J Bone Miner Metab (2017) 35:464–471
DOI 10.1007/s00774-016-0777-4

ORIGINAL ARTICLE

Maternal vitamin D supplementation in pregnancy and offspring outcomes: a double-blind randomized placebo-controlled trial

Saroj Kumar Sahoo[1] · Kishore Kumar Katam[1] · Vinita Das[2] · Anjoo Agarwal[2] · Vijayalakshmi Bhatia[1,3]

Received: 3 June 2016 / Accepted: 8 August 2016 / Published online: 14 September 2016

Abstract We studied bone mineral content (BMC), bone mineral density (BMD), and body composition in offspring of women supplemented with vitamin D during pregnancy. Pregnant women were randomized to receive oral cholecalciferol 60,000 units 4 weekly (group 1), 8 weekly (group 2), or placebo (group 3). All received 1 g calcium daily (groups 1 and 2 without, and group 3 with 400 units vitamin D). Offspring at 12–16 months underwent dual-energy X-ray absorptiometry. Maternal hypovitaminosis D at recruitment was common (serum 25OHD <50 nmol/L in 88 %) and severe (25OHD <25 nmol/L in 46 %). Groups 1 and 2 ($n = 23$ and 13, median age 14 months) had higher cord blood 25OHD (47.8 ± 13.8 and 31.0 ± 14.0 nmol/L) versus group 3 ($n = 16$, median age 16 months, 17.8 ± 13.5 nmol/L, $p < 0.001$). Babies in group 3 had higher whole-body BMC (250.8 ± 42.5 gm) and BMD (0.335 ± 0.033 gm/cm^2) compared to group 1 (213.1 ± 46.2 gm and 0.295 ± 0.041 gm/cm^2) and group 2 (202.9 ± 29.9 gm and 0.287 ± 0.023 gm/cm^2) ($p = 0.006$ and 0.001, respectively). In multivariate analysis, age, weight z score, and lean body mass remained significant contributors to BMC. Parameters of body composition were comparable among the groups. Vitamin D supplementation to pregnant women with severe deficiency in doses that improved cord blood 25OHD did not result in improved bone health or body composition in offspring at 12–16 months, compared to a dose too small to improve 25OHD levels.

Keywords Maternal vitamin D supplementation · Offspring · Bone health · Body composition · Infections

Introduction

Maternal vitamin D (25OHD) status during pregnancy may have immediate, as well as long-term implications for both skeletal and extra-skeletal health of the offspring. Poor musculoskeletal growth in fetal life may have an adverse effect on adult bone mass and body composition [1]. However, though initial studies suggested that low maternal 25OHD during pregnancy may affect offspring bone mass adversely at birth [2], 9 years [3], and 20 years of age [4], other studies showed a null association at birth [5, 6], 14 months [7], and 9 years [8], or an inverse association [9]. There was no association between maternal vitamin D status and offspring fracture, the final outcome of a low bone mass [10]. In all three interventional studies to date, maternal vitamin D supplementation did not result in a better bone mass in the offspring. The first study was not randomized and used a less accurate technique, single photon absorptiometry, to assess the bone mineral content (BMC) [11]. In the second study [12], the dose of vitamin D supplemented (200 IU/d), was much below the recommended dietary allowance [13], and the supplementation

The study protocol can be found at http://www.icmr.nic.in/ and http://ctri.nic.in/Clinicaltrials/login.php.

Electronic supplementary material The online version of this article (doi:10.1007/s00774-016-0777-4) contains supplementary material, which is available to authorized users.

✉ Vijayalakshmi Bhatia
bhatiaviji@gmail.com

[1] Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

[2] King George's Medical University, Lucknow, India

[3] Department of Endocrinology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

was limited to the last trimester, whereas vitamin D and calcium transfer to the fetus is known to be active in the middle trimester itself. In the third study, the MAVIDOS trial, there was no beneficial effect of maternal vitamin D supplementation (at a dose of 1000 IU/d) on neonatal BMC [14]. However, the findings from this study could not be generalized as the study population did not include non-white populations or subjects with severe vitamin D deficiency (serum 25OHD <25 nmol/L).

Conflicting results have also been described regarding association of maternal 25OHD and offspring body composition [9, 15–17] or extra-skeletal diseases such as atopic dermatitis, wheeze, and respiratory infections [17–21]. In the lone interventional study evaluating the latter outcomes, vitamin D supplementation in late pregnancy did not reduce the incidence of wheeze, respiratory infections and eczema in the offspring at the age of 3 years [22].

