Trial Report: MAVIDOS Maternal Vitamin D Osteoporosis Study

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Related publications

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, Reid DM, Javaid MK; MAVIDOS Study Group. Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): a multicentre, double-blind, randomised placebo-controlled trial. Lancet Diabetes Endocrinol. 2016 May;4(5):393-402.

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Introduction

Osteoporosis is a devastating disease and its high prevalence makes it a suitable choice for population-wide public health interventions aimed at optimising bone health[1,2]. Evidence is accruing that early growth, and factors acting in utero or during early infancy, may influence the trajectory of long-term skeletal accrual to peak bone mass [3]. In particular, maternal serum 25(OH)-vitamin D [25(OH)D] concentrations in pregnancy have been associated with offspring bone morphology [4-7] and bone mass [8,9] up to young adulthood [10]. In most populations, the main determinant of 25(OH)D concentrations is UVB exposure to the skin, which varies markedly by season in temperate climates [11]. Seasonal differences in neonatal BMC have been demonstrated [12,13], with effects potentially modified by vitamin D supplementation, and maternal UVB exposure during pregnancy has been positively associated with bone mass in childhood [8,14]. Not all studies have demonstrated a benefit of higher maternal 25(OH)D levels in pregnancy on childhood skeletal health, however [15,16], and no high quality intervention studies exist [17].

Whole body bone mineral content (BMC) is the recommended measure of bone mass in children; although it is of limited clinical utility in neonate due to the lack of normative data [18]. Infant DXA been widely used in research studies, where comparisons are internal. Childhood BMC is inversely related to childhood fracture risk [19]; although data spanning from conception to peak bone mass in a single cohort are lacking, the current evidence-base supports tracking of bone mineral content over this time [20,21]. The magnitude of peak bone mass achieved has also been shown to be an important determinant of future fracture risk[22].

The aim of the MAVIDOS trial was therefore to test the hypothesis that neonates born to mothers supplemented with vitamin D during pregnancy would have greater whole body bone mineral content (BMC) at birth than those of mothers who had not received supplementation [23]. Given the previously documented importance of season for both 25(OH)D concentrations and childhood bone mass, we further hypothesised that an interaction between season of birth and treatment effect would be present.

The original trial protocol specified follow up to age 4 years as a trial endpoint, however funding to perform follow-up to age 6-8 years (under the same research ethics committee approval) was obtained. This report therefore is reflective of the original trial protocol using 4 years as the endpoint, meanwhile follow-up of children (at around 7 years old) born to the original trial participants is ongoing.

Methods

MAVIDOS is a multicentre, double-blind, randomised, placebo-controlled trial of vitamin D supplementation in pregnancy in the United Kingdom.[1,2] The study was conducted in accordance with guidelines laid down in the Declaration of Helsinki and was approved by the Southampton and South West Hampshire Research Ethics Committee. MAVIDOS was registered prospectively (ISRCTN:82927713; EUDRACT:2007-001716-23); full approval from UK MHRA was granted, and

written, informed consent was obtained from all participants. A summary of the trial is shown in Figure 1.

