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Pregnancy vitamin D supplementation and offspring bone mineral density in childhood

Follow-up of a randomised controlled trial

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Declaration of interests

RJM has received travel bursaries from Kyowa Kirin unrelated to this work. EMC has received travel bursaries or lecture fees from Eli Lilly, Pfizer, Thornton and Ross and UCB, unrelated to this work. KMG has received reimbursement for speaking at conferences sponsored by companies selling nutritional products, and is part of an academic consortium that has received research funding from Abbott Nutrition, Nestec, BenevolentAI Bio Ltd. and Danone, outside the submitted work. MKJ reports consultancy and speaker fees from UCB, Amgen, Kyowa Kirin. CC reports personal fees from ABBH, Amgen, Eli Lilly, GSK, 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, Theramex, Shire, Consilient Healthcare, Kyowa Kirin

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Abbreviations

25(OH)D, 25-hydroxyvitamin D

BA, bone area

BMAD, bone mineral apparent density

BMC, bone mineral content

BMD, bone mineral density

COPSAC₂₀₁₀, Copenhagen Prospective Studies on Asthma in Childhood

DXA, dual-energy X-ray absorptiometry

WBLH, whole body less head

.

Abstract

Background: Findings from the MAVIDOS trial demonstrated a positive effect of gestational cholecalciferol supplementation on offspring bone mineral density (BMD) at age 4 years. Demonstrating persistence of this effect is important to understanding whether maternal vitamin D supplementation could be a useful public health strategy to improving bone health.

Objective: We investigated whether gestational vitamin D supplementation increases offspring BMD at 6-7 years in an exploratory post-hoc analysis of an existing trial.

Methods: In the MAVIDOS randomised controlled trial, pregnant females <14 weeks' gestation with a singleton pregnancy and serum 25-hydroxyvitamin D [25(OH)D] 25-100nmol/l at three UK hospitals (Southampton, Sheffield and Oxford) were randomised to either 1000 IU/day cholecalciferol or placebo from 14-17 weeks gestation until delivery. Offspring born at term to participants recruited in Southampton were invited to the childhood follow-up at 4 and 6-7 years. The children had a dual-energy X-ray absorptiometry (DXA, Hologic discovery) scan of whole-body-less-head (WBLH) and lumbar spine, from which bone area [BA], bone mineral content [BMC], BMD and bone mineral apparent density [BMAD]) were derived. Linear regression was used to compare the two groups adjusting for age, sex, height, weight, duration of consumption of human milk and vitamin D use at 6-7 years.

Results: 454 children were followed up at age 6-7 years, of whom 447 had a usable DXA scan. Gestational cholecalciferol supplementation resulted in higher WBLH BMC (0.15 SD, 95%CI 0.04, 0.26), BMD (0.18 SD, 95%CI 0.06,0.31), BMAD (0.18

SD, 95%CI 0.04,0.32,) and lean mass (0.09 SD, 95%CI 0.00,0.17) compared to placebo. The effect of pregnancy cholecalciferol on bone outcomes was similar at ages 4 and 6-7 years.

Conclusions and relevance: Supplementation with cholecalciferol 1000 IU/day during pregnancy resulted in greater offspring BMD and lean mass in mid-childhood versus placebo in this exploratory post-hoc analysis. These findings suggest that pregnancy vitamin D supplementation may be an important population health strategy to improve bone health.

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Keywords

Bone mineral density; Cholecalciferol; Developmental programming; Pregnancy; Randomised controlled trial; Vitamin D

Introduction

Vitamin D has a recognized role in calcium homeostasis and skeletal health. There is increasing evidence that also suggests the importance of vitamin D to skeletal development during fetal and early postnatal life.(1, 2) In observational studies, maternal 25-hydroxyvitamin D [25(OH)D] status has been positively associated with offspring bone mineral density (BMD) and/or bone mineral content (BMC) at birth(3, 4), during childhood(5, 6) and at peak bone mass(7), although these findings are not consistent across all cohorts.(1, 8-10)

Results from intervention studies also suggest beneficial effects of gestational vitamin D supplementation on offspring BMD in early childhood.(2) In the MAVIDOS randomized placebo-controlled trial of pregnancy vitamin D supplementation in the United Kingdom(11), we demonstrated a positive effect of 1000 IU/day cholecalciferol during pregnancy on offspring whole-body-less-head (WBLH) BMD at age 4 years.(12) Interestingly, there was no difference in offspring whole body BMC or BMD at birth between the two groups.(13) This complemented the findings of the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC₂₀₁₀) trial in Denmark, in which high-dose maternal vitamin D supplementation (2800 IU/day) increased offspring whole body BMC and BMD at age 6 years compared to low-dose supplementation (400 IU/day), with similar but weaker effects at age 3 years in a subset of children.(14) Together, these findings suggest that an effect of gestational vitamin D supplementation on the offspring skeleton might evolve over childhood.