There are no controlled studies that evaluated childhood bone mass and body composition after supplementing pregnant women of non-European ethnic groups, especially with severe vitamin D deficiency. We report whole-body BMC, bone mineral density (BMD), and body composition in 12–16-month-old offspring of Indian women supplemented with vitamin D during their second and third trimesters of pregnancy. Results in groups who received doses that improved maternal term and cord blood 25OHD were compared with that in a group who received 400 IU daily, a dose commonly used in India, which is smaller than current international standard-of-care doses. We also studied the prevalence of atopic dermatitis, wheeze, and infections in these subjects.

Materials and methods

Study design and subjects

A double-blind, randomized, placebo-controlled, multi-arm parallel study of vitamin D supplementation in pregnancy was conducted at King George's Medical University, Lucknow, India (clinical trial registry CTRI/2012/02/002395). We had the opportunity to study prospectively the children born to the participants of the above supplementation study. The evaluation of offspring, conduct of laboratory assays and DXA scans were done at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. These are two government run tertiary care centers which provide services to people of all socioeconomic strata.

Pregnant women were eligible for the study if they were more than 18 years of age, had a singleton pregnancy, gestational age less than 20 weeks, and gave consent to participate in the study. Those with known metabolic bone disease, complicated pregnancy such as renal disease, liver disease, and those using medication for medical conditions such as tuberculosis or epilepsy were excluded from the study, as were those had received vitamin D supplementation in the previous 3 months. Among the 300 women who met inclusion and exclusion criteria, 160 delivered at our institution. These 160 mothers were invited to participate in the current offspring study, 1 year after delivery. Of the 160, only 150 were contactable. Fifty-two of the 150 gave consent and participated in the current study (Fig. 1). The study was approved by the institutional ethics committee and written informed consent was obtained from all participants.

Randomization and intervention

Pregnant women ($n = 300$) in their early second trimester (14–20 weeks) were randomized into three groups in 1:1:1 ratio to receive, under observation, oral cholecalciferol 60,000 units (in sachets) every 4 weeks (group 1) or 8 weeks (group 2) or placebo sachets (group 3) until delivery. All had been provided 1 g elemental calcium daily (groups 1 and 2 without added vitamin D and group 3 with 400 units vitamin D). Calcium tablets were provided for a month at a time. The participants were asked to bring back the empty blisters to check the compliance. Randomization was done by a computer-generated sequence in randomly permuted blocks of hundred (by VD, AA). All the medications were dispensed in sequentially numbered, identical, opaque, sealed packs (carrying the name of the participant) by a research assistant, who was blinded to intervention. The allocation sequence was also concealed from all participants; the researcher enrolling and assessing mothers (KKK), the researcher assessing offspring and DXA scan images (SKS), and performing data analysis (VB). All the women received standard antenatal care.

Maternal evaluation

Maternal history of smoking and alcohol intake during pregnancy was obtained. Parent's socioeconomic status was assessed using modified Kuppuswamy's Socioeconomic Status Scale [23, 24]. Maternal height and weight at recruitment, along with child's birth-weight and birth-length were noted from the records. Maternal serum at recruitment and at term and cord blood were collected for assay of 25OHD, which was measured by radio-immunoassay (Diasorin, Stillwater, USA). This assay measured all forms of vitamin D2 and D3. The analytical sensitivity of the assay was 3.75 nmol/L. The intra-assay and inter-assay coefficient of variation was 8.6–12.5 % and 8.2–11 % at different concentrations of 25OHD.

