Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial

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Summary

Background Maternal vitamin D status has been associated with bone mass of offspring in many, but not all, observational studies. However, maternal vitamin D repletion during pregnancy has not yet been proven to improve offspring bone mass in a randomised controlled trial. We aimed to assess whether neonates born to mothers supplemented with vitamin D during pregnancy have greater whole-body bone mineral content (BMC) at birth than those of mothers who had not received supplementation.

Methods The Maternal Vitamin D Osteoporosis Study (MAVIDOS) was a multicentre, double-blind, randomised, placebo-controlled trial that recruited pregnant women from three study sites in the UK (Southampton, Oxford, and Sheffield). Eligible participants were older than 18 years, with a singleton pregnancy, gestation of less than 17 weeks, and a serum 25-hydroxyvitamin D (25[OH]D) concentration of 25–100 nmol/L at 10–17 weeks' gestation. Participants were randomly assigned (1:1), in randomly permuted blocks of ten, to either cholecalciferol 1000 IU/day or matched placebo, taken orally, from 14 weeks' gestation (or as soon as possible before 17 weeks' gestation if recruited later) until delivery. Participants and the research team were masked to treatment allocation. The primary outcome was neonatal whole-body BMC, assessed within 2 weeks of birth by dual-energy x-ray absorptiometry (DXA), analysed in all randomly assigned neonates who had a usable DXA scan. Safety outcomes were assessed in all randomly assigned participants. This trial is registered with the International Standard Randomised Controlled Trial registry, ISRCTN 82927713, and the European Clinical Trials Database, EudraCT 2007–001716–23.

Findings Between Oct 10, 2008, and Feb 11, 2014, we randomly assigned 569 pregnant women to placebo and 565 to cholecalciferol 1000 IU/day. 370 (65%) neonates in the placebo group and 367 (65%) neonates in the cholecalciferol group had a usable DXA scan and were analysed for the primary endpoint. Neonatal whole-body BMC of infants born to mothers assigned to cholecalciferol 1000 IU/day did not significantly differ from that of infants born to mothers assigned to placebo (61·6 g [95% CI 60·3–62·8] *vs* 60·5 g [59·3–61·7], respectively; p=0·21). We noted no significant differences in safety outcomes, apart from a greater proportion of women in the placebo group with severe post-partum haemorrhage than those in the cholecalciferol group (96 [17%] of 569 mothers in the placebo group *vs* 65 [12%] of 565 mothers in the cholecalciferol group; p=0·01). No adverse events were deemed to be treatment related.

Interpretation Supplementation of women with cholecalciferol 1000 IU/day during pregnancy did not lead to increased offspring whole-body BMC compared with placebo, but did show that 1000 IU of cholecalciferol daily is sufficient to ensure that most pregnant women are vitamin D replete, and it is safe. These findings support current approaches to vitamin D supplementation in pregnancy. Results of the ongoing MAVIDOS childhood follow-up study are awaited.

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Introduction

Osteoporosis is a devastating disease, and its high prevalence makes it eminently suitable for population-wide public health interventions aimed at optimising bone health.[1] There is increasing evidence that early growth, and factors acting in utero or during early infancy, can affect the trajectory of long-term skeletal accrual to peak bone mass.[1] In particular, maternal serum 25-hydroxyvitamin D (25[OH]D) concentrations in pregnancy have been associated with offspring bone morphology[2–5] and bone mass[6,7] up to young adulthood.[8] The main determinant of 25(OH)D concentrations in most populations is ultraviolet B (UVB) exposure to the skin, which varies markedly by season in temperate climes.[9] Seasonal differences in neonatal bone mineral content (BMC) have been reported,[10,11] with effects potentially modified by vitamin D supplementation,[11] and maternal UVB exposure during pregnancy has been

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Research in context

Evidence before this study

We did a systematic review of studies relating maternal vitamin 25-hydroxyvitamin D (25[OH]D) concentrations, UVB exposure, dietary vitamin D intake, or use of vitamin D supplements during pregnancy to maternal and offspring health outcomes.[1] Major electronic databases (including, but not limited to, PubMed, Embase, and Web of Science) were searched for articles published from the databases' inception until June, 2012. This search was complemented by interrogation of grey literature and manual searching of reference lists. Two independent reviewers undertook all assessments, and the review was done in accordance with PRISMA guidelines. We identified eight observational studies relating maternal gestational vitamin D status to offspring bone mass, all of which were assessed as having a medium to low risk of bias. Of these, five reported a significant positive relation between maternal vitamin D status and offspring bone outcomes, which included whole-body, lumbar, femoral, and tibial bone mineral content (BMC), and whole-body and lumbar spine bone mineral density (BMD). Of the remaining studies, no significant association was reported between maternal vitamin D status and offspring radial and whole-body BMC. Differences in study design did not permit meta-analysis. We identified one small intervention study, judged to be at high risk of bias, which found no difference in offspring forearm BMC (measured within 5 days of birth) between supplemented and unsupplemented mothers. We subsequently updated the search in August, 2014, identifying two further observational studies, both judged to have a low to medium risk of bias; one, using the Avon Longitudinal Study of Parents and Children cohort, found no association between maternal 25(OH)D concentrations in pregnancy and offspring bone mass at 9 years. By contrast, the second study, from the Western Australian Pregnancy Cohort (RAINE), documented positive associations between maternal gestational 25(OH)D concentrations and offspring bone mass at 20 years.

Added value of this study

We found no difference in the primary outcome (neonatal whole-body BMC) between offspring born to mothers assigned to a vitamin D supplement during pregnancy compared with mothers assigned to placebo. However, among the prespecified secondary analyses, we noted an interaction between treatment and season, with the suggestion of a benefit for offspring neonatal BMC with treatment for deliveries during winter months. Although biologically plausible, this intriguing finding clearly needs to be replicated in further studies before it can provide a basis for alterations to clinical care. This study thus complements previous observational data and is the first randomised trial to show a potential benefit of vitamin D supplementation during pregnancy for offspring bone mass.