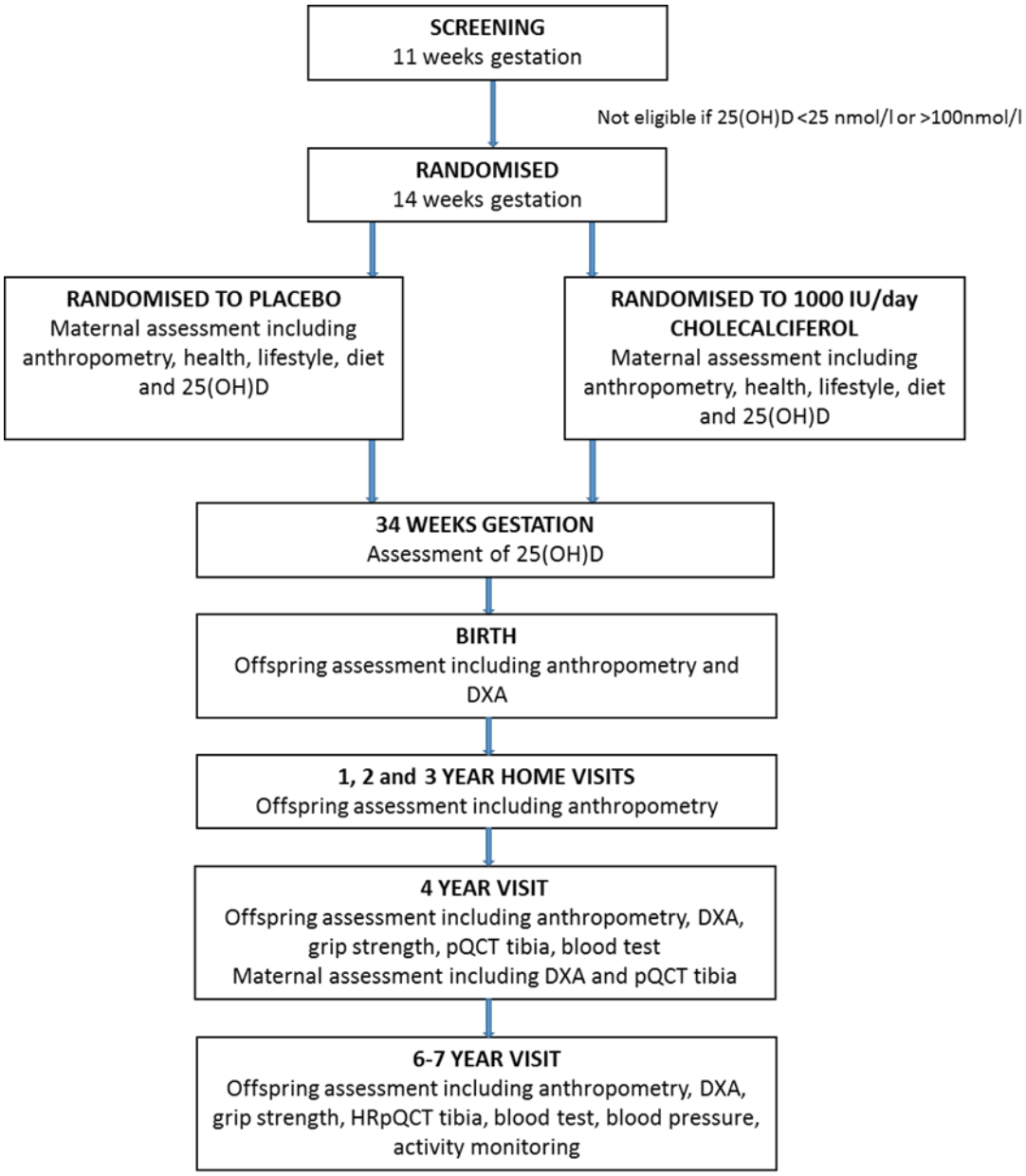


Figure 1 MAVIDOS trial overview

Participants

Pregnant women were recruited when attending for early pregnancy ultrasound screening at three study sites [University Hospital Southampton NHS Foundation Trust, Southampton, UK; Oxford University Hospitals NHS Trust, Oxford, UK; Sheffield Hospitals NHS Trust (University of Sheffield), Sheffield, UK] between 06/10/2008 and 11/02/2014. Inclusion criteria were: age over 18 years, singleton pregnancy, gestation less than 17 weeks based on last menstrual period (LMP) and ultrasound measurements, and aiming to give birth at the local maternity hospital. Women with known metabolic bone disease, renal stones, hyperparathyroidism or hypercalciuria, those with a diagnosis of cancer in the last 10 years, those unable to give informed consent or comply with the protocol, those taking medication known to interfere with fetal growth, those with fetal anomalies on ultrasonography and women already using >400IU/day vitamin D supplementation were excluded. A

screening blood sample was obtained and analysed on the local NHS platforms and only 25 and 100nmol/l and serum calcium<2.75mmol/l were eligible to enrol in the study. All three laboratories (Southampton, Oxford, Sheffield) were accredited by the Vitamin D External Quality Assessment Scheme (DEQAS) (http://www.deqas.org/).

Trial assessments: maternal and neonatal

Questionnaire and anthropometry: All participants received standard antenatal care, and were able to continue self-administration of antenatal multivitamins containing up to 400IU/day vitamin D. Women were assessed in detail at 14 and 34 weeks' gestation including assessments of diet (including calcium and vitamin D intake), smoking, alcohol consumption, health, physical activity, medications, supplements (all interviewer-led questionnaires) and anthropometry. Anthropometric measurements of the newborns were obtained, and information on obstetric complications was extracted from maternity records.

Biochemical: Study blood samples were collected from the mother at 14 and 34 weeks' gestation, and stored at -80°C after processing. Measurement of plasma 25(OH)D (Liaison RIA automated platform, Diasorin, Minnesota, 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. Details of assay performance and quality control through participation in DEQAS, NIST and NEQAS are given elsewhere [24].

DXA: The baby underwent DXA assessment at whole body and lumbar spine sites [Hologic Discovery (Hologic Inc., Bedford, MA, USA) or GE-Lunar iDXA (GE-Lunar, Madison, Wisconsin, US) with neonatal software] within 2 weeks after birth. In order to maximise scan quality, the infant was undressed, and clothed in a standard towel, fed and pacified before the assessment. Each instrument underwent daily QC, with cross-calibration between sites. The total radiation dose was estimated as 0.04mSv, equivalent to approximately 7 days' exposure to background radiation in the UK. All DXA images were reviewed by two operators for movement artefacts and quality.

Interventions, randomisation and blinding: Women were randomised at 14 weeks' gestation (or as soon as possible before 17 weeks' gestation if recruited later) to either cholecalciferol 1000IU/day or matched placebo [Merck KGaA, (Darmstadt, Germany)/ Sharp Clinical Services (Crickhowell, UK; previously DHP-Bilcare)]. Packs were randomised in a 1:1 ratio in randomly permuted blocks of 10, starting randomly midway through the block, and sequentially numbered, by Sharp Clinical Services Ltd prior to 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 blind to treatment allocation throughout the study duration.

Trial assessments: 1, 2 and 3 years of age

Mothers recruited in the Southampton arm of the study were invited to participate in further follow-up of their children at 1, 2 and 3 years of age. The children were reviewed during a home visit, including an interviewer-led questionnaire assessment of diet, health and lifestyle and anthropometry (crown heel length at 1 year of age, standing height at age 2 years using the same protocol as previously described, mid upper arm circumference, triceps and subscapular skinfold thickness was performed following the same protocol as used in adult study participants.