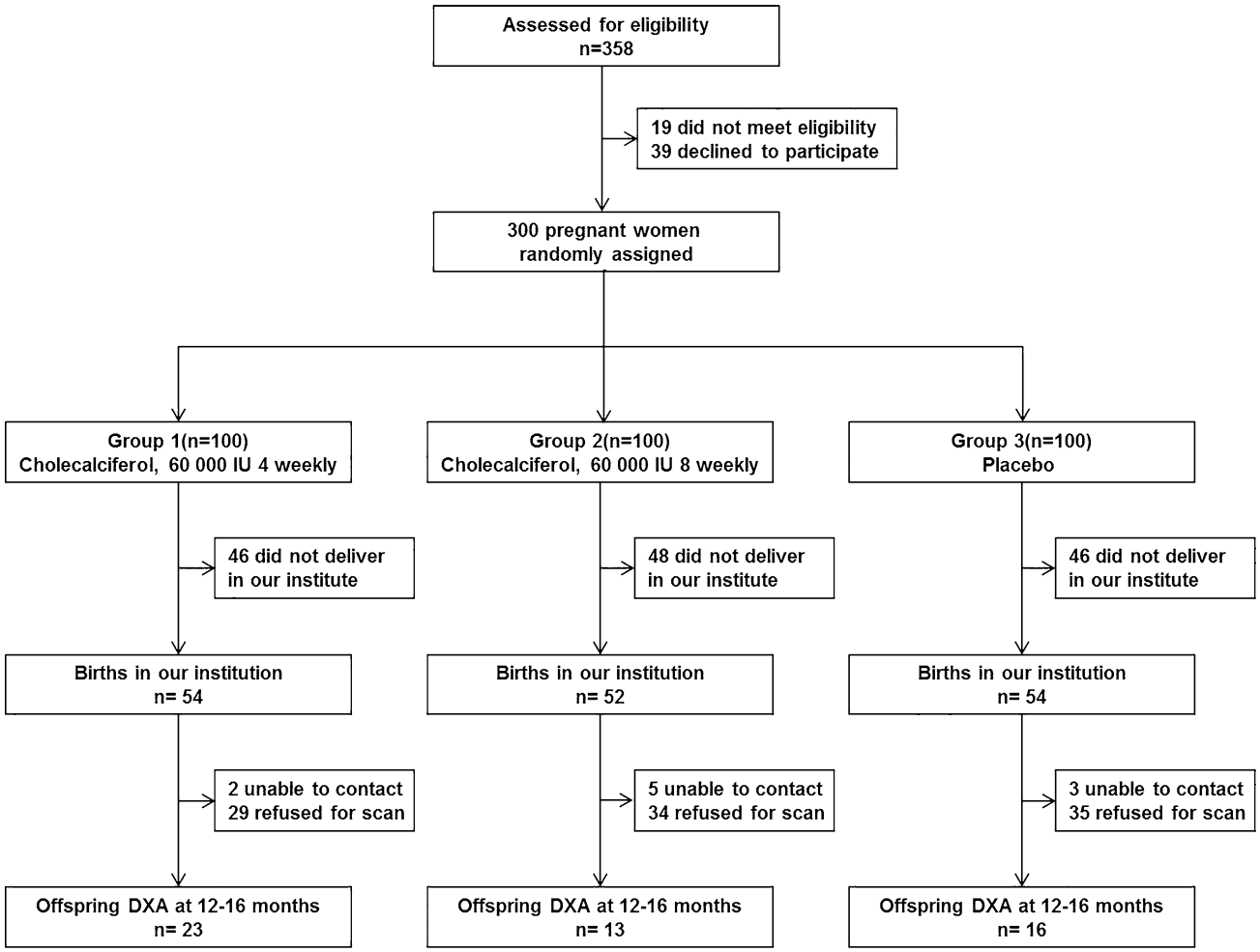

Fig. 1 Flow-chart of participation in the study

Offspring evaluation

Offspring between the ages of 12 and 16 months underwent dual-energy X-ray absorptiometry (DXA) scan using the infant whole-body software (Hologic Inc., Bedford, MA, USA). The infants were dressed with light hospital clothing to enhance scan quality. To minimize movement of the child during DXA scan, oral sedation using triclofos sodium was used and scans were performed only when the child was asleep. All DXA images were examined for movement artifacts and no image had a movement artifact. The mean coefficient of variation of the instrument was 0.296 %.

Whole-body BMC, BMD, and parameters of body composition (lean mass, fat mass, and percentage fat mass) were assessed by DXA. Anthropometric parameters of the child i.e., length, weight, mid upper-arm circumference, and head circumference were measured using standard procedure. Triceps and subscapularis skin-fold thickness was measured using a Holtain calliper (Crosswell, Crymych, UK). An average of three readings was taken at each site. *Z* scores were derived for the anthropometric parameters and skin-fold thickness using the WHO calculator (WHO Anthro, version 3.2.2). Dentition was assessed for dental age, enamel hypoplasia, and dental caries. Delayed tooth eruption was defined if emergence of a tooth occurred more than two SDs from the mean of established norms for eruption times [25]. The child was examined for evidence of visible atopic eczema. Parents were also asked whether the child had dry or itchy skin since birth. Modified UK Working Party's diagnostic criteria were used to make a diagnosis of atopic dermatitis [26]. Any history of wheeze, need for nebulization and their frequency any time since birth were noted. A history of diarrhoeal illness (lasting for more than 2 days), respiratory infection (either upper or lower) and their frequency since birth were noted from parents and past medical records. A mean of three systolic and diastolic blood pressures measured while the child was quiet, was recorded. The child was examined for any evidence of rachitic deformity and vitamin deficiency. Feeding methods

since birth were obtained and daily dietary calcium and vitamin D intake of the baby was calculated from 3-day food record.