Implications of all the available evidence

Vitamin D supplementation during pregnancy is already recommended in many countries, including the UK. Observational studies have provided conflicting evidence regarding associations between maternal 25(OH)D status and offspring intrauterine bone development. The MAVIDOS study, although negative for its primary outcome, has shown that 1000 IU of cholecalciferol daily is sufficient to ensure that most pregnant women are replete in 25(OH)D, and that such a strategy is safe.

positively associated with bone mass in childhood.[6,12] However, not all studies have shown a benefit of higher maternal 25(OH)D concentrations in pregnancy on childhood skeletal health.[13–15]

Whole-body BMC is the recommended measure of bone mass in children. Although dual-energy x-ray absorptiometry (DXA) is of limited clinical utility in neonates because of the absence of normative data,[16] infant DXA has been widely used in research studies[17–20] in which comparisons are done within the same study. Childhood BMC is inversely related to childhood fracture risk,[21] and although data spanning from conception to peak bone mass in a single cohort are yet to be obtained, the evidence base supports tracking of BMC over this time period,[22–25] and the magnitude of peak bone mass achieved is an important determinant of future fracture risk.[26]

The aim of the Maternal Vitamin D Osteoporosis Study (MAVIDOS) was therefore to test the hypothesis that neonates born to mothers supplemented with vitamin D during pregnancy would have greater whole-body BMC at birth than those of mothers who had not received supplementation.[27] Given the previously documented importance of season for both 25(OH)D concentrations and childhood bone mass, we further hypothesised that there would be an interaction between season of birth and treatment effect.

Methods

Study design and participants

A detailed description of the aims and methods of MAVIDOS has been previously published.[27] Briefly, MAVIDOS is a multicentre, double-blind, randomised, placebo-controlled trial of vitamin D supplementation in pregnancy in the UK. Pregnant women were recruited when attending early pregnancy ultrasound screening at three study sites (University Hospital Southampton National Health Service [NHS] Foundation Trust, Southampton, UK; Oxford University Hospitals NHS Trust, Oxford, UK; Sheffield Hospitals NHS Trust [University of Sheffield], Sheffield, UK). Women were eligible if they were older than 18 years, had a singleton pregnancy, had gestation of less than 17 weeks based on last menstrual period and ultrasound measurements, and were aiming to give birth at the local maternity hospital.

Women were excluded if they had known metabolic bone disease, renal stones, hyperparathyroidism, or hypercalciuria, had been diagnosed with cancer in the previous 10 years, were unable to give informed consent or comply with the protocol, were taking drugs known to interfere with fetal growth, had fetal anomalies on ultrasonography, or were taking more than 400 IU/day vitamin D supplementation. A screening blood sample was obtained and analysed at local NHS laboratories and only women with a serum 25-hydroxyvitamin D (25[OH]D) concentration of 25–100 nmol/L and serum calcium of less than 2·75 mmol/L were eligible to enrol in the study. All three laboratories (Southampton, Oxford, and Sheffield) were accredited by the Vitamin D External Quality Assessment Scheme (DEQAS).

The study was done in accordance with guidelines laid down in the Declaration of Helsinki and was approved by the Southampton and South West Hampshire Research Ethics Committee. The study protocol has been published previously.[27] Full approval from the UK Medicines and Healthcare Products Regulatory Agency was granted, and written, informed consent was obtained from all participants.

Randomisation and masking

Women were randomly assigned at 14 weeks' gestation (or as soon as possible before 17 weeks' gestation if recruited later) to either cholecalciferol 1000 IU/day or matched placebo (Merck KGaA, Darmstadt, Germany). Packs of study treatment were randomly assigned in a 1:1 ratio by Sharp Clinical Services (Crickhowell, UK; previously DHP-Bilcare) by a computer-generated sequence in randomly permuted blocks of ten, starting randomly midway through the block, and sequentially numbered, before delivery to the study sites, and then dispensed in order by each study pharmacist. Each pack contained sufficient capsules for the study duration and both the participant and research team were masked to treatment allocation throughout the study duration.

Procedures

Women assigned to cholecalciferol took 1000 IU/day, orally as a single capsule until delivery; women assigned to placebo also took matched capsules orally until delivery. All participants received standard antenatal care, and were able to continue self-administration of antenatal multivitamins containing up to 400 IU/day of vitamin D. Women were assessed in detail at 14 weeks' and 34 weeks' gestation to investigate diet (including calcium and vitamin D intake), smoking status, alcohol consumption, change in health status, physical activity, medication intake, supplement intake (all by interviewer-led questionnaires), and anthropometric variables.[27] Anthropometric measurements of the neonates were obtained within 14 days after delivery, and information about obstetric complications was extracted from maternity records.

Study blood samples were collected from the mother at 14 weeks' and 34 weeks' gestation, and stored at –80°C after processing. Measurement of plasma 25(OH)D (Liaison RIA automated platform, Diasorin, Stillwater, MN, USA), calcium, alkaline phosphatase, and albumin was undertaken centrally (MRC Human Nutrition Research, Cambridge, UK) in a single batch at the end of the study. Measurement of vitamin D binding protein is ongoing and the results will be published separately. Details of assay performance and quality control through participation in the DEQAS, National Institute of Standards and Technology, and UK National External Quality Assessment Service are presented elsewhere.[28,29]

All neonates underwent DXA assessment at whole-body and lumbar spine sites (Hologic Discovery, Hologic Inc, Bedford, MA, USA, or GE-Lunar iDXA, GE-Lunar, Madison, WI, USA, with neonatal software) within 2 weeks after birth. To maximise scan quality, the infant was undressed, clothed in a standard towel, fed, and pacified before the assessment. Each instrument underwent daily quality control, with cross-calibration between sites. The total radiation dose was estimated to be 0·04 mSv, equivalent to about 7 days' exposure to background radiation in the UK. All DXA images were reviewed for movement artefacts and quality by two operators (NCH and RJM), who were masked to treatment allocation.

