit 2) | 28–32 weeks | Delivery (visit 3) | 37–42 weeks | 1 month post partum (visit 4) |
|------------------------------------------------------------------------|-------------------------------|----------------------|----------|-----------------------|-------------|--------------------|-------------|--------------------------|
| Maternal height                                                        | ✓                             |                      |          |                       |             |                    |             |                          |
| Maternal weight, blood pressure                                       | ✓                             | ✓                    |          | ✓                     | ✓           | ✓                  |             |                          |
| Maternal health and diet assessment                                    | ✓                             |                      |          |                       |             |                    |             |                          |
| Maternal 25(OH)D, Crea, Ca                                             | x                             |                      |          |                       |             |                    |             |                          |
| Maternal cell count, ph, mg, alb, TSH, 1,25(OH)₂D                      | x                             |                      |          |                       |             |                    |             |                          |
| PTH                                                                    | x                             |                      |          |                       |             |                    |             |                          |
| Maternal glucose (1 h)                                                 | x                             |                      |          | ✓                     | ✓           |                    |             |                          |
| Maternal 24 h (or spot) urine collection for calcium and creatinine    | x                             |                      |          |                       |             |                    |             |                          |
| Maternal vitamin D binding protein                                     | x                             |                      |          |                       |             |                    |             |                          |
| Maternal genetic pathways of vitamin D metabolism                      | x                             |                      |          |                       |             |                    |             |                          |
| 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                                                  |                               |                      |          |                       |             |                    |             | x                        |
| 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, V13.5.3.1, 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 \( \chi^2 \) 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 \( \geq 50 \text{ nmol/L} \): binary outcome; the percent of women achieving 25(OH)D \( \geq 50 \text{ nmol/L} \) in the low dose will be compared to those in the high dose using \( \chi^2 \), 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 \( \chi^2 \) 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 \( \chi^2 \), 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 \( \leq 0.025 \).

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 \( \geq 50 \text{ nmol/L} \) and the Endocrine Society targets a level of \( \geq 75 \text{ nmol/L} \). Subgroup analysis based on a 25(OH)D level at study entry (\( \leq 50 \text{ nmol/L} \) vs \( \geq 50 \text{ nmol/L} \)) 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. 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. 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.