Statistical analysis

The primary outcome was the whole-body BMC while whole-body BMD, body composition, and infection rates were the secondary outcomes. Statistical analyses were performed using SPSS software version 18.0. All data were expressed as mean ± SD unless mentioned otherwise. To compare the means between the groups, ANOVA and Kruskal–Wallis tests were used for parametric and nonparametric data respectively. Chi square test was used to assess differences in the proportions. Spearman correlation was used to establish the correlation between different parameters. To predict the contribution of each covariate to BMC and BMD, a general linear model (GLM) was used. Covariates that had a p value <0.1 in the univariate model were considered for final analysis in GLM. A p value <0.05 was considered as significant.

Results

Mothers of offspring who took part in the current study ($n = 52$) had similar characteristics compared to those who did not ($n = 108$), with respect to age, gravidity, gestational age, weight, and height at recruitment, socioeconomic status, average number of sachets consumed in each group and mean serum 25OHD level achieved at term in each group (Supplementary Table 1).

The baseline characteristics of the current mother-offspring cohort ($n = 52$ pairs) is shown in Table 1. The maternal age, gravidity, weight, height, smoking habit, and socioeconomic class were not different among the groups. The median maternal serum 25OHD, as well as prevalence of vitamin D deficiency (serum 25OHD <50 nmol/L) at recruitment were comparable among the groups. At term, there was a substantial improvement in serum 25OHD levels in group 1 and 2, but not in group 3. The birth-length and birth-weight in the offspring were comparable among the groups. The offspring in group 3 had higher median

Table 1 Baseline characteristics of mothers and children in the study cohort

	Group 1 ($n = 23$)	Group 2 ($n = 13$)	Group 3 ($n = 16$)	p
Maternal characteristics				
Age (years) (median and IQR)	25 (22–28)	25 (23–28)	25 (23–30)	0.83
Height (cm)	152 ± 6	152 ± 4	152 ± 5	0.96
Weight (kg) (median and IQR)	54 (48–66)	49 (47–56)	52 (48–55)	0.44
Number of cholecalciferol sachets consumed[a]	4.7 ± 1.5	2.6 ± 0.7	0 ± 0	<0.001
Smokers	0	0	0	
Gravidity (median and IQR)	2 (1–2)	1 (1–3)	2 (1–2)	0.80
Serum 25OHD at recruitment (nmol/L)	30.3 (16.5–41.0)	24.3 (11.5–34.0)	28.5 (16.0–54.5)	0.35
Serum 25OHD <50 nmol/L at recruitment (%)	91	92	77	0.22
Serum 25OHD at term (nmol/L)	59.8 ± 22.9	47.3 ± 15.3	24.5 ± 17.3	<0.001[b]
Serum 25OHD <50 nmol/L at term (%)	38	38	92	0.006[c]
Characteristics of offspring				
Age (months) (median and IQR)	14 (13–15)	14 (13–15)	16 (15–16)	0.001
Birth-weight (kg)	2.67 ± 0.45	2.78 ± 0.46	2.67 ± 0.43	0.73
Birth-length (cm)	47.2 ± 2.3	47.1 ± 1.9	47.2 ± 2.1	0.99
Cord blood 25OHD (nmol/L)	47.8 ± 13.8	31.0 ± 14.0	17.8 ± 13.5	<0.001[d]
Neonatal calcium (mmol/L)	2.2 ± 0.3	2.5 ± 0.1	2.3 ± 0.3	0.038[e]
Dietary calcium intake/d (mg)	845 ± 555	790 ± 501	803 ± 495	0.96

Data presented as mean ± SD unless mentioned otherwise. *IQR* inter-quartile range

Groups 1 and 2 mothers were supplemented with 60,000 units of cholecalciferol 4 weekly and 8 weekly, respectively, while group 3 mothers were supplemented with placebo sachets

[a] 1 sachet contained 60,000 units of cholecalciferol. Number of cholecalciferol sachets consumed during pregnancy varied among the same group because of difference in time of delivery

[b] $p < 0.001$ between groups 1 and 3, $p = 0.02$ between groups 2 and 3, and non-significant between groups 1 and 2 ($p = 0.23$)

[c] $p = 0.001$ between groups 1 and 3, $p = 0.002$ between groups 2 and 3, and non-significant between 1 and 2 ($p = 0.33$)

[d] $p < 0.001$ between groups 1 and 3, $p = 0.004$ between groups 1 and 2, and non-significant between group 2 and 3 ($p = 0.07$)