(2). This is supported by a study in a small subset of children born into the MAVIDOS study that showed greater bone anabolic response to stimulation in those born to mothers randomised to vitamin D supplementation.(15) We therefore sought

to establish persistence and/or evolution of the effect of gestational vitamin D supplementation on offspring BMD at 6-7 years in the MAVIDOS trial.

Methods

MAVIDOS was a double-blind randomized placebo-controlled trial of gestational vitamin D supplementation.⁽¹¹⁾ The trial and subsequent follow-up phases were approved by the Southampton and South-West Hampshire Research Ethics Committee and registered prospectively (ISRCTN:82927713; EUDRACT:2007-001716-23); full approval from UK Medicines and Healthcare products Regulatory Agency (MHRA) was granted. All participants gave written consent, and an adult with parental responsibility consented on behalf of their child for the offspring follow-up.

Pregnancy phase

Individuals attending for early pregnancy (11-14 weeks' gestation) ultrasound scanning at three UK hospitals (University Hospital Southampton NHS Foundation Trust, Oxford University Hospitals NHS Foundation Trust and Sheffield Hospitals NHS Trust) were invited to participate. Inclusion criteria were ≥ 18 years, singleton pregnancy and gestational age < 17 weeks. Exclusion criteria were known metabolic bone disease, renal stones, hyperparathyroidism or hypercalciuria, taking medication known to interfere with fetal growth, fetal anomalies on ultrasonography and individuals wishing to continue taking > 400 IU/day vitamin D supplementation. A blood sample was collected, and serum 25(OH)D analyzed on the local hospital platform; those with a 25(OH)D between 25 and 100 nmol/l were eligible to enrol in the study.

Participants were randomized 1:1 to either oral cholecalciferol 1000 IU/day or placebo from 14-17 weeks' gestation until delivery, as detailed previously.(13) All participants received standard antenatal care delivered by health professionals blinded to the study allocation. Participants could continue taking up to 400 IU/day vitamin D supplementation.

Assessments of lifestyle, health and nutrition by interviewer-led questionnaire and anthropometry were performed at randomization and 34 weeks' gestation. Participants were asked to self-report their ethnicity from the following categories: White, Black Caribbean, Black African, Black Other, Indian, Pakistani, Bangladeshi, Chinese, Other Asian, Other as specified by the participant. Blood samples were also collected at these study visits. Serum was stored at -80°C . 25(OH)D concentration was assessed by chemiluminescence immunoassay (Liaison automated platform, Diasorin, Minnesota, USA). All samples were analysed in a single batch at Medical Research Council (MRC) Human Nutrition Research, Cambridge, UK. Within- and between-assay coefficients of variation were 4.1 and 6.1%.

Offspring follow-up

Gestational age and birthweight were collected by a research nurse from participants' medical records. Children born to participants recruited in Southampton were eligible to continue in the offspring follow-up. Duration of consumption of human milk was established in an interviewer-led questionnaire during a home-visit at 1 year of age. At ages 4 and 6-7 years, milk intake, use of vitamin D supplementation, physical activity, and medical diagnoses were established by an interviewer-administered questionnaire. Standing height was measured using a

portable stadiometer (Leicester height measurer, Seca Ltd, Birmingham, UK), to the nearest 0.1 cm. Weight was measured in light clothing using calibrated electronic scales (Seca Ltd, Birmingham, UK) to the nearest 0.1 kg. Height, weight and body mass index (BMI) z-scores for age and sex were calculated using British reference data.(16, 17)