REFERENCES

1. Kovacs CS, Fuleihan Gel-H. Calcium and bone disorders during pregnancy and lactation. Endocrinol Metab Clin North Am 2006;35:21–51.
2. Kovacs CS. Calcium and bone metabolism disorders during pregnancy and lactation. Endocrinol Metab Clin North Am 2011;40:795–826.
3. Parkes I, Schenker JG, Shufaro Y. Parathyroid and calcium metabolism disorders during pregnancy. Gynecol Endocrinol 2013;29:515–19.
4. Bouillon R, Van Assche FA, Van Baelen H, et al. Influence of the vitamin D-binding protein on the serum concentration of 1, 25-dihydroxyvitamin D: significance of the free 1, 25-dihydroxyvitamin D3 concentration. J Clin Invest 1981;67:589.
5. Van Doel HJ, de Sévaux RG, Van Baelen H, et al. Relationship between free and total 1, 25-dihydroxyvitamin D in conditions of modified binding. Eur J Endocrinol 2001;144:391–6.
6. Zerenweh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr 2008;87:1087S–91S.
7. Kovacs CS. Maternal vitamin D deficiency: fetal and neonatal implications. Semin Fetal Neonatal Med 2013;18:129–35.
8. Arabi A, El Rasii R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nat Rev Endocrinol 2010;6:500–8.
9. Nassar N, Halligan GH, Roberts CL, et al. Systematic review of first-trimester vitamin D normative levels and outcomes of pregnancy. Am J Obstet Gynecol 2011;205:208.e1–7.
10. Sahu M, Bhatia V, Aggarwal A, et al. Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. Clin Endocrinol 2009;70:680–4.
11. Molla AM, Al Badawi M, Hammoud MS, et al. Vitamin D status of mothers and their neonates in Kuwait. Pediatr Int 2005;47:469–52.
12. Hossain N, Khanani R, Hussain-Kanani F, et al. High prevalence of vitamin D hypovitaminosis in Pakistani mothers and their newborns. Int J Gynaecol Obstet 2011;112:229–33.
13. Ergüç AT, Berberoğlu M, Atasay B, et al. Vitamin D deficiency in Turkish mothers and their neonates and in women of reproductive age. J Clin Res Pediatr Endocrinol 2009;1:256.
14. Bialik D, Raïsane M, Hoteit M, et al. Hypovitaminosis D in the Middle East and North Africa: prevalence, risk factors and impact on outcomes. Dermatoendocrinol 2013;5:274.
15. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol 2010;121:297–300.
16. Van Der Meer IM, Middelkoop BJ, Boeke AJ, et al. Prevalence of vitamin D deficiency among Turkish, Moroccan, Indian and sub-Saharan African populations in Europe and their countries of origin: an overview. Catecholamines 2011;12:1009–21.
17. Van Der Meer IM, Koovangam NS, Boeke AJ, et al. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. Am J Clin Nutr 2006;84:350–3.
18. Hollis BW, Wagner CL. Vitamin D and pregnancy: skeletal effects, nonskeletal effects, and birth outcomes. Calcif Tissue Int 2013;92:126–39.
19. Aghajari F, Nagulesapillai T, Ronksley PE, et al. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis. JAMA 2013;310:2340–7.
20. Poel YH, van der Meer IM, Lips P, et al. Vitamin D and gestational diabetes: a systematic review and meta-analysis. Eur J Intern Med 2012;23:465–9.
21. Wei SQ, Qi HP, Luo ZC, et al. Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2013;98:3165–73.
22. Tabesh M, Salehi-Abargouei A, Tabesh M, et al. Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis. J Clin Endocrinol Metab 2013;98:3165–73.
23. Thorne-Lyman A, Fawzi WW. Vitamin D during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. Paediatr Perinat Epidemiol 2012;26(Suppl 1):75–90.
24. Javadi MK, Crozier SR, Harvey NC, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 2006;367:36–43.
25. Harvey NC, Moon RJ, Sayer AA, et al. Southampton Women’s Survey Group. Maternal antenatal vitamin D status and offspring muscle development: findings from the Southampton Women’s Survey. J Clin Endocrinol Metab 2013;98:330–7.
26. Martin R, Harvey NC, Crozier SR, et al. Placental calcium transporter (PMCA3) gene expression predicts intratropical bone mineral accrual. Bone 2007;40:1203–8.
27. Cleal JK, Day PE, Sinner CL, et al. Placental amino acid transport may be regulated by maternal vitamin D and vitamin D-binding protein: results from the Southampton Women’s Survey. Br J Nutr 2015;113:1903–10.
28. Javadi MK, Lekamwasam S, Clark J, et al. Infant growth influences proximal femoral geometry in adulthood. J Bone Miner Res 2006;21:508–12.
29. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. Public Health Nutr 2011;14:938–9.
30. 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;96:1911–30.
31. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 495: vitamin D: screening and supplementation during pregnancy. Obstet Gynecol 2011;118:197.
32. WHO Guideline. Vitamin D supplementation in pregnant women. 2012. http://apps.who.int/iris/bitstream/10665/85313/1/9789241504935_eng.pdf?ua=1 (accessed Nov 2015).
33. De-Regil LM, Palacios C, Ansary A, et al. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2012;CD008873.
34. NICE Guidelines. 2008. http://www.nice.org.uk/guidance/PH11 (accessed Nov 2015).
35. Hollis BW, Johnson D, Hulse TC, et al. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res 2011;26:2341–57.
36. Cooper C, Harvey N, Bishop N, et al. MAVIDOS Study Group. Maternal Gestational Vitamin D Supplementation and Offspring Bone Mass: a Multicentre Randomised, Double-Blind, Placebo-Controlled Trial (MAVIDOS). Abstract Presented at the American Society for Bone and Mineral Research (ASBMR) Annual Meeting; 9–12 October 2015; Seattle; 2015.
37. Sablok A, Batra A, Thariani K, et al. Supplementation of vitamin D in pregnancy and its correlation with feto-maternal outcome. J Clin Diagn Res 2013;7:2389–92.
38. Dawodu A, Saadi HF, Bekdache G, et al. Randomized controlled trial (RCT) of vitamin D supplementation in pregnancy in a population with endemic vitamin D deficiency. J Clin Endocrinol Metab 2013;98:2337–46.
39. Sabet Z, Ghazi A, Tohidi M, et al. Vitamin D supplementation in pregnant Iranian women: effects on maternal and neonatal vitamin D and parathyroid hormone status. Acta Endocrinol 2012;8:59–66.
40. Soheilykhai S, Mojibad M, Moghadam MJ, et al. The effect of different doses of vitamin D supplementation on insulin resistance during pregnancy. Gynecol Endocrinol 2013;29:396–9.
41. Shakiba M, Irannamesh MR. Vitamin D requirement in pregnancy to prevent deficiency in neonates: a randomised trial. Singapore Med J 2012;54:285–8.
42. ClinicalTrials.Gov. https://clinicaltrials.gov/ (accessed Nov 2015).
43. College of American Pathologists. http://www.cap.org (accessed Nov 2015).
44. DEQAS. http://www.deqas.org (accessed Nov 2015).
45. The World Bank definition of Middle East countries. http://data.worldbank.org/about/country-and-lending-groups (accessed Nov 2015).
46. Skinner AM, Cieslak Z, MacWilliam L, et al. The measurement of knee-heel length in newborn infants using a simple vernier calipers. Lancet 2006;367:36–43.
47. Bender R, Lange S. Adjusting for multiple testing—when and how? J Clin Epidemiol 2001;54:343–9.
48. Online sample size calculation. http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html (accessed Nov 2015).
49. Heaney RP, Davies KM, Chen TC, et al. Hypovitaminosis D in the Middle East population with endemic vitamin D deficiency. J Bone Miner Res 2009;1:266.
50. Cooper C, Harvey N, Bishop N, et al. MAVIDOS Study Group. Maternal Gestational Vitamin D Supplementation and Offspring Bone Mass: a Multicentre Randomised, Double-Blind, Placebo-Controlled Trial (MAVIDOS). Abstract Presented at the American Society for Bone and Mineral Research (ASBMR) Annual Meeting; 9–12 October 2015; Seattle; 2015.
51. Bender R, Lange S. Adjusting for multiple testing—when and how? J Clin Epidemiol 2001;54:343–9.
52. Online sample size calculation. http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html (accessed Nov 2015).
53. Skinner AM, Cieslak Z, MacWilliam L, et al. The measurement of knee-heel length in newborn infants using a simple vernier calipers. Lancet 2006;367:36–43.