[e] $p = 0.036$ between groups 1 and 2, non-significant between group 1 and 3 ($p = 1.00$), group 2 and 3 ($p = 0.22$)

Table 2 Anthropometric parameters of offspring aged 12-16 months

Anthropometric parameters	Group 1 ($n = 23$)	Group 2 ($n = 13$)	Group 3 ($n = 16$)	p
Length z score	−0.54 ± 1.01	−0.92 ± 1.04	−0.64 ± 1.23	0.62
Weight z score	−0.85 ± 1.24	−1.06 ± 0.91	−0.78 ± 1.32	0.81
Head circumference (z score)	−0.99 ± 0.73	−0.97 ± 1.27	−1.16 ± 1.33	0.86
MUAC (z score)	0.14 ± 1.20	−0.21 ± 0.70	0.20 ± 1.1	0.55
Triceps SFT (z score)	−0.71 ± 1.31	−0.67 ± 0.71	−0.38 ± 0.99	0.63
Subscapularis SFT (z score)	−0.45 ± 1.52	−1.02 ± 1.24	−0.35 ± 1.16	0.37

Data presented as mean ± SD. *Z* scores obtained using WHO anthropometric calculator (WHO Anthro, version 3.2.2)

MUAC mid upper-arm circumference, *SFT* skin-fold thickness

Table 3 Parameters of bone health and body composition as measured by DXA in offspring aged 12–16 months

DXA Parameters	Group 1 ($n = 23$)	Group 2 ($n = 13$)	Group 3 ($n = 16$)	p
BMC (gm)	213.1 ± 46.2	202.9 ± 29.9	250.8 ± 42.5	0.006[a]
BMD (gm/cm^2)	0.295 ± 0.041	0.287 ± 0.023	0.335 ± 0.033	0.001[b]
Lean mass (gm)	6807.9 ± 744.1	6642.4 ± 712.4	7079.0 ± 969.0	0.34
Fat mass (gm)	2184.0 ± 1036.5	1920.7 ± 637.1	2194.3 ± 829.7	0.65
% fat	22.99 ± 8.00	21.69 ± 6.46	22.59 ± 6.44	0.87

Data presented as mean ± SD

[a] $p = 0.02$ between group 1 and group 3, $p = 0.01$ between group 2 and group 3

[b] $p = 0.003$ between group 1 and group 3, $p = 0.002$ between group 2 and group 3

Table 4 Multiple regression analysis by GLM for contribution of various parameters to BMC and BMD

Variable	BMC		BMD	
	F value	p	F value	p
Age	4.4	0.04	2.9	0.10
Vitamin D dose supplemented	0.6	0.46	1.7	0.21
Weight z score	9.9	0.003	2.4	0.13
Height z score	0.7	0.41	1.8	0.19
Lean body mass	5.8	0.02	1.6	0.21
Dietary calcium intake of baby	1.0	0.33	0.6	0.43

GLM general linear model, *BMC* bone mineral content, *BMD* bone mineral density

age in comparison to other two groups. All the children were immunized as per the national immunization program. All the infants were on exclusive breast feeding for the first 6 months of life. Breast feeding was continued in all the infants until the time of the study. After 6 months, all infants received animal (cow or buffalo) milk in addition. Further, a proportion of infants in groups 1 ($n = 7$), 2 ($n = 4$), and 3 ($n = 5$) also received formula feed ($p = 0.97$). Complementary semisolid feed was given to all infants after 6 months of age. The mean dietary vitamin D intake in group 1 (102 ± 49 IU), 2 (117 ± 71 IU), and 3 (82 ± 38 IU) was comparable ($p = 0.60$). Dietary calcium intake between the groups was comparable. None of the children were on calcium or vitamin D supplements since birth. There were no significant differences in various anthropometric parameters among the groups at age of 12–16 months (Table 2). None of the mothers or children had hypercalcemia or serum 25OHD level >250 nmol/L.

The offspring in group 3 had significantly higher whole-body BMC and BMD when compared to that of groups 1 or 2 (Table 3). Age, vitamin D supplemented during pregnancy, weight z score, height z score, lean body mass, and daily dietary calcium intake of the baby were the contributors to the BMC and BMD in the univariate model ($p < 0.1$), while pre-pregnancy weight, gravidity, gestational age at delivery, birth-weight, maternal 25OHD at term, cord blood 25OHD, neonatal calcium, socioeconomic status, and sex were not. In the multivariate general linear model, age, weight z score, and lean body mass remained significant contributors to BMC (Table 4). For the primary outcome (offspring BMC), the power of the study was 76 %, with an assumed common SD of 46 (which is the BMC SD of group 1) with two-sided alpha of 0.05 and a Cohen's effect size of 0.99 (eta squared) [an assumed common BMC SD of 40 gave 87 % power, while a SD of 43 gave 82 % power]. Various parameters of body composition (lean body mass, fat mass, percentage fat mass, and skin-fold thickness) were not different among the groups (Table 3).