Whole body and lumbar spine dual-energy X-ray absorptiometry (DXA) scans were obtained using a Hologic Discovery instrument (Hologic Inc., Bedford, MA) in paediatric scan mode within two weeks of birth and at 4 and 6-7 years. Outcomes of interest were bone area (BA), BMC, BMD, bone mineral apparent density (BMAD)(18), fat and lean mass. Two researchers masked to treatment allocation reviewed the scans and those with substantial movement artefact affecting the whole body and/or both legs/both arms were excluded. In scans with movement artefact in one limb, the region of interest (ROI) of the unaffected limb was transposed into the limb with movement artefact. The DXA instrument underwent daily calibration using a spine phantom. The experimental coefficient of variation for this instrument when a spine phantom was repeatedly scanned in the same position 16 times, in a single session with no repositioning, was 0.68%.

All participants, children, and researchers remain blinded to the treatment allocation.

Statistical analysis

The primary analysis was limited to children born at term ($>37^{+0}$ weeks^{+days} gestation) as these children had received full exposure to the study intervention. In a

further sensitivity analysis, all children were included, irrespective of their gestation at birth.

Between group comparisons on the effects of gestational vitamin D supplementation (maternal 25(OH)D, offspring outcomes) and comparing maternal characteristics for those included versus not included in this follow-up were performed using t-tests, Mann-Whitney U tests and Chi-squared tests for normally distributed continuous, non-normally distributed continuous and categorical variables, respectively. Results are presented as mean (standard deviation [SD]), median (interquartile range [IQR]) and n (%), respectively.

At 4 and 6-7 years, WBLH scans were used for the primary analysis(19); at birth, whole body scans were used as isolating the skull ROI is not possible at this age. In secondary analysis whole body scans were used to assess whether the use of WBLH DXA accounted for the different findings at birth and 6-7 years.

Although DXA outcomes were normally distributed, these were transformed to a standard deviation scale using Fisher-Yates normal scores for ease of comparison of effect sizes in regression models. Offspring sex and age at DXA were included in the models to increase the precision of the effect size estimates.(20) Height and weight were included to minimise the effect of bone size on BMD measured by DXA.(21) In a further model, duration of consumption of human breast milk and use of vitamin D supplementation at 6-7 years were included as these differed between the two groups and may be associated with BMD in childhood (22, 23). Assumptions of normality and homoscedasticity of residuals were assessed after fitting linear regression models. Adjustment for multiple comparisons was not undertaken as the DXA outcomes are associated with each other (Supplementary table 1) and applying formal adjustments to account for multiple comparisons can sabotage the

interpretation of findings when the outcomes are associated (24). Whilst this is statistically appropriate, the post-hoc exploratory nature of the analysis of course provides less robust statistical evidence than would findings from a prespecified primary analysis.”

We assessed for an interaction between the intervention and (i) child’s sex and (ii) maternal 25(OH)D at randomisation (using a threshold of 50 nmol/l).

Additionally, we examined the differences in the effect of pregnancy vitamin D supplementation in the children with scans at 4 and 6-7 years and at all three follow-up phases. Sex, age, height and weight at DXA were included in these models, and in a further model duration of human milk consumption and use of vitamin D supplements were additionally included only for the outcomes at 4 and 6-7 years.

All analysis was performed using Stata V17.0 (StataCorp LP, College Station, TX, USA).

Results

Between 10th October 2008 and 11th February 2014, 1134 individuals agreed to participate in the original trial. 965 continued in the study until delivery, of which 767 were born in Southampton (Figure 1). 723 of these infants were born at term and 477 had a usable DXA scan (either at the whole body or spine) at age 4 years between 4th April 2013 and 25th October 2018. Between 22nd November 2016 and 12th April 2022, 454 (63% of eligible children) attended the 6-7 year visit, of whom 447 had a usable DXA scan (Figure 1). Five children (1.1% of attendees; 2 placebo, 3 cholecalciferol) were aged between 8.0 and 8.1 years due to delays in attendance resulting from the COVID-19 pandemic.

Maternal characteristics for the children attending at 6-7 years were similar between the randomization groups (Table 1). Compared with those not participating in the follow-up, children who attended this visit were born to participants who were older, less likely to smoke in pregnancy and had achieved a higher educational level (Supplementary Table 2). The children in the two groups were similar in age, sex, height, weight and BMI z-score at the 6-7-year visit (Table 2). Children in the cholecalciferol group, on average, consumed human breast milk for longer and a somewhat greater proportion were taking vitamin D supplements at 6-7 years (Table 2). Medical diagnoses were similar for each group (Supplementary Table 3).