For more on **DEQAS** see http://www.deqas.org/

Follow-up assessments of the children at 1, 2, 3, and 4 years are ongoing.

Outcomes

The primary outcome was whole-body BMC of the neonate, assessed within 2 weeks of birth by DXA. Although we had originally planned to use whole-body BMC adjusted for age, after further statistical review (before completion of the trial), in this randomised controlled trial setting, it was judged to be more appropriate to include offspring age in a sensitivity analysis, rather than as the primary outcome. Secondary outcomes included neonatal whole-body bone area, bone mineral density (BMD), size-corrected BMC, and body composition. To preserve statistical power, rather than do separate analyses (as planned in the original protocol) for participants who completed the protocol, complied with treatment, and had a rise in 25(OH)D, and for stratification by baseline 25(OH)D, we explored these potential effect modifiers via their incorporation as interaction terms in regression models.

Safety outcomes included the frequency of adverse events, such as infection, nausea and vomiting, diarrhoea, abdominal pain, headache, hypertension, and hypercalcaemia (≥2·75 mmol/L) in the mother at 34 weeks' gestation, as well as intrauterine growth restriction, preterm birth (<37 weeks' gestation), instrumental delivery, severe post-partum haemorrhage, stillbirth or neonatal death, and congenital abnormalities.

Statistical analysis

We estimated the required sample size using results from the Princess Anne Hospital Study,[6] in which a difference of 0·42 SD in whole-body BMC was reported between the infants of mothers who had been vitamin D deficient and those of mothers who had been vitamin D replete during pregnancy. Given this was a single observational study, we powered the trial conservatively, calculating that to detect 50% of this difference in whole-body BMC at birth between the neonates of mothers who were deficient in vitamin D versus those replete in pregnancy (0·21 SD or 3·5 g), at the 5% significance level with 90% power, would require recruitment of 477 neonates in each group.

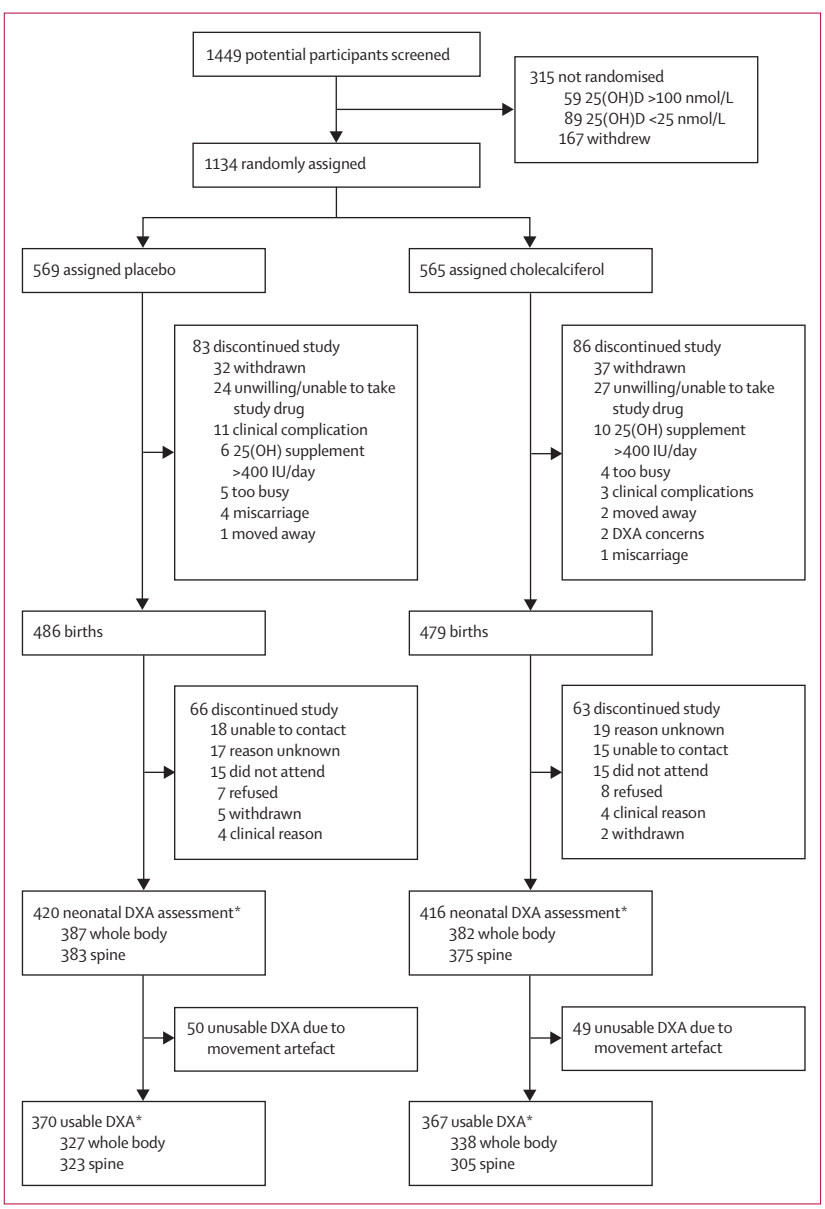


Figure 1: **Trial profile**
25(OH)D=25-hydroxyvitamin D. DXA=dual-energy x-ray absorptiometry. *Assessments could be done in both the whole body and spine within the same neonate.

For the primary analyses, we analysed all participants with a neonatal DXA assessment and a usable DXA scan (without movement artefact); although our original protocol, published before unblinding of the study,[27] stipulated that the analysis would be on an intention-to-treat basis, we subsequently revised the terminology used (before unblinding) because the analysis had to be based on available neonatal DXA assessments, a continuous outcome, and methods to account for missing data would not have been applicable in this setting. Safety outcomes were analysed in all randomly assigned participants.