BMC had a positive correlation with triceps ($r = 0.453$, $p = 0.01$) and subscapularis skin-fold thickness z score ($r = 0.299$, $p = 0.05$), as well as lean body mass ($r = 0.628$, $p = 0.01$) and fat mass ($r = 0.598$, $p = 0.01$). BMD correlated positively with lean body mass ($r = 0.342$, $p = 0.05$) and fat mass ($r = 0.325$, $p = 0.05$). Neither maternal term 25OHD nor cord blood 25OHD had any correlation with BMC, BMD, and anthropometric parameters.

There was no difference in the frequency of respiratory infections or diarrheal illness between different groups; the frequency of respiratory infections being 3, 5, 4 episodes in groups 1, 2, and 3, respectively ($p = 0.384$) and mean frequency of diarrheal illness being 2 episodes in each group. There was no association of respiratory or diarrhoeal infection with term 25OHD ($r = -0.153$, $p = 0.31$ and $r = -0.021$, $p = 0.89$, respectively) and cord blood 25OHD ($r = -0.057$, $p = 0.71$ and $r = -0.104$, $p = 0.49$, respectively). Only one offspring in group 3 had atopic dermatitis defined using modified UK Working Party's criteria. One child in each group required therapy for wheezes. In the three groups, the mean systolic and diastolic blood pressure were similar. Delayed tooth eruption was seen in 4, 2, and 1 offspring in groups 1, 2, and 3 respectively. None had enamel hypoplasia or dental caries. No child had biochemical or radiological rickets nor signs of vitamin deficiency.

Discussion

In this study of a non-white population, with severe vitamin D deficiency, we documented the effects of maternal vitamin D supplementation (in doses sufficient to improve mean maternal and cord blood 25OHD) on offspring bone health and body composition. Three important results were demonstrated. First, vitamin D supplementation in pregnancy in doses that achieved good maternal term and cord blood 25OHD did not result in better bone health in the offspring compared to that in a group receiving a lower dose that is commonly used in India. In fact, BMC and BMD were influenced only by age, and BMC was age-appropriate in all three groups [27]. The relatively higher BMC and BMD in the un-supplemented group can be ascribed to the higher age in that group and the fact that bone mineral accrual occurs at a rapid rate in infancy [27]. Secondly, maternal vitamin D supplementation did not lead to better body composition and anthropometric parameters in the offspring. Finally, there was no impact on occurrence and frequency of respiratory and diarrhoeal illness in children.

There are three interventional studies to date mentioning the effects of maternal vitamin D supplementation on offspring bone health, all of them showing a null effect. The first two studies suffered from the methodological limitations mentioned above [11, 12]. In the third study, the recently published MAVIDOS trial, the primary outcome (BMC at birth) was quite similar to our results [14]. However, pregnant women who had severe vitamin D deficiency (serum 25OHD <25 nmol/L) were excluded from this study, thus limiting the generalization of the study result. In contrast to the MAVIDOS trial, our study population included pregnant women who had severe vitamin D deficiency at recruitment. In addition, we also supplemented one group (group 1) with a relatively higher dose of vitamin D compared to that used in the MAVIDOS trial, which also failed to obtain a better bone health in the offspring. Finally, the results of these studies, including ours, corroborate the findings from an animal study, where fetal skeleton mineralization was independent of vitamin D [28].

There are a few observational studies mentioning conflicting results on the association of maternal 25OHD with offspring BMC [2–9]. The design of these studies may help reconcile the conflicting results. Firstly, they are observational studies; therefore, it is difficult to implicate a causal association. Secondly, the time period for measurement of 25OHD in mother and BMC in offspring varied from study to study and extended right up to offspring age of 20 years. Thus, confounding factors such as nutrition and lifestyle measures may hamper interpretation of results and a comparison with each other. Except the study by Lawlor et al. [8], all had the limitation of small sample size. In the largest of these studies, which included ~4000 mother–child pairs, Lawlor et al. did not find any association of maternal vitamin D with offspring BMC at 9 years of age.

Our results did not show any beneficial effect of vitamin D supplementation in pregnancy on offspring anthropometry and body composition at the age of 12–16 months, which is similar to the findings from MAVIDOS, where maternal vitamin D supplementation did not result in better body composition [14].