WBLH BMD and BMAD were greater in the cholecalciferol group than the placebo group at 6-7 years (Table 2). WBLH BA, BMC and lean mass were also numerically greater in the cholecalciferol group, but this difference was not of statistical significance (Table 2). There was less evidence of an effect on lumbar spine parameters.

Figure 2 shows the effect of gestational cholecalciferol supplementation compared with placebo on offspring bone outcomes with adjustment for age, sex, height, weight, duration of human milk consumption and use of vitamin D supplementation at age 6-7 years. This displays the positive effect of gestational cholecalciferol on WBLH BMC (0.15 SD, 95%CI 0.04, 0.26), BMD (0.18 SD, 95%CI 0.06,0.31) and BMAD (0.18 SD, 95%CI 0.04,0.32), with similar direction of effects at the lumbar spine (data shown in Supplementary Tables 4 and 5). This model included 384 children (201 cholecalciferol, 183 placebo) due to missing covariates (47 duration of human milk consumption, 14 vitamin D supplementation at age 6-7 years, 13 height/weight). In the fully adjusted model, WBLH lean mass was also greater in the cholecalciferol group (0.09 SD, 95%CI 0.00,0.17, $p=0.05$). The findings were

unchanged when whole body rather than WBLH scans were used (Supplementary Tables 4 and 5).

No significant statistical interaction between randomisation and either (i) child's sex or (ii) maternal 25(OH)D at randomisation with any of the WBLH or lumbar spine DXA outcomes was present ($p>0.05$ for all).

WBLH and lumbar spine DXA data were available at all of birth, 4 and 6-7 years for 263 and 236 children, respectively. In analysis of this subset, with adjustment for age, sex, height (length at birth) and weight, no effect of gestational cholecalciferol on offspring WBLH bone outcomes at birth, but a positive effect of similar magnitude at 4 and 6-7 years (Figure 3, Supplementary Table 6). At the lumbar spine, there was a difference in BA and BMC noted at 4 years of age (although they did not reach statistical significance), which disappeared at 6-7 years, but a suggestion of greater lumbar spine BMAD at 6-7 years (Figure 3). Additional adjustment for vitamin D supplementation use in childhood and duration of consumption of human milk did not fundamentally change these findings (Supplementary Table 7).

An additional 24 children (13 placebo, 11 cholecalciferol) who had been born preterm (median 36.1 weeks, range 32.3-36.9 weeks) participated in the 6-7 year follow-up. The inclusion of these children in the analysis did not change the overall findings (Supplementary Table 8).

Discussion

In this follow-up of the MAVIDOS randomised placebo-controlled trial, pregnancy supplementation with 1000 IU/day cholecalciferol increased offspring WBLH BMC and BMD at 6-7 years, with a similar direction of effect at the lumbar spine. This strengthens inference from previous MAVIDOS data, by showing persistence of the

previously demonstrated positive effect of pregnancy vitamin D supplementation on offspring BMD at age 4 years.(12)

These findings are consistent with those from the COPSAC₂₀₁₀ study in Denmark, in which 2800 IU/day compared with 400 IU/day cholecalciferol from mid-pregnancy until 1 week after delivery resulted in higher whole body BMD and BMC adjusted for age, sex, height and weight at age 6 years in 383 children, with similar effects for WBLH measurements.(14) The observed effect sizes (0.15-0.20 SD) in that study were of comparable magnitude to our results (0.15-0.18 SD). In contrast, O'Callaghan et al found no differences in WBLH BMD or BMC at 4 years of age in offspring of children born to mothers randomised to either placebo (n=114), 4200 IU/week (n=126), 16800 IU/week (n=120), or 28000 IU/week (n=121) cholecalciferol in Bangladesh(25), but that trial was performed in a very different geographical location and population to the MAVIDOS and COPSAC₂₀₁₀ studies and the competing effects of other pre and post-natal environmental factors, such as malnutrition, micronutrient and calcium deficiency, infections and healthcare accessibility on skeletal development are important to consider when comparing the studies. Furthermore, it incorporated weekly supplementation, compared with the daily supplementation used in the two European studies; weekly supplementation may lead to greater fluctuations in maternal 25(OH)D status (26). There are currently no intervention studies of pregnancy vitamin D supplementation with DXA assessment at an older age than the children in the MAVIDOS trial,(2) but data from an Australian observational mother-offspring cohort study showed a positive association between maternal 25(OH)D at 18 weeks' gestation and offspring whole body BMD and BMC at 20 years of age.(7)