Trial assessments: 4 years of age

At age 4 years, the children born in Southampton were invited to attend the Osteoporosis Centre at Southampton General Hospital for a detailed assessment. A diet, health and lifestyle questionnaire was completed and anthropometric measurements were obtained including weight, height, OFC,

MUAC, triceps and subscapular SFT following the previously described standard protocols. Bone indices and body composition were assessed by DXA of the whole body, lumbar spine and hip using a Hologic Discovery DXA instrument (Hologic Discovery, Hologic Inc., Bedford MA, USA). Hand grip strength was measured using a Jamar hand dynamometer (Promedics, Blackburn, UK), again using the previously described protocols. A pQCT scan of the right tibia was obtained using a Stratec XCT 2000 instrument (Stratec Inc., Pforzhein, Germany). Maternal DXA and pQCT of the tibia were also performed. Where consent was provided, a venous blood sample was collected from the child.

Trial assessments: 6-8 years of age

At age 6-8, the children of the MAVIDOS study have been invited for further assessment at the Osteoporosis Centre at Southampton General Hospital; these visits are ongoing. The diet, health and lifestyle questionnaire and anthropometry are performed as per protocol and the children undergo whole body DXA using Hologic Discovery DXA instrument (Hologic Discovery, Hologic Inc., Bedford MA, USA). Hand grip strength is measured using a Jamar Plus + digital hand dynamometer (Promedics, Blackburn, UK), again using the previously described protocols. HRpQCT measurements are obtained (Xtreme CT, Scanco Medical Ag, Bruettisellan, CH) of bone microarchitecture at the tibia. Blood pressure is measured, and when consent is given a venous blood sample and buccal swab is collected from the child and stored at -70°C. Physical activity is being measured in a subset of children using the GeneActiv tri-axial accelerometer (Activinsights, UK), worn on the non-dominant wrist (posted back in a reply paid envelope). The device has been validated in both paediatric and adult populations [25-29].

Outcomes

The primary outcome was whole body BMC of the neonate. Although we had originally planned to use whole body BMC adjusted for age, it was judged following further statistical review, in this randomised controlled trial setting, appropriate to include offspring age in a sensitivity analysis, rather than as the primary outcome. Secondary outcomes included maternal 25(OH)D concentration at 34 weeks' gestation; change in 25(OH)D between 14 and 34 weeks' gestation, neonatal whole body bone area and bone mineral density, and neonatal bone indices at the spine. In order to preserve statistical power, rather than perform separate analyses (as planned in original protocol) for those who completed the protocol, complied with treatment, demonstrated a rise in 25(OH)D, and stratification by baseline 25(OH)D, we explored these potential effect modifiers via their incorporation as interaction terms in regression models. Safety analyses examined the frequency of adverse outcomes including: infection, nausea/vomiting, diarrhoea, abdominal pain, headache, hypertension, hypercalcaemia (greater than equal to 2.75mmol/l) at 34 weeks' gestation, intrauterine growth restriction (IUGR), preterm birth (less than 37 weeks' gestation), instrumental delivery, severe postpartum haemorrhage, stillbirth or neonatal death, congenital abnormalities.

Sample size

We estimated the sample size using the results from the Princess Anne Hospital Study [8], in which a difference of 0.42 SD in whole body BMC was found 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 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 (0.21SD or 3.5g), at the 5% significance level with 90% power, would require recruitment of 477 neonates in each arm.

Statistical analysis: neonatal assessment

We undertook analyses on an intention to treat (ITT) basis for all those with a neonatal DXA assessment; the analysis plan was published prior to unblinding of the study [23]. At the request of the

Data Monitoring Committee, an interim safety analysis of serum calcium concentration was requested after two years of recruitment, but no analysis of DXA outcomes was undertaken until follow-up of all participants had been completed. All data were checked for normality by visual inspection of histograms. Data were assumed to be missing at random. Comparison was made between treatment groups using Student t-test and Mann-Witney U test for normally and non-normally distributed outcomes respectively. Categorical outcomes were compared using χ^2 test. DXA indices included neonatal whole body bone area, bone mineral content, bone mineral density, lean and fat. In order to assess bone mass independent of body size, we used bone mineral content adjusted for birth length in a regression model. Given the seasonal change in the 25(OH)-vitamin D observed in many previous studies, we hypothesised, *a priori*, that there might be an interaction between treatment and season of birth. We defined season of birth using the UK Meteorological Office classification, as winter (December-February), spring (March-May), summer (June-August) and autumn (September-November) [www.metoffice.gov.uk], and secondly explored differences in treatment effect by individual month of birth. We also investigated pre-specified interactions between treatment and offspring sex, and between treatment and ethnicity, as both these factors have been associated with variations in vitamin D metabolism. Since there is clear evidence of differences in body composition between first and subsequent offspring [3], and an inverse relationship between body mass index and 25(OH)D concentration, we hypothesised interactions between each of these 2 variables and treatment. Finally we reasoned that treatment might be more effective in those who fully complied with the protocol and were compliant with medication, who had low concentrations of 25(OH)D at baseline, or in those with a greater change in 25(OH)D from 14 to 34 weeks, and that for a combination of reasons given above, there might be differences by study centre, providing the basis for the final 5 interaction analyses. In summary, the interactions tested were with: study centre, maternal ethnicity, parity, compliance, protocol completion, baseline BMI, baseline 25(OH)D and change in 25(OH)D from 14 to 34 weeks; and offspring sex and season of birth. All these interactions were explored in multivariable linear regression (with the independent variables, for example: treatment; season; treatment*season, and inclusion of no other covariates). In further analyses we adjusted bone outcomes for postnatal age at DXA. Given that the secondary analyses were pre-specified and hypothesis-based, and that the study was powered for the primary outcome, it was not judged appropriate to undertake correction for multiple testing, recognising that any statistically significant results from the secondary analysis would require further confirmation in future studies. With 10 analyses and an alpha of 0.05, we calculated that the probably of observing one or more false positive associations was 40% [equal to (1-0.95^10)*100]. All analysis was performed using Stata v13.1 (Statacorp, College Station, Texas, USA). A p value of <0.05 was accepted as statistically significant.