We could not find any effect of maternal vitamin D supplementation on occurrence and frequency of childhood respiratory and diarrhoeal infections, and recurrent wheeze, similar to the study findings of Goldring et al. [22]. There are a few studies mentioning variable results on the association of maternal vitamin D status and offspring wheeze, respiratory infections and/or diarrheal illness [17–21]. However, all except one are observational studies, so a causal association cannot be established. Additionally, they suffer from considerable heterogenicity in terms of study design, timing of maternal 25OHD measurement, outcome definition, and interval between measurement of 25OHD and outcomes. The results need to be confirmed from ongoing, large-scale randomized studies.

The major limitation of our study is the high dropout rate. However, the power of the study was calculated to be in excess of 76 % for the primary outcome (offspring BMC).

Second, the median age among the groups differed, with group 3 having a higher median age, thereby making them more likely to have a greater bone mass, pushing the outcome of the study towards the null hypothesis. Third, mothers in all three groups were provided 1 g calcium daily as a part of their obstetrics care. The results of our study may not be generalized to situations of very low calcium intake. Finally, our control group, which was given 400 IU vitamin D daily as standard-of-care at the time, was merely documented to have low maternal serum 25OHD at all-time points during pregnancy (unpublished data). We have made the assumption that a dose which did not improve maternal or cord blood 25OHD did not make a difference to the fetal outcomes. Therefore, our findings should be regarded as preliminary. Nevertheless, being a randomized supplementation study using a liberal dose of vitamin D, it contradicts the concept of vitamin D supplementation in pregnancy resulting in better bone health in the offspring, even in a population with extremely low baseline 25OHD. To conclude, maternal vitamin D supplementation in doses that improved maternal and cord blood 25OHD did not result in an improved bone health, body composition in the offspring at 12–16 months, neither did it protect from respiratory and diarrhoeal infections, compared to a dose too small to improve 25OHD levels.

Acknowledgments SKS was responsible for planning, conduct of the study, offspring recruitment and assessment, and manuscript writing. KKK was responsible recruitment of mothers and their assessment. VD and AA were responsible for conception and design of the study, while VB was responsible for conception, design and planning of the study, data analysis and drafting, revision and final approval of the manuscript. We acknowledge the grant support from Department of Biotechnology (BT/PR/13985/SPD/11/1297/2010) to V. Bhatia, intramural grant to S. K. Sahoo and V. Bhatia from SGPGIMS, Indian Council for Medical Research grant (manpower development scheme) to S. K. Sahoo. We thank Dr Joshua Richman, UAB School of Medicine, Birmingham, USA and Dr Ashish Awasthi, Department of Biostatistics, SGPGIMS, Lucknow for helpful discussions in statistical analysis. We extend our thanks to Arjun Singh, Pallavi Tiwari, Manoj Dubey and Rajesh Srivastava for their technical support in this project.

Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest.

Financial disclosure Nothing to declare.