The primary outcome of the MAVIDOS trial was offspring BMC at birth. There was no difference between the randomisation groups, although in stratified analyses a positive effect of cholecalciferol supplementation on BMC in infants born in winter was observed.(13) It is therefore interesting that an effect across the whole cohort on BMD adjusted for age and sex of similar magnitude was observed at 4 years (0.17 SD, 95%CI 0.00, 0.35 (12)) and 6-7 years (0.16 SD, 95% CI -0.01, 0.34). Notably, the analysis at 4 years included all children irrespective of gestation at birth, whereas in this analysis we excluded a small number of children born preterm as they would have had less exposure to the pregnancy intervention and prematurity is recognised as a risk factor for low BMD in childhood.(27) However, sensitivity analysis including these children did not alter the overall findings. Adjustment for height attenuated the observed effect on BMD at 4 years of age, but not at 6-7 years. Given the recognised bias of greater body size on DXA measured BMD (18), this difference may reflect subtle differences in height of the two groups at the follow-up ages, with children in the cholecalciferol group being on average taller at age 4 years, but shorter at age 6-7 years. High resolution peripheral quantitative computed tomography (HRpQCT), which has been undertaken on a subset of these children at 6-7 years, is less subject to influence by height and may provide further insight into the effect of gestational vitamin D supplementation on offspring bone microarchitecture and true volumetric BMD (analysis in progress).

Similarly to MAVIDOS, in the COPSAC₂₀₁₀ trial, there was no difference in offspring DXA outcomes at 3 years of age.(28) It is possible that this represents reduced statistical power in the COPSAC₂₀₁₀ given the smaller subset of children with successfully obtained DXA at age 3 years (n=244) compared with 6 years (n=383), although this is in contrast to MAVIDOS where DXA was available on more infants at

birth than at ages 4 or 6-7 years. WBLH scans are the preferred site for DXA in childhood as the relatively large size and greater BMD of the skull can mask effects on the remainder of the skeleton.(19, 29) However, at 6-7 years, the effect of the intervention on whole body outcomes were very similar to WBLH. It is therefore unlikely that this methodological difference in scan parameters at birth and 6-7 accounts for the differing effects at the two ages. Additionally, subgroup analysis of the children with DXA at all three timepoints, which were broadly similar to the whole cohort analysis, suggests that the changing effect is not due to inclusion of different children at each age studied.

Our findings suggest that the effect of gestational cholecalciferol on offspring BMD may not result directly from increased calcium availability to the fetus as a difference in bone measures would have been expected in the neonatal period. We have previously reported in this trial that maternal supplementation resulted in an increase in umbilical cord blood 25(OH)D concentration, considered to reflect neonatal vitamin D status.(30) The circulating half-life of 25(OH)D is 2-3 weeks.(31) Pregnancy vitamin D supplementation has been shown in one study in Bangladesh to improve infant 25(OH)D during the first 2 months of life(32), thus the higher 25(OH)D at birth may allow for increased intestinal fractional calcium absorption during the first few months of postnatal life. Furthermore, whilst the vitamin D content of breast milk is low, risk factors associated with a lower breast milk anti-rachitic activity (the sum of vitamin D₂, D₃, 25(OH)D₂ and 25(OH)D₃) are similar to those for vitamin D deficiency (e.g. lack of supplementation, season, darker skin pigmentation).(33) As such, improving maternal 25(OH)D status in the early postnatal period through pregnancy supplementation could be having an indirect effect on offspring bone development via increased breast milk vitamin D content.(34) Thus, mechanisms related to early

postnatal vitamin D status might account for the evolution of an effect of pregnancy vitamin D supplementation on skeletal mineralisation between birth and age 4 years, although previous studies of postnatal vitamin D supplementation in infancy have not shown an effect on BMD.(25, 35) There are no reliable data on BMD between birth and 3-4 years in trials of gestational vitamin D supplementation(2) to elucidate at what point an effect becomes apparent.