Statistical analysis: 4 year follow-up

DXA outcomes and grip strength were transformed to Z scores (representing standard deviations) for ease of comparison of outcomes, grip strength was measured three times in each hand; both a maximum value and a mean value of the six attempts was analysed.

Owing to differences in body composition between boys and girls, DXA outcomes were adjusted for sex using linear regression. DXA outcomes were also further adjusted for age. Grip strength was adjusted for height and sex.

Owing to chance differences in the baseline mean BMI of the randomly assigned pregnant women in the MAVIDOS trial [placebo n = 523, mean (SD) 25.7 (23.0-30.0) kg/m^2; cholecalciferol n = 533, 24.7 (22.3-28.6) kg/m^2], maternal BMI was also adjusted for [2]. Higher maternal BMI at 14 weeks gestation has been shown to be associated with attainment of a lower late pregnancy 25(OH)D status following

cholecalciferol supplementation [30]. Higher maternal BMI is also relevant to offspring bone mass [31-33].

Model building by iterative forward selection was used to determine which covariates should remain in a multivariate regression model examining the association between randomisation group and offspring bone or lean mass outcomes, or grip strength. The following covariates, chosen due to evidence from previous studies suggesting an association with offspring bone mass, 25(OH)D status, or both, were tested: child's sex, child's age, child's height, offspring gestational age at birth, maternal age at delivery, maternal parity, maternal BMI in early pregnancy, maternal smoking in early or late pregnancy, maternal moderate to strenuous physical activity in late pregnancy, maternal educational attainment (A-level or above), offspring season of birth, child's weekly milk intake at age 4 years as a proxy for calcium intake, and child's weekly "screen time" (watching TV or computer screen) as an inverse measure of physical activity (sedentary time assessed by questionnaire has been shown to be negatively associated with physical activity assessed by accelerometry in primary school children [34,35]).

Multivariate linear regression was subsequently performed including all variables with a $p < 0.2$ from the univariate model, in order to assess their importance after adjustment for other important covariates.

In the primary trial analysis, season of birth was classified according to the UK Meteorological office recommendations, (www.metoffice.gov.uk) as winter (December-February), spring (March-May), summer (June-August), and autumn (September-November). As 25(OH)D concentrations are not linearly associated with season, and for ease of comparison, and to maximise power, this classification has been into two groups, "winter/spring" (the months in which 25(OH)D concentrations tended to be lowest, December – May) and "summer/autumn" (the months in which 25(OH)D concentrations tended to be highest, June – November).

In sensitivity analyses, associations between randomisation group and DXA outcomes and grip strength were examined, stratifying baseline 25(OH)D using a threshold of ≤ 30 nmol/l (a threshold advised by the Institute of Medicine [36] and the Global Consensus Recommendations on the Prevention and Management of Nutritional Rickets [37] to define vitamin D deficiency. The influence of adjustment for maternal late pregnancy moderate to strenuous physical activity was examined in a separate multivariate model (missing data reduced the overall numbers substantially). In further sensitivity analyses, firstly data from children who were born preterm (< 37 weeks) was excluded, then analysis was limited to children of mothers of white ethnicity, and finally analysis was limited to scans without any movement artefact.

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 other role in the study.

Results

Participants (baseline)

A total of 1449 women were initially eligible after screening and consented to a blood test to determine early pregnancy 25(OH)D status.[2] Of these, 148 were ineligible to participate due to either 25(OH)D<25nmol/l (n=59) or 25(OH)D>100nmol/l (n=89). None had a plasma calcium >2.75 mmol/L. A further 167 women withdrew prior to randomisation. Thus, 1134 were randomised, of whom 965 (85.1%) remained in the study until delivery. A total of 836 (73.7%) neonates had a DXA scan. After

excluding scans with significant movement artefact, DXA scan data were available for 737 neonates (65.0%), comprising 665 assessments at the whole body (intervention, n=338; placebo, n=327) and 628 at the lumbar spine (intervention, n=305; placebo, n=323), meaning that numbers were somewhat lower than specified in the original power calculation.

Participants (4 year follow-up)

564 attended the 4 year visit (Southampton participants only) (286 in the placebo group, 278 in the cholecalciferol group), 78.0 % of eligible children. 561 children (99.5% of attendees) underwent anthropometric measurement, and 515 (91.3% of attendees) underwent at least one measurement of grip strength. Numbers of DXA scans were lower, either due to participant refusal or technical issues. 508 children (90.1% of attendees) underwent DXA scanning, and 452 children had a useable DXA scan (89.0% of all DXAs). 90 DXAs (19.9% of the useable DXAs) had movement artefact in one leg, one arm, or one leg and one arm and underwent transposition of data from the ROI of the opposite side. The MAVIDOS trial consort diagram is shown in Figure 2.