At the request of the data monitoring committee, an interim safety analysis of serum calcium concentration was requested after 2 years of recruitment (April 23, 2010), but no analysis of DXA outcomes was undertaken until follow-up of all participants had been completed. This analysis showed that no participant had serum calcium concentrations above 2·75 mmol/L, permitting study progression.

We checked all data for normality by visual inspection of histograms. Missing data were assumed to be missing at random. We compared treatment groups using the Student's *t* test and Mann-Witney *U* test for normally and non-normally distributed outcomes, respectively. We compared categorical outcomes using the χ^2 test.

DXA indices included neonatal whole-body bone area, BMC, BMD, lean mass, and fat mass. To assess bone mass independent of body size, we used BMC adjusted for birth length in a regression model. Given the seasonal change in 25(OH)D concentrations reported in many previous studies, we hypothesised, a priori, that an interaction might be noted between treatment effect and season of birth. We defined season of birth using the UK Meteorological Office classification, as winter (December to February), spring (March to May), summer (June to August), and autumn (September to November), and also explored differences in treatment effect by individual month of birth. We also investigated prespecified interactions between treatment effect and offspring sex, and between treatment effect and ethnic origin, because both these factors have been associated with variations in vitamin D metabolism. Since the evidence of differences in body composition between first and subsequent offspring is clear,[1] and BMI has been shown to be inversely related to 25(OH)D concentration, we hypothesised that interactions might be apparent between each of these two variables and treatment. Finally, we reasoned that treatment might be more effective in mothers who fully complied with the protocol and were compliant with study treatment, who had low concentrations of 25(OH)D at baseline, or who had a greater change in 25(OH)D from 14 weeks to 34 weeks, and that for a combination of reasons, differences by study centre might be apparent, providing the basis for further interaction analyses. In summary, the interactions

tested were with study centre, maternal ethnic origin, parity, treatment compliance, protocol completion, baseline maternal BMI, baseline maternal 25(OH)D, change in 25(OH)D from 14 weeks to 34 weeks, offspring sex, and season of birth. All these interactions were explored in multivariable linear regression (with the independent variables—eg, treatment; season; treatment*season—and inclusion of no other covariates).

In further prespecified sensitivity analyses we adjusted bone outcomes for postnatal age at DXA. Given that the secondary analyses were prespecified and hypothesis-based, and that the study was powered for the primary outcome, correction for multiple testing was not judged to be appropriate, recognising that any statistically significant results from the secondary analyses would require further confirmation in future studies. With ten analyses and an α of 0·05, we calculated that the probability of observing one or more false positive associations was 40% (equal to $[1–0·95^{10}]\times100$). SD'A, SRC, and HMI undertook all analyses using Stata, version 13.1. A p value of less than 0·05 was accepted as statistically significant.

This trial is registered with the International Standard Randomised Controlled Trial registry, ISRCTN 82927713, and the European Clinical Trials Database, EudraCT 2007–001716–23.

Role of the funding source

The study was funded by Arthritis Research UK, UK Medical Research Council, UK National Institute for Health Research, and the Bupa Foundation. The original protocol incorporated suggestions from the Arthritis Research UK Clinical Trials Collaboration. The funders had no role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 6, 2008, and Feb 11, 2014, we recruited 1449 women who were initially eligible after screening and who consented to a blood test to determine early pregnancy 25(OH)D status. Of these, 148 were ineligible to participate because they had a 25(OH)D concentration of less than 25 nmol/L (n=89) or greater than 100 nmol/L (n=59). No participants had a plasma calcium concentration of greater than 2·75 mmol/L. A further 167 women withdrew before randomisation. Thus, 1134 pregnant women were randomised (figure 1), of whom 965 (85%) remained in the study until delivery. 836 (87%) of 965 neonates had a DXA scan. After excluding scans with substantial movement artefact, DXA scan data were available for 737 (76%) neonates, consisting of 665 assessments of the whole body and 628 at the lumbar spine (figure 1), meaning that numbers were somewhat lower than specified in the original power calculation. 317 (56%) women in the placebo group and 312 (55%) in the cholecalciferol group completed the full study protocol; 411 (72%) and 401 (71%) women, respectively, took 75% or more of the study medication.

Women in the cholecalciferol and placebo groups at randomisation were of similar age, and a similar proportion were nulliparous, had educational attainment to A level or higher, were current smokers, participated

	Placebo (N=569)	Cholecalciferol 1000 IU/day (N=565)
Age, years	30·5 (5·2)	30·5 (5·2)
White ethnic origin	497/527 (94%)	499/531 (94%)
Nulliparous	230/524 (44%)	232/532 (44%)
Current smoker	43/526 (8%)	44/533 (8%)
Educational attainment ≥A level	393/522 (75%)	414/531 (78%)
Walking speed at least fairly brisk	205/500 (41%)	193/505 (38%)
Strenuous exercise ≥once per week	70/499 (14%)	79/503 (16%)
Height, cm*	165·8 (6·6)	165·6 (6·4)
Weight, kg†	71·4 (63·3–81·8)	68·4 (60·9–79·5)
BMI, kg/m²‡	25·7 (23·0–30·0)	24·7 (22·3–28·6)
Sum of skinfold thicknesses, mm§	84·0 (27·8)	79·8 (27·9)
25(OH)D, nmol/L¶	45·9 (17·0)	46·7 (17·7)
25(OH)D >50 nmol/L	199/533 (37%)	218/535 (41%)