Alternatively, epigenetic mechanisms may be implicated in the evolving effect of pregnancy vitamin D on offspring skeletal development observed in our trial. Data from studies in both animals and humans, including intervention studies of gestational vitamin D supplementation,(12, 36) support a role for vitamin D status in epigenetic programming.(37) Indeed, in a small trial of gestational vitamin D supplementation in pregnancy [3800 IU (n=3) vs 400 IU (n=7)], methylation differences in a number of genes, including those involved in bone and metabolic functions, were identified in offspring leucocytes.(36) Epigenetic mechanisms could underlie our previous observation that gestational vitamin D supplementation improves the anabolic response of the offspring's bone to mechanical loading,(15, 38) which would explain the evolving effect of gestational vitamin D supplementation on the skeleton during childhood. Further replication of the epigenetic findings in larger studies is needed, alongside detailed biochemical studies to try to establish further potential mechanistic pathways.

There are no previous data relating maternal vitamin D status to offspring lumbar spine DXA measurements, but in an observational birth cohort study, Javaid et al reported no association between maternal 25(OH)D status in late pregnancy and offspring lumbar spine BA, but positive associations with lumbar spine BMC and BMD, at age 9 years.(5) Overall, the effect of gestational cholecalciferol on offspring

lumbar spine BMC and BMD at 6-7 years were weaker than for WBLH, but with a similar magnitude of effect for BMAD. Interestingly, the data on children with longitudinal DXA measurements suggests that the intervention resulted in greater lumbar spine bone area and BMC at 4 years, but by 6-7 years these parameters did not differ between the two randomisation groups, but BMAD (and to a lesser extent BMD) were greater in the children born to mothers randomised to cholecalciferol. This suggests that early life vitamin D exposure may have an early positive effect on spinal growth coinciding with the period of rapid spinal growth in infancy,(39) with a greater effect on spine mineralisation from later in childhood. However, no effect of pregnancy vitamin D supplementation on offspring height was statistically apparent in our study or has been shown to persist beyond early infancy in other published trials.(14, 25, 40)

The observed positive effect sizes are likely to be of clinical significance. Although increased physical activity in childhood may be associated with both greater BMD and higher fracture risk (41), on the whole, the evidence supports the notion that increasing BMD in childhood will reduce fracture risk (42); in a study of over 6000 children, a one SD reduction in WBLH BMD at age 9 years was associated with a 1.12 increased odds of fracture over the subsequent 2 years.(21) The 0.18 SD difference in WBLH BMD between the two randomisation groups would therefore be expected to reduce offspring fracture risk, and indeed a lower fracture incidence was observed at age 6 years in post-hoc analysis of the COPSAC₂₀₁₀ trial.(14) Furthermore, whilst the reduction in odds of fracture in childhood may be small (~2%), if this effect size on BMC and/or BMD were sustained into adult life, it would similarly be expected to translate to a clinically meaningful reduction in the burden of fracture in later life given the high frequency of fragility fracture in the population.(43)

Further follow-up of this cohort of children during early adolescence is ongoing (commenced May 2023) to establish persistence of this effect and to obtain biological samples to undertake further work to elucidate mechanisms underlying the observed effects. Considering the low cost of pregnancy vitamin D supplementation, if these findings can be replicated and persist through puberty, increasing the currently recommended pregnancy supplementation guidance (44-46) to 1000 IU/day should be considered, particularly considering the other suggested benefits for maternal and offspring health.(47-49)

The MAVIDOS study is the largest study of pregnancy vitamin D supplementation to assess offspring BMD and has the furthest duration of follow-up, but is not without limitations. Due to an ethical stipulation, only individuals with a baseline 25(OH)D between 25-100 nmol/l were eligible to take part in the trial. Thus, individuals who were very deficient in vitamin D who would perhaps be expected to derive the greatest benefit from supplementation were excluded. This limitation would be expected to favour the null hypothesis, yet despite this, a positive effect of vitamin D supplementation has been shown. However, replication of these findings in individuals with vitamin D deficiency is needed. The participants were predominately of White ethnicity, reflective of the local population, tended to be well-educated and when considering the BMI distribution for both the mothers and offspring, overweight was common. This may limit the generalisability of our findings to other populations, and indeed the differences between our findings and those of the study in Bangladesh(25) highlight that effects may differ depending on the presence of other risk factors for poor bone health such as poor nutrition. Only 47% of the original cohort participated in this follow-up phase and this post-hoc exploratory follow-up was not included in the original trial design or statistical analysis plan. There were