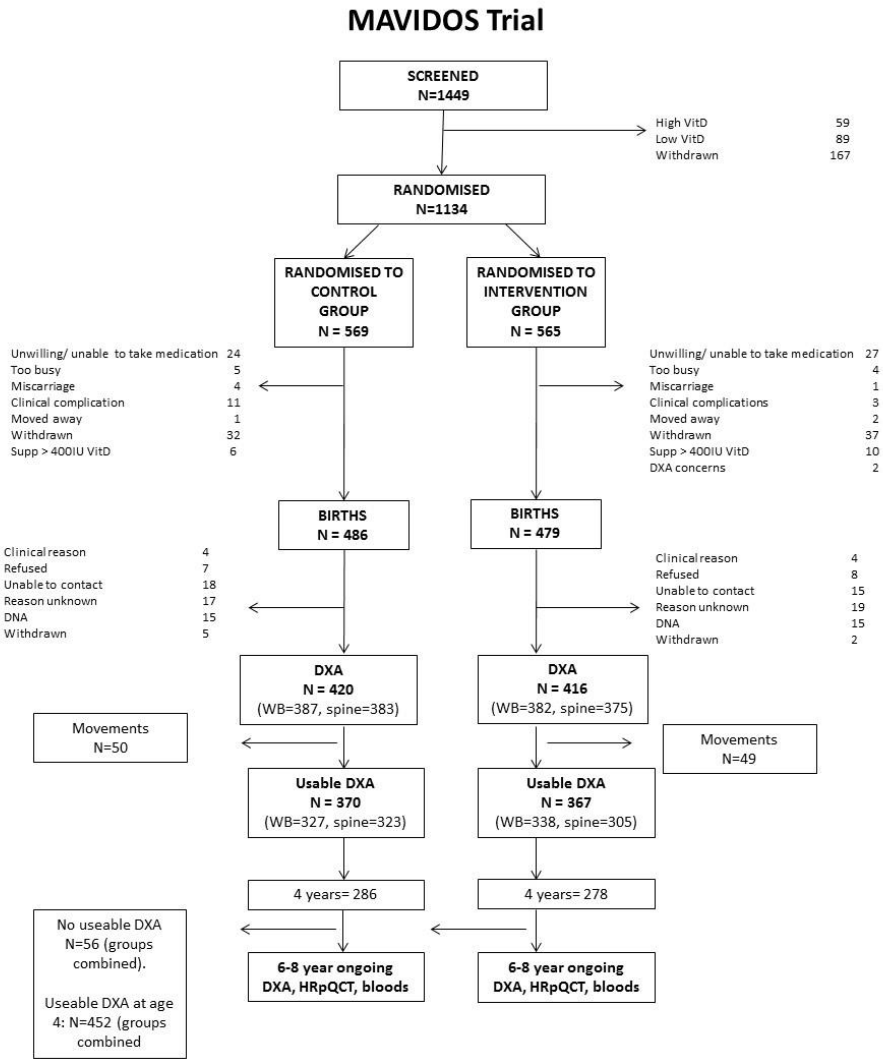


Figure 2 MAVIDOS Trial Consort diagram up to complete 4 year follow-up

Maternal characteristics (baseline)

Women in the treatment and placebo group at randomisation were of similar age, parity, educational attainment, smoking, exercise participation and ethnicity. Height was also similar between the two groups, but weight, BMI and sum of skinfold thicknesses were greater in the placebo arm. The women who remained in the trial until their baby was born were older (30.7 vs 28.9 years, p<0.001) and more likely to be white Caucasian (94.8% vs 89.2%, p=0.01), than those who withdrew. Women whose infants underwent DXA scanning tended to be older, and be less likely to smoke and have lower skinfold thicknesses, than women whose infants did not undergo DXA assessment.

Bone indices, anthropometry and body composition (Baseline, neonatal)

There was no difference in neonatal whole body bone mineral content (BMC) of infants born to mothers randomised to 1000IU/day cholecalciferol compared with infants born to mothers randomised to placebo [61.6g (95%CI: 60.3, 62.8g) vs 60.5g (95%CI: 59.3, 61.7g) respectively, p=0.21]. Similarly, there was no difference in bone area, bone mineral density, BMC adjusted for birth length, fat or lean mass of the neonate by treatment allocation (Table 2). There was no significant difference in neonatal bone indices at the spine, or birth weight, length, head or abdominal circumference between the two treatment groups (Table 2).

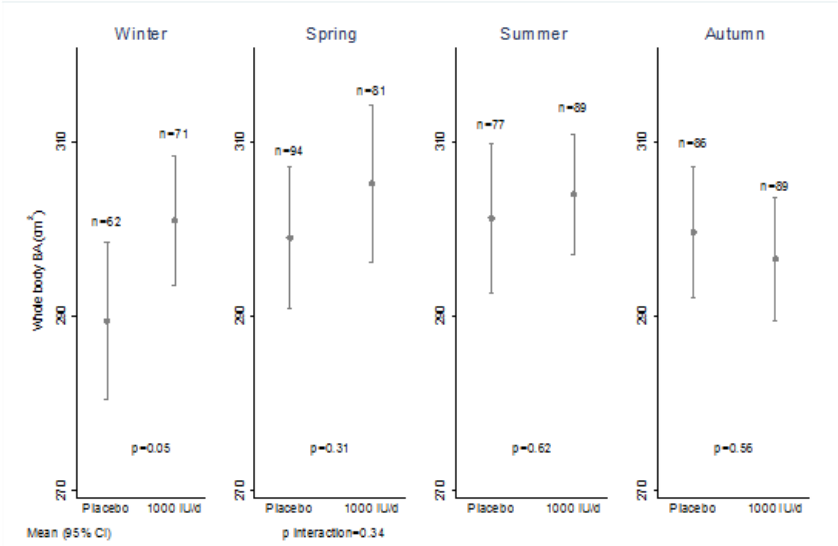
Table 2 Anthropometry, whole body bone mineralisation and body composition in neonates born to mothers randomised to 1000 IU/day cholecalciferol or placebo from 14 weeks' gestation until delivery. Data displayed as mean±SD, unless otherwise stated