References

1. Harvey N, Dennison E, Cooper C (2014) Osteoporosis: a life-course approach. J Bone Miner Res 29:1917–1925
2. Viljakainen HT, Saarnio E, Hytinantti T, Miettinen M, Surcel H, Makitie O, Andersson S, Laitinen K, Lamberg-Allardt C (2010) Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab 95:1749–1757
3. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, Arden NK, Godfrey KM, Cooper C (2006) Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 367:36–43
4. Zhu K, Whitehouse AJ, Hart PH, Kusel M, Mountain J, Lye S, Pennell C, Walsh JP (2014) Maternal vitamin D status during pregnancy and bone mass in offspring at 20 years of age: a prospective cohort study. J Bone Miner Res 29:1088–1095
5. Dror DK, King JC, Durand DJ, Fung EB, Allen LH (2012) Feto-maternal vitamin D status and infant whole-body bone mineral content in the first weeks of life. Eur J Clin Nutr 66:1016–1019
6. Ioannou C, Javaid MK, Mahon P, Yaqub MK, Harvey NC, Godfrey KM, Noble JA, Cooper C, Papageorghiou AT (2012) The effect of maternal vitamin D concentration on fetal bone. J Clin Endocrinol Metab 97:E2070–E2077
7. Viljakainen HT, Korhonen T, Hytinantti T, Laitinen EKA, Andersson S, Makitie O, Lamberg-Allardt C (2011) Maternal vitamin D status affects bone growth in early childhood—a prospective cohort study. Osteoporos Int 22:883–891
8. Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH (2013) Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. Lancet 381:2176–2183
9. Weiler H, Fitzpatrick-Wong S, Veitch R, Kovacs H, Schellenberg J, McCloy U, Yuen KC (2005) Vitamin D deficiency and whole-body and femur bone mass relative to weight in healthy newborns. CMAJ 172:757–761
10. Petersen SB, Olsen SF, Molgaard C, Granstrom C, Cohen A, Vestergaard P, Strom M (2014) Maternal vitamin D status and offspring bone fractures: prospective study over two decades in Aarhus city, Denmark. PLoS One 9:e114334
11. Congdon P, Horsman A, Kirby PA, Dibble J, Bashir T (1983) Mineral content of the forearms of babies born to Asian and white mothers. Br Med J (Clin Res Ed) 286:1233–1235
12. Diogenes MEL, Bezerra FF, Rezende EP, Donangelo CM (2015) Calcium plus vitamin D supplementation during the third trimester of pregnancy in adolescents accustomed to low calcium diets does not affect infant bone mass at early lactation in a randomized controlled trial. J Nutr 145:1515–1523
13. Committee to review dietary reference intakes for vitamin D and calcium, Institute of Medicine (2011) In: Ross AC, Taylor CL, Yaktine AL, Valle HBD (eds) Dietary reference intakes for calcium and vitamin D. The National Academies Press, USA
14. Cooper C, Harvey NC, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I, Fraser R, Gandhi SV, Carr A, D'Angelo S, Crozier SR, Moon RJ, Arden NK, Dennison EM, Godfrey KM, Inskip HM, Prentice A, Mughal MZ, Eastell R et al (2016) Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. Lancet Diabetes Endocrinol 4:393–402
15. Krishnaveni GV, Veena SR, Winder NR, Hill JC, Noonan K, Boucher BJ, Karat SC, Fall CHD (2011) Maternal vitamin D status during pregnancy and body composition and cardiovascular risk markers in Indian children: the Mysore Parthenon study. Am J Clin Nutr 93:628–635
16. Crozier SR, Harvey NC, Inskip HM, Godfrey KM, Cooper C, Robinson SM, the SWS study group (2012) Maternal vitamin D status in pregnancy is associated with adiposity in the offspring: findings from the Southampton Women's Survey. Am J Clin Nutr 96:57–63
17. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C, the Princess Anne Hospital study group (2008) Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 62:68–77
18. Morales E, Romieu I, Guerra S, Ballester F, Rebagliato M, Vioque J, Tardon A, Rodriguez Delhi C, Arranz L, Torrent M, Espada M, Basterrechea M, Sunyer J (2012) Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology 23:64–71

19. Pike KC, Inskip HM, Robinson S, Lucas JS, Cooper C, Harvey NC, Godfrey KM, Roberts G, Southampton Women's Survey study group (2012) Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes. Thorax 67:950–956
20. Baiz N, Dargent-Molina P, Wark JD, Souberbielle JC, Annesi-Maesano I, EDEN mother-child cohort study group (2014) Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. J Allergy Clin Immunol 133:147–153
21. de Jongh RT, Crozier SR, D'Angelo S, Pike KC, Roberts G, Lucas JSA, Inskip H, Godfrey KM, Cooper C, Harvey NC, Southampton Women's Survey study group (2014) Maternal 25-hydroxyvitamin D levels in relation to offspring respiratory symptoms and infections. Eur Respir J 43:1181–1183
22. Goldring ST, Griffiths CJ, Martineau AR, Robinson S, Yu C, Poulton S, Kirkby JC, Stocks J, Hooper R, Shaheen SO, Warner JO, Boyle RJ (2013) Prenatal vitamin D supplementation and child respiratory health: a randomised controlled trial. PLoS One 8:e66627
23. Kuppuswamy B (1981) Manual of socioeconomic status (urban), 1st edn. Manasayan, Delhi, pp 66–72
24. Indian Labour Journal (2012) Labour Bureau, Shimla. Government of India
25. Suri L, Gagari E, Vastardis H (2004) Delayed tooth eruption: pathogenesis, diagnosis, and treatment. A literature review. Am J Orthod Dentofacial Orthop 126:432–445
26. Williams HC, Burney PG, Pembroke AC, Hay RJ, atopic dermatitis diagnostic criteria working party (1994) The UK Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. Br J Dermatol 131:406–416
27. Gallo S, Vanstone CA, Weiler HA (2012) Normative data for bone mass in healthy term infants from birth to 1 year of age. J Osteoporos. doi: 10.1155/2012/672403
28. Kovacs CS, Woodland ML, Fudge NJ, Friel JK (2005) The vitamin D receptor is not required for fetal mineral homeostasis or for the regulation of placental calcium transfer in mice. Am J Physiol Endocrinol Metab 289:E133–E144