differences between the participants that continued in the study compared to those that did not, in that they tended to be born to mothers who were older, less likely to smoke and more highly educated. This may introduce bias into the analysis and affect the generalisability of the study. Whilst it would not be expected that allocation to the intervention or placebo (to which the participants remain blinded) would influence the likelihood of non-participation, the possibility of non-random dropout remains, with the associated potential to influence the results. Furthermore, due to missing covariates on duration of human milk consumption and use of vitamin D supplementation, the number of children included in the fully adjusted model reduced by 15% compared to the unadjusted model. Nonetheless, the effect size estimates were similar in the minimally and fully adjusted models.

In conclusion, we have demonstrated in a randomised placebo-controlled trial that supplementation with 1000 IU/day cholecalciferol from 14-17 weeks' pregnancy until delivery results in higher offspring BMD at age 6-7 years. These findings suggest that pregnancy vitamin D supplementation may represent a population health strategy to improve bone health, although further work is needed to demonstrate persistence of this effect into adulthood, together with, ideally replication in additional studies.

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Contributor statement

RJM, EMC, KAW, IS, MKJ, NJB, KMG, CC and NCH and all members of the MAVIDOS study group designed the research. RJM, EMC and IS conducted research. SD and SRC analyzed data or performed statistical analysis. RJM, SD and NCH wrote the paper. NCH has primary responsibility for the final content. All authors have read and approved the final manuscript.

Data sharing plan

Data described in the manuscript, code book, and analytic code will be made available upon request pending application to and approval by the trial steering committee. Proposals should be directed to nch@mrc.soton.ac.uk. To gain access, data requestors will need to sign a data access agreement.

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Figure Legends

Figure 1: Participant flow diagram

(DXA, dual energy X-ray absorptiometry; LS, lumbar spine; WB, whole body; WBLH, whole body less head)

Footnote: (a) Offspring born preterm in Southampton in control group: DXA at birth: WB n=15 LS n=12; 4 years WBLH N=12 LS N=12; 6-7 years WBLH N=13 LS N=13.
(b) Offspring born preterm in Southampton in intervention group: DXA at birth: WB n=12 LS n=9; 4 years WBLH N=6 LS N=6 ; 6-7 years WBLH N=9 LS N=10.

Figure 2: The effect of maternal pregnancy cholecalciferol supplementation compared with placebo on offspring whole-body-less-head (WBLH) (n=384) and lumbar spine (LS) (n=384 for bone area and 382 for other outcomes) bone area (BA), bone mineral content (BMC), bone mineral density (BMD) and bone mineral apparent density (BMAD) at age 6-7 years. The point estimate shows the beta coefficient (95% confidence interval) for cholecalciferol group compared to placebo (effectively the mean difference in the measure between the two groups). A confidence interval that does not cross y=0 demonstrates a statistically significant ($p<0.05$) difference between the two randomisation groups. Beta coefficients for standardised variables have been generated using linear regression and including adjustment for age at DXA, sex, height, weight, use of vitamin D supplementation at age 6-7 years and duration of consumption of human milk. * $p<0.05$

Figure 3: The effect of maternal pregnancy cholecalciferol supplementation compared with placebo on bone area (BA), bone mineral content (BMC), bone

mineral density (BMD) and bone mineral apparent density (BMAD) in children who had DXA assessment at birth, 4 and 6-7 years for (A) whole body (birth)/whole-body-less head (4 and 6-7 years) (n=263) and (B) lumbar spine (n=236). Shown as beta (95% confidence interval) for cholecalciferol group compared to placebo. Beta coefficients for standardised variables have been generated using linear regression and including adjustment for age at DXA, sex, height (length at birth), weight at all ages, and additionally use of vitamin D supplementation at time of DXA and duration of consumption of human milk at ages 4 and 6-7 years.