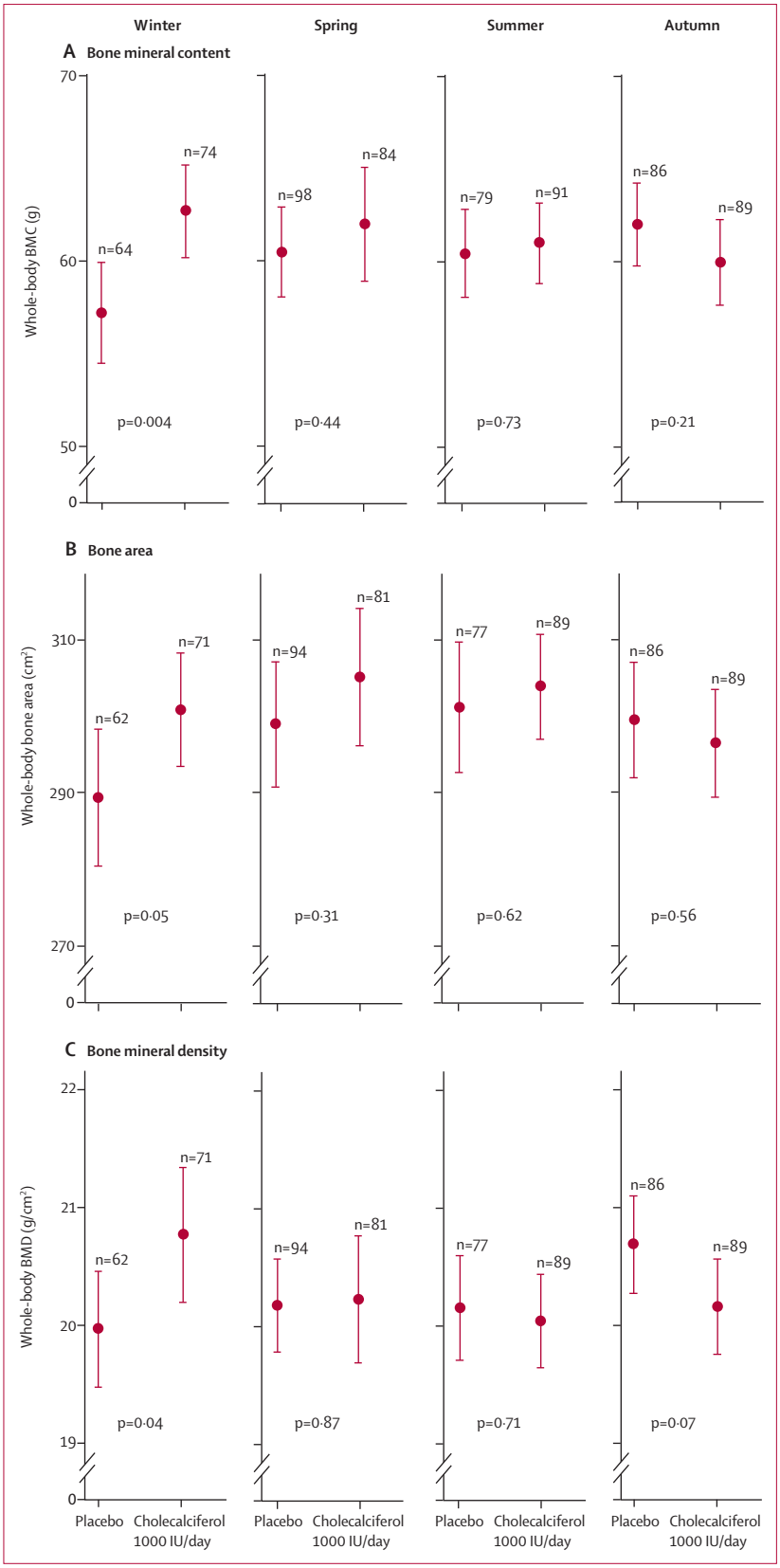
Data are mean (SD), number (%), or median (IQR). Percentages calculated accounting for missing observations. 25(OH)D=25-hydroxyvitamin D. *N=523 (placebo); N=533 (cholecalciferol). †N=528 (placebo); N=534 (cholecalciferol). ‡N=523 (placebo); N=533 (cholecalciferol). §N=472 (placebo); N=473 (cholecalciferol). ¶N=533 (placebo); N=535 (cholecalciferol).

Table 1: **Baseline characteristics in randomly assigned pregnant women**

	Placebo	Cholecalciferol 1000 IU/day	p value
Neonatal characteristics*			
N	486	479	
Male	251 (51%)	258 (54%)	0·49
Birthweight, g	3518 (3472–3564)	3481 (3432–3530)	0·28
Crown–heel length, cm	50·8 (50·6–51·0)	50·6 (50·4–50·8)	0·31
Head circumference, cm	35·5 (35·3–35·6)	35·4 (35·3–35·5)	0·62
Abdominal circumference, cm	32·7 (32·4–32·9)	32·9 (32·7–33·1)	0·16
Whole-body DXA results			
N	327	338	
Age at DXA, days	7 (6·1–7·4)	8 (6.8–8.4)	0·12
BMC, g	60·5 (59·3–61·7)	61·6 (60·3–62·8)	0·21
Bone area, cm²	297·8 (293·7–301·9)	301·6 (297·8–305·4)	0·18
BMD, g/cm²	0·203 (0·200–0·205)	0·203 (0·200–0·205)	0·96
Lean mass, g	3014 (2965–3062)	3055 (3008–3101)	0·23
Median fat mass, g (IQR)	374 (244–517)	355 (235–564)	0·97

Data are N, n (%), or mean (95% CI), unless otherwise stated. DXA=dual-energy x-ray absorptiometry. BMC=bone mineral content. BMD=bone mineral density. *Data obtained within 14 days of delivery.