	Placebo	***Cholecalciferol (1000 IU/day)***	***p***
Obstetric data			
N	486	479	
Male, N (%)	251 (51.7)	258 (53.9)	0.49
Birth weight (g)	3518 ± 517	3481 ± 543	0.28
Crown-heel length (cm)	50.8 ± 2.3	50.6 ± 2.6	0.31
Head circumference (cm)	35.5 ± 1.5	35.4 ± 1.4	0.62
Abdominal circumference (cm)	32.7 ± 2.3	32.9 ± 2.2	0.16
DXA			
Whole body			
N	327	338	
Age at DXA (days)	7 ± 6	8 ± 7	0.12
Bone Area (cm^2)	297.8 ± 37.3	301.6 ± 34.7	0.18
BMC (g)	60.5 ± 11.1	61.6 ± 11.7	0.21
BMD (g/cm^2)	0.203 ± 0.019	0.203 ± 0.022	0.96
Lean (g)	3014 ± 435	3055 ± 423	0.23
Fat (g), median (IQR)	374 (244-517)	355 (235-564)	0.97

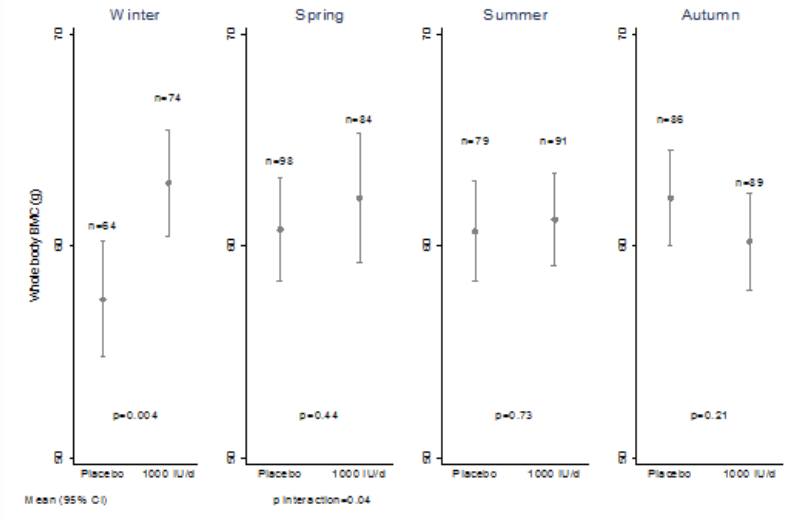
Pre-specified secondary analyses

A priori, we hypothesized an interaction between treatment group and offspring season of birth. The formal interaction term between treatment group and season of birth on offspring BMC was statistically significant (p=0.04) and the effect of treatment was of substantially greater magnitude [mean difference 5.5g (95%CI: 1.8, 9.1, p=0.004)] in winter months (December to February inclusive) than in the remaining seasons (Figure 3). A similar winter effect was observed for offspring whole body bone area (mean difference by group=11.5cm^2, p=0.05), bone mineral density (mean difference by group=0.01g/cm^2; p=0.04), bone mineral content adjusted for length (mean difference by group=3.7g, p=0.03) and indices of body composition. Results were similar for each of the 3 winter months, albeit with the statistical significance limited by the reduced sample size when stratified by individual month of delivery. Results were little changed after bone indices were adjusted for postnatal age at DXA. There was no evidence of a treatment season interaction for offspring birth length (p=0.95) or birth weight (p=0.19). Further pre-specified interactions for neonatal BMC between treatment and offspring sex (p=0.92); maternal BMI (p=0.91); maternal parity (p=0.95); recruitment centre (p=0.67); ethnicity (p=0.12); protocol completion (p=0.60); treatment compliance (p=0.70); baseline 25(OH)D status (p=0.67) and change in 25(OH)D (p=0.91) were not statistically significant. A further analysis of the effect of baseline 25(OH)D status on treatment efficacy within the winter group also demonstrated no statistically significant interaction (p=0.31).

a) Bone area



b) Bone mineral content



c) Bone mineral density

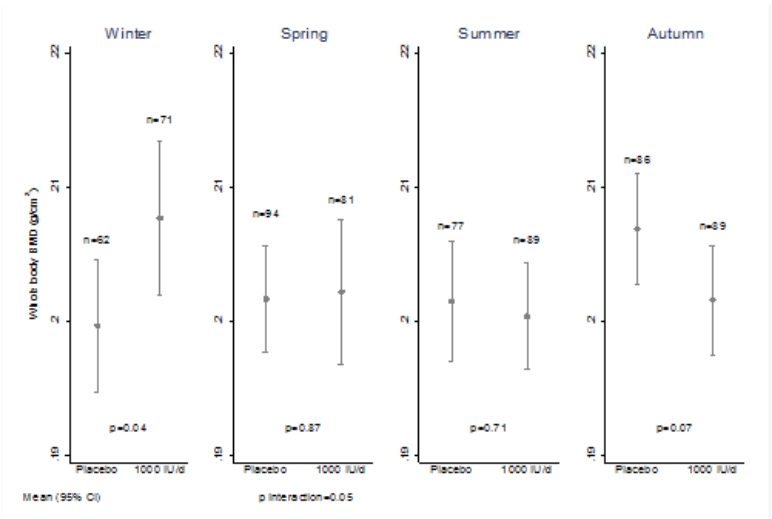


Figure 3 Neonatal whole body a) bone area, b) bone mineral content and c) bone mineral density by intervention group and season of birth. [Winter = December to February]. Data shown are mean and 95%CI.