Table 2: **Anthropometry and whole-body bone mineralisation and composition in neonates**

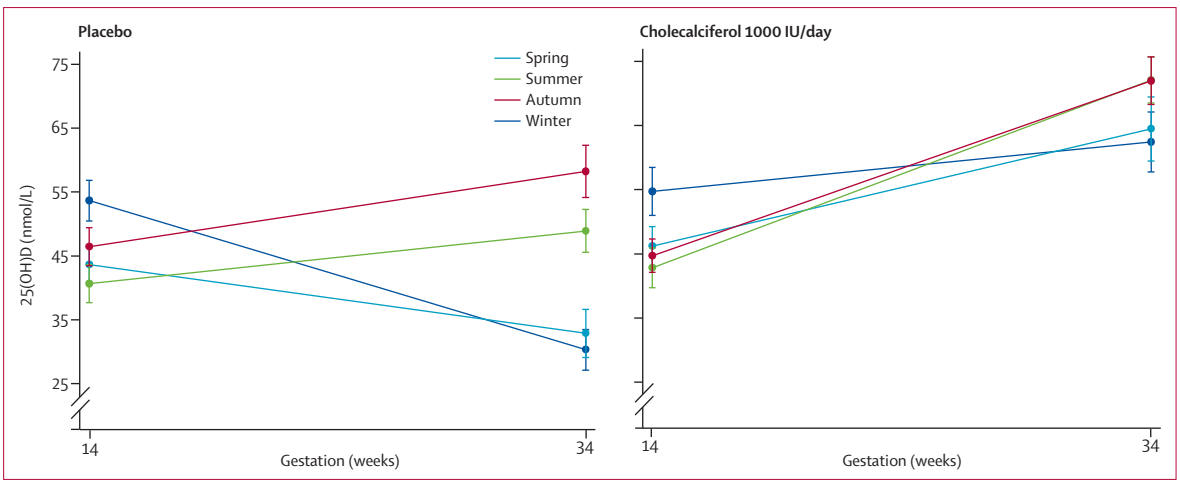


in strenuous exercise at least once per week, and were white (table 1). Height was also similar between the two groups, but median weight, median BMI, and mean sum of skinfold thicknesses were nominally greater in the placebo group than in the cholecalciferol group (table 1). Compared with those who withdrew, the women who remained in the trial until their baby was born were significantly older (mean 30·7 years [SD 5·2] in women who stayed in the study *vs* 28·9 years [5·1] in those who withdrew, p=0·0002) and more likely to be white (880 [95%] of 928 *vs* 116 [89%] of 130, p=0·01; denominators exclude women with no ethnic origin recorded). Women whose infants underwent DXA scanning tended to be older, less likely to smoke, and have lower skinfold thicknesses than mothers of infants who did not undergo DXA (appendix p 2).

Neonatal whole-body BMC of infants born to mothers assigned to cholecalciferol 1000 IU/day, measured within 2 weeks of birth, was no different to that of infants born to mothers assigned to placebo (mean 61·6 g [95% CI 60·3–62·8] *vs* 60·5 g [59·3–61·7], respectively; p=0·21). Similarly, we found no difference between treatment groups with respect to bone area, BMD, fat mass, or lean mass of the neonate (table 2), or neonatal BMC adjusted for birth length (appendix p 11). We found no significant difference in neonatal bone indices at the spine (data not shown), or birthweight, length, or head or abdominal circumference between the two treatment groups (table 2).

Maternal baseline characteristics by treatment group and season of offspring delivery are presented in the appendix, p 3. The formal interaction term between treatment group and season of birth on offspring BMC was statistically significant ($p_{interaction}$=0·04) and the effect of treatment was substantially greater for winter births (mean difference in BMC between treatment groups of 5·5 g [95% CI 1·8–9·1]; p=0·004) than in the remaining seasons (figure 2A). A similar winter-birth effect was observed for offspring whole-body bone area (mean difference 11·5 cm² [95% CI 0·1–22·9]; p=0·05; figure 2B), BMD (mean difference 0·01 g/cm² [0·00–0·02]; p=0·04; figure 2C), BMC adjusted for length (mean difference 3·7 g [0·3–7·2]; p=0·03; appendix p 12) and whole-body fat mass (mean difference 113·6 g [30·7–196·4]; p=0·008; appendix p 13), but not whole-body lean mass (appendix p 14). BMC results were similar in each of the three winter months, and differences were still noted between treatment groups (appendix p 5), albeit with the statistical significance limited by the reduced sample sizes when stratified by individual month of delivery. Results did not change substantially after bone indices were adjusted for

Figure 2: **Neonatal whole-body bone mineral content (A), bone area (B), and bone mineral density (C) by intervention group and season of birth**
Data shown are mean and 95% CI. Winter is December to February, spring is March to May, summer is June to August, and autumn is September to November. BMC=bone mineral content. BMD=bone mineral density.



See Online for appendix

Figure 3: **Maternal 25(OH)D status at baseline (14 weeks' gestation) and 34 weeks' gestation by intervention group and season of birth**
Data shown are mean and 95% CI. Winter is December to February, spring is March to May, summer is June to August, and autumn is September to November. 25(OH)D=25-hydroxyvitamin D.

postnatal age at DXA (data not shown). No interaction between treatment effect and season was noted for offspring birth length ($p_{interaction}$=0·95) or birthweight ($p_{interaction}$=0·19).

Further prespecified interactions for neonatal BMC between treatment and offspring sex ($p_{interaction}$=0·92), baseline maternal BMI ($p_{interaction}$=0·91), maternal parity ($p_{interaction}$=0·95), recruitment centre ($p_{interaction}$=0·67), maternal ethnic origin ($p_{interaction}$=0·12), protocol completion ($p_{interaction}$=0·60), treatment compliance ($p_{interaction}$=0·70), baseline maternal 25(OH)D concentration ($p_{interaction}$=0·67; appendix p 6), and change in maternal 25(OH)D concentration from 14 weeks to 34 weeks ($p_{interaction}$=0·91) were not statistically significant. A further post-hoc analysis of the effect of baseline maternal 25(OH)D concentration on treatment efficacy for neonates born in the winter also had no statistically significant interaction ($p_{interaction}$=0·31).