Maternal pregnancy 25(OH)D status

Baseline 25(OH)D status was similar in both groups and varied by season. Maternal 25(OH)D at 34 weeks' gestation was significantly higher in the women who received 1000IU/day (68.2±21.9nmol/l) compared with placebo (43.4±22.4nmol/l), p<0.001. The percentage of participants with insufficient 25(OH)D (<50nmol/l) was similar at baseline, but significantly lower at 34 weeks' gestation in the intervention compared with placebo group (16.6% vs 63.5%, p<0.001). Furthermore, when the effect of vitamin D supplementation on maternal 25(OH)D status was explored by season of birth, the decline in 25(OH)D from 14 to 34 weeks' gestation observed in placebo group women who delivered in winter and spring was not evident in the women, delivering in these same months, who received the vitamin D supplement (Figure 4). Frequency of non-protocol vitamin D containing supplement use did not vary by treatment group or season and there was no effect of treatment on maternal adiposity (weight or skinfold thicknesses) at 34 weeks regardless of season.

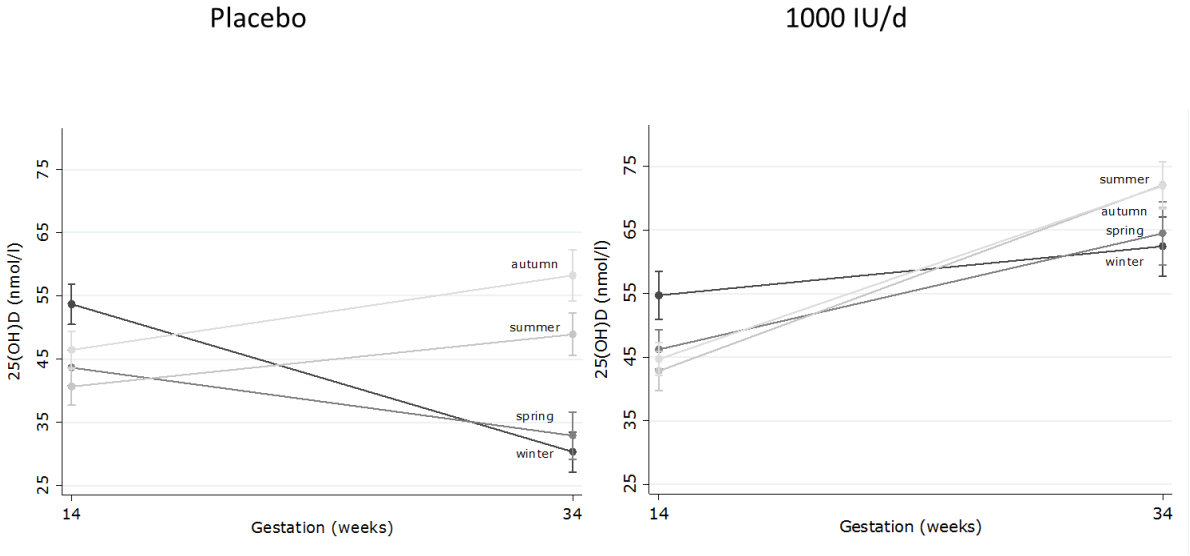


Figure 4 Maternal 25(OH)D status at baseline (14 weeks gestation) and 34 weeks' gestation by randomisation to either 1000IU/day cholecalciferol or placebo and season of birth. [Winter = December to February]. Data shown are mean and 95%CI.

Safety analysis

The absolute numbers and percentages of adverse events by treatment group are documented in the trial outcome publication [(2)]. Other than a greater proportion of women in the placebo group who had a report of severe postpartum haemorrhage, there were no differences in these safety measures.

Maternal characteristics (4 year follow-up)

Maternal characteristics did not differ between the two randomisation groups for those attending the 4 year visit (all p > 0.05). No statistically significant differences were observed in early pregnancy plasma 25(OH)D concentrations between the groups. Plasma 25(OH)D concentrations obtained in late pregnancy were significantly higher in the mothers in the cholecalciferol supplemented group (p < 0.001). Late pregnancy 25(OH)D was significantly greater in the cholecalciferol supplemented groups regardless of season of delivery. Late pregnancy 25(OH)D was greater in the placebo group in "summer/autumn" births than "winter/spring" births, therefore the increase in 25(OH)D attributable to supplementation was greater in "winter/spring" births ("winter/spring" placebo group late

pregnancy mean 25(OH)D 24.55 nmol/l, compared with "summer/autumn" placebo group late pregnancy mean 25(OH)D 52.8 nmol/l). In comparison to the mothers who remained in the study to delivery, mothers attending the 4 year follow-up visit were older at time of delivery, of higher educational attainment and a smaller proportion smoked in early pregnancy (all $p < 0.001$) or late pregnancy ($p = 0.009$).

Characteristics of children (4 year follow-up)

The median (IQR) age of children attending the 4 year visit was 4.08 (4.03, 4.16) years in the placebo group and 4.07 (4.03, 4.15) years in the cholecalciferol group. Characteristics and anthropometry of the children attending the four year visit did not differ significantly, numerically there were more boys in the cholecalciferol group, and weight and height were greater. Subscapular SFT was lower in the cholecalciferol group though the difference was of borderline statistical significance ($p = 0.09$).

Table 3 Characteristics of the children of the Southampton arm of the MAVIDOS trial attending the four year follow-up visit. Shown as mean (SD), n (%) or median (IQR)+*

	n	*Placebo*	*n*	*Cholecalciferol 1000 IU/day*	*p difference*
Age (years)+	284	4.08 (4.03, 4.16)	276	4.07 (4.03, 4.15)	0.61
Gestational age at birth (weeks)+	285	40.29 (39.29, 41.00)	278	40.29 (39.29, 41.00)	0.95
*Male sex**	286	144 (50.3)	278	161 (57.9)	0.08
Weight (kg)	285	17.20 (2.06)	275	17.37 (2.20)	0.34
Height (cm)	281	104.78 (4.43)	274	105.19 (4.38)	0.27
BMI (kg/m^2)	281	15.65 (1.26)	274	15.66 (1.24)	0.91
OFC (cm)	274	50.88 (1.59)	266	50.98 (1.57)	0.49
MUAC (cm)	274	17.42 (1.23)	263	17.44 (1.27)	0.88
Triceps SFT (mm)	251	10.13 (2.26)	234	9.95 (2.35)	0.38
Subscapular SFT (mm)+	240	6.08 (5.37, 7.13)	231	5.97 (5.07, 7.00)	0.09
Screen time (TV/computer) (hrs/day)+	286	1.5 (1.5, 2.5)	277	1.5 (1.5, 2.5)	0.14

Offspring bone indices, lean mass and grip strength (4 year followup)

Whole body (less head) bone mineral density was statistically significantly greater in the children of gestational cholecalciferol supplemented mothers (a difference of 0.007 g/cm^2, $p = 0.048$), as shown in a recent abstract at the American Society for Bone Mineral Research, not yet published (Curtis et al., Abstract, ASBMR, Orlando 2019). Whole body (less head) BMC and BA did not differ between the two groups, though BMC was numerically greater in the cholecalciferol supplemented group. Lumbar spine BMC and BA were greater in the children of cholecalciferol supplemented mothers, though these differences did not reach statistical significance. In terms of body composition, whole body (less head) lean mass was 241.98 g greater in the 4 year old offspring of the cholecalciferol supplemented group, of borderline statistical significance ($p = 0.051$). A trend towards a slightly greater lean mass percentage (0.85% greater in the cholecalciferol vs. placebo group) was observed ($p = 0.091$). There were no statistically significant differences in fat mass, though it was numerically greater in the children of placebo group mothers.

Grip strength, recorded as maximum grip strength and mean of six attempts (three in each hand) did not differ significantly between the two groups ($p = 0.271$ and $p = 0.328$ respectively), though was

numerically greater in the children of cholecalciferol supplemented mothers (a difference of 0.18 kg maximum grip strength).

Table 4 Whole body less head DXA, lumbar spine DXA and grip strength assessed at age 4 years in the children born to mothers randomised to placebo or cholecalciferol 1000 IU/day in pregnancy. Outcomes are unadjusted. Values are mean (95% confidence interval) unless otherwise stated.

	n	*Placebo*	*n*	*Cholecalciferol 1000 IU/day*	*p difference*
Bone outcomes:					
Whole body (less head):					
BA (cm^2)	246	756.7 (750.2, 763.2)	248	756.0 (749.3, 762.7)	0.878
BMC (g)	246	356.7 (351.2, 362.2)	248	361.2 (355.7, 366.7)	0.252
BMD (g/cm^2)	246	0.470 (0.466, 0.475)	248	0.477 (0.472, 0.481)	**0.048**
Lumbar spine:					
BA (cm^2)	248	24.8 (24.3, 25.3)	248	25.3 (24.8, 25.9)	0.153
BMC (g)	248	14.3 (13.9, 14.6)	248	14.6 (14.2, 15.0)	0.212
BMD (g/cm^2)	248	0.576 (0.570, 0.583)	248	0.576 (0.569, 0.583)	0.930
Body composition:					
Whole body (less head):					
Lean (g)	248	9006.3 (8830.2, 9182.4)	248	9248.3 (9080.0, 9416.5)	**0.051**
Fat (g) (median, IQR)	248	4516.9 (3882.8, 5360.0)	248	4447.0 (3779.8, 5276.2)	0.172
Lean mass percentage	248	64.3 (63.5, 65.0)	248	65.1 (64.4, 65.8)	0.091
Fat mass percentage	248	33.1 (32.4, 33.9)	248	32.4 (31.7, 33.0)	0.124
Grip strength:					
Maximum (kg)	262	5.74 (5.52, 5.97)	253	5.92 (5.69, 6.15)	0.271
Mean (of 6 attempts) (kg)	262	4.53 (4.34, 4.73)	253	4.67 (4.48, 4.86)	0.328

Conclusion

In the MAVIDOS RCT of maternal gestational vitamin D supplementation, offspring whole body BMC and BMD were greater in neonates born to mothers who had been supplemented with vitamin D in pregnancy compared with those of mothers allocated to placebo, for deliveries occurring in winter months. Whole body less head bone mineral density and lean mass at 4 years old were greater in the children of intervention than placebo mothers. Our findings suggest that maternal cholecalciferol supplementation may have lasting benefits for offspring musculoskeletal health. The influence of this intervention in later childhood is being assessed at 6-8 years in the follow-up of the whole multicentre MAVIDOS population.

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