Baseline maternal 25(OH)D concentration was similar in both groups (table 1) and varied by season (appendix p 7, 15–16). Mean maternal 25(OH)D concentration at 34 weeks' gestation was significantly higher in the women who received cholecalciferol (67·8 nmol/L [SD 22·1]) than in those who received placebo (43·3 nmol/L [22·3]; p<0·0001). The proportion of pregnant women with insufficient 25(OH)D (≤50 nmol/L) was similar at baseline (table 1), but was significantly lower at 34 weeks' gestation in the cholecalciferol group than in the placebo group (73 [17%] of 425 *vs* 281 [64%] of 440, respectively; p<0·0001). Furthermore, when the effect of cholecalciferol on maternal 25(OH)D concentration was explored by season of birth, the decline in 25(OH)D from 14 weeks' to 34 weeks' gestation noted in women in the placebo group who delivered in winter and spring was not evident in the women, delivering in these same months, who received cholecalciferol (figure 3). The frequency of participants taking a non-protocol vitamin D-containing supplement did not vary by treatment group or season (appendix p 8) and we noted no effect of treatment on maternal adiposity (weight or skinfold thicknesses) at 34 weeks, irrespective of season (appendix p 9).

A greater proportion of women in the placebo group had severe post-partum haemorrhage than did those in the cholecalciferol group (96 [17%] of 569 mothers in the placebo group *vs* 65 [12%] of 565 mothers in the cholecalciferol group; p=0·01; appendix p 10). We noted no other significant differences in safety outcomes (appendix p 10). No adverse events were deemed to be treatment related.

Discussion

Overall, we found no effect of maternal supplementation with cholecalciferol 1000 IU/day during pregnancy on the primary outcome of offspring neonatal BMC. However, the intervention clearly achieved maintenance of vitamin D repletion, and was safe. Furthermore, in a prespecified secondary analysis we showed that there was an interaction between treatment effect and season of birth such that, for births in the winter, neonatal BMC, bone area, BMD, and body fat, but not birthweight or birth length, were greater in offspring of mothers who had received cholecalciferol than in the offspring of mothers who had not. To our knowledge, this is the first published randomised controlled trial of vitamin D supplementation in pregnancy to include objective measures of offspring neonatal bone mass by DXA.

However, our study had some limitations that must be considered. First, we could not, as a result of stipulations made during the ethics approval process, include participants with baseline 25(OH)D concentrations of less than 25 nmol/L. Additionally, our study population did not include many women who were not white. If anything, both of these considerations are likely to bias towards the null hypothesis, but might reduce the

generalisability of our findings. Furthermore, DXA assessment in neonates presents some difficulties, because newborn babies are prone to move and have low absolute BMC. However, appropriate software was used on each DXA instrument, DXA indices were cross-calibrated, and the validity of the technique in small animals has been documented.[30] Additionally, although we cannot exclude the possibility that some participants were taking vitamin D in addition to the study drug, supplement use was recorded at interview and did not differ between the treatment groups. Fourth, although the secondary analyses were prespecified and the interaction between treatment effect and season that we noted is consistent with previous medical literature and biologically plausible, the possibility of false positive results remains. This finding should therefore be interpreted with caution pending replication in other populations.

We identified only one previously published intervention study in which neonatal bone outcomes were measured,[31] although its null result is difficult to interpret given the small sample size (n=64) and methodological limitations.[7] Results of a study by Javaid and colleagues[6] showed that whole-body bone area, BMC, and BMD, but not height or weight, at 9 years of age in children born to mothers with a 25(OH)D concentration of less than 25 nmol/L in late pregnancy, were lower than those of children born to 25(OH)D-replete mothers. Following this report, other observational studies documented positive associations between 25(OH)D status in pregnancy and newborn bone indices assessed by DXA,[32,33] peripheral quantitative CT at birth[4] and 18 months,[5] and ultrasound measures of fetal femoral morphology.[2,3] The persistence of such associations into adulthood has been shown in the Western Australian Pregnancy Cohort (RAINE).[8] By contrast, four other studies[15,34–36] reported no association between maternal 25(OH)D and infant bone mass, highlighting the need for our randomised controlled trial.

Variation of 25(OH)D with season has been well documented[37,38] and was also observed in our cohort (appendix). UVB exposure to the skin is a major determinant of circulating 25(OH)D concentrations in temperate climates such as the UK, and since 25(OH)D has a half-life of around 3 weeks, the nadir occurs in late winter or early spring.[37,38] In the present study, we observed a distinct fall in 25(OH)D concentration from 14 weeks to 34 weeks of pregnancy in the placebo group, but a rise in the treatment group, when delivery occurred during winter or spring. Indeed, cholecalciferol 1000 IU/day had a statistically significant effect on neonatal bone area, BMC, and BMD for births between December and February, consistent with relations observed between maternal gestational UVB exposure and infant bone mass in our previous cohort study.[6] Although fat mass was greater in neonates born in winter months to mothers in the cholecalciferol group than those born to mothers in the placebo group, we noted no treatment-by-season interaction for birth length or birthweight; although we did note a treatment-by-season effect for BMC adjusted for birth length, suggesting that cholecalciferol 1000 IU/day had a specific effect on bone development rather than simply a generalised effect on birth size.

Most calcium mineral is accrued during the last trimester of pregnancy,[1] and our previous work has suggested that maternal factors (eg, adiposity, physical activity, smoking, 25[OH]D status) within the last trimester are associated with offspring BMC, by contrast with exposures in early pregnancy, for which associations tend to be much weaker.[1] Furthermore, data from the Southampton Women's Survey show a seasonal difference in neonatal whole-body BMC by season of birth, with winter births associated with lower neonatal BMC than summer births.[39] Low 25(OH)D is therefore likely to be most important during the period of rapid bone mineral accrual in late pregnancy, consistent with effects of supplementation observed in the current study when delivery occurred in the months when maternal 25(OH)D concentrations are lowest. We therefore hypothesise that vitamin D supplementation, which reversed the drop in maternal 25(OH)D concentrations from 14 weeks to 34 weeks in women who gave birth in spring and, particularly, winter, therefore ameliorates the adverse effect of this decline in maternal 25(OH)D on offspring BMC, with the overall effect being one of removal of deficit rather than overall improvement. However, conversely, we did not find a statistically significant effect of treatment among spring births, which would have been expected from the timing of the 25(OH)D nadir. Given the relatively arbitrary nature of seasonal definitions in relation to objectively measured UVB exposure, we feel that the best reconciliation of the findings relating to late pregnancy 25(OH)D concentrations in spring and winter with those relating to offspring bone in these seasons is that vitamin D supplementation has the greatest effect in participants with an absolute decline in 25(OH)D above a notional threshold. Overall, our findings should be interpreted with caution, and replication of the treatment-by-season interaction in further studies will be needed to delineate any messages for clinical care. Results of the ongoing MAVIDOS childhood follow-up study might also help to clarify this issue.

Notwithstanding, our findings inform public health policy, providing the first data from a large, blinded, randomised controlled trial with bone outcomes assessed by DXA, and showing that overall, gestational supplementation with 1000 IU/day of vitamin D does not benefit neonatal bone mass of offspring. The intervention seemed to be safe, and although vitamin D supplementation seemed to be associated with a reduced incidence of severe post-partum haemorrhage, we suspect that this is a false positive finding as a result of misclassification, since these events were not adjudicated

and it is very difficult to accurately assess post-partum blood loss in the typical clinical situation. This finding will therefore be the subject of further investigation. Although neonatal BMD was lower in births occurring in autumn in the cholecalciferol group than in the placebo group, the difference was not statistically significant (p=0·07) and such differences were not consistent across other DXA indices. Finally, although the dose used in our study is 2·5 times the standard UK recommendation of 400 IU daily in pregnancy, it is much lower than the highest doses used in several US studies (up to 4000 IU daily)[7] and our results clearly show that such high doses are not needed to achieve good levels of 25(OH)D repletion.

In conclusion, we found that supplementation of pregnant women with cholecalciferol 1000 IU/day from 14 weeks' gestation until delivery of the baby does not lead to increased offspring neonatal BMC overall. Our demonstration of an interaction between treatment effect and season of delivery is consistent with previous data and biologically plausible, and suggests the potential for beneficial effects of supplementation in pregnant women due to deliver in winter months. This finding should be replicated in further populations before its significance for public health can be fully appreciated. The overall safety of vitamin D supplementation during pregnancy is supported by our results.

Contributors

CC, NCH, NJB, SK, ATP, IS, AC, NKA, EMD, KMG, AP, HMI, RE, DMR, and MKJ contributed to the study design. CC and NCH were responsible for study oversight and execution. NJB, CC, RF, SVG, NCH, MKJ, SK, and ATP collected the data. RJM was responsible for management of the trial dataset. SD'A and SRC undertook the statistical analysis. HMI was responsible for overseeing the statistical analysis. IS was responsible for the biochemical analyses. All authors contributed to data interpretation, and preparation of the draft and final versions of the report. CC is the chief investigator. NCH is the principal investigator at the Southampton site and lead-principal investigator of the study. SVG and RF are principal investigators at the Sheffield site. MKJ, ATP, and SK are principal investigators at the Oxford site. DMR is chair of the trial steering committee. All members of the MAVIDOS Study Group contributed to report preparation, study design, and study execution.

Declaration of interests

CC reports personal fees, consultancy, lecture fees, and honoraria from Alliance for Better Bone Health, Amgen, Eli Lilly, GlaxoSmithKline, Medtronic, Merck, Novartis, Pfizer, Roche, Servier, and Takeda, outside the submitted work. NCH reports personal fees, consultancy, lecture fees, and honoraria from Alliance for Better Bone Health, AMGen, MSD, Eli Lilly, Servier, Shire, Consilient Healthcare, and Internis Pharma, outside the submitted work. NJB reports remuneration from Internis Pharmaceuticals, outside the submitted work. ATP reports grants from the Arthritis Research Council, during the conduct of the study. NKA has received honoraria, held advisory board positions (which involved receipt of fees), and received consortium research grants from Merck, grants from Roche, Bioiberica, and Novartis, personal fees from Smith & Nephew, Nicox, Flexion, Bioventus, and Freshfields, outside the submitted work. KMG reports reimbursement for speaking at Nestle Nutrition Institute conferences, and grants from Abbott Nutrition and Nestec, outside the submitted work. KMG also has a patent pending for phenotype prediction, a patent pending for predictive use of CpG methylation, and a patent pending for maternal nutrition composition, not directly related to this work. HMI reports grants from the Medical Research Council (MRC), Arthritis Research UK, and European Union's Seventh Framework Programme, during the conduct of the study; and while not directly receiving funding from other bodies, members of her team have received funding from the following companies from other work: Danone, Nestec, and Abbott Nutrition. RE reports grants and personal fees from Amgen and Alexion; grants from the Department of Health, AstraZeneca, ARUK/MRC Centre for Excellence in Musculoskeletal Ageing Research, National Institute for Health Research, MRC/AZ Mechanisms of Diseases Call, and the MRC; grants, personal fees, and non-financial support from Immunodiagnostic Systems; grants and membership of a clinical and scientific committee from the National Osteoporosis Society; grants, personal fees, and advisory board membership from Roche; personal fees from Otsuka, Novartis, Merck, Bayer, Johnson & Johnson, Fonterra Brands, Janssen Research, Ono Pharma, Alere (Unipath), Chronos, Teijin Pharma Limited, D-STAR, and GSK Nutrition; personal fees and advisory board membership from Eli Lilly, and CL Biosystems; and advisory board membership from the European Calcified Tissue Society, IOF CSA, and the American Society for Bone and Mineral Research, outside the submitted work. MKJ reports personal fees from Stirling Anglia, Consilient Health, and Internis, outside the submitted work. All other authors declare no competing interests.

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