Tables

Table 1: Characteristics of the mothers whose children had DXA data at 6-7 years

	Placebo (n=216)		Cholecalciferol (n=231)	
	n		n	
Age at randomization, mean (SD), years	201	31.3 (4.8)	221	31.5 (4.7)
Height, mean (SD), cm	198	166.4 (6.4)	221	165.5 (6.3)
Weight, mean (SD), kg	201	73.8 (13.6)	221	71.8 (14.1)
BMI, median (IQR), kg/m ²	198	25.7 (23.1,29.6)	221	25.0 (22.4,28.5)
Smoking in pregnancy, n (%)	183	10 (5.5)	203	12 (5.9)
White ethnicity, n (%)	201	197 (98.0)	219	211 (96.4)
Nulliparous, n (%)	200	85 (42.5)	221	92 (41.6)
Educated to degree level or higher, n (%)	199	162 (81.4)	219	184 (84.0)
25(OH)D in early pregnancy, mean (SD), (nmol/l)	211	45.0 (15.9)	228	46.3 (16.8)
25(OH)D in late pregnancy, mean (SD), (nmol/l)	196	43.4 (21.5)	216	68.1 (18.7)

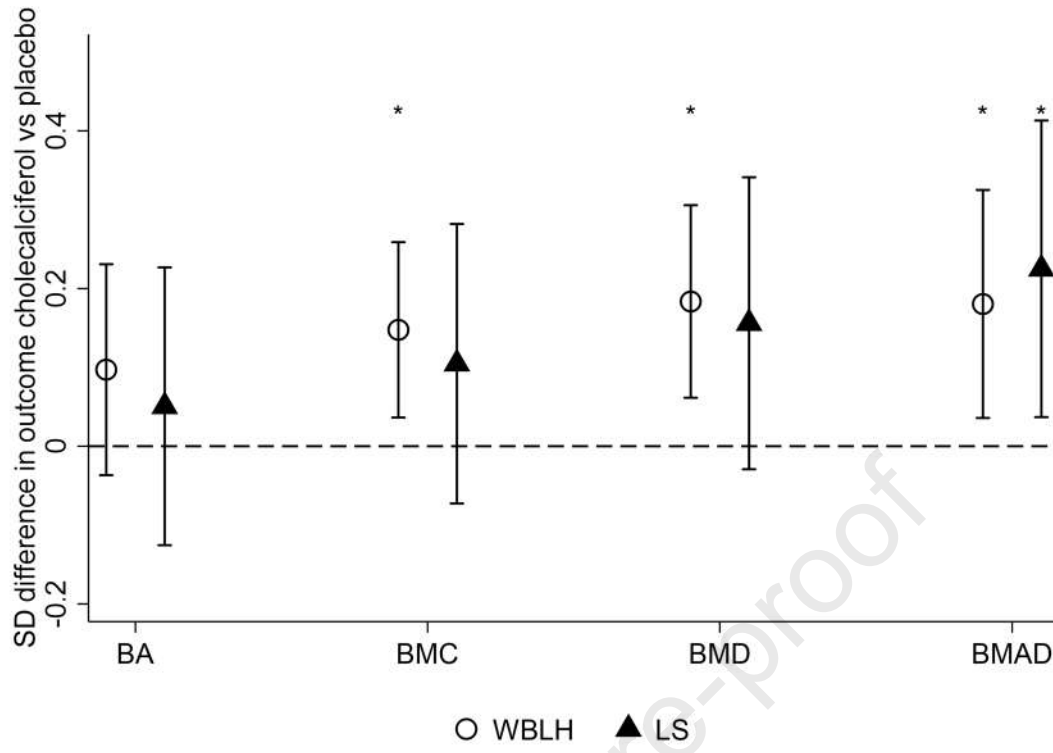
Table 2: Anthropometry, bone densitometry and body composition at age 6-7 years by maternal randomization to placebo or 1000 IU/day cholecalciferol

		Placebo		Cholecalciferol	p*
	n		n		
Age, mean (range, SD), years	216	7.0 (range 6.1-8.1, SD 0.4)	231	7.1 (range 6.2-8.2, SD 0.5)	0.64
Male sex, n (%)	216	104 (48.2)	231	129 (55.8)	0.10
Birthweight, mean (SD), g	216	3592 (452)	231	3586 (468)	0.88
Gestation at birth, median (IQR), weeks	216	40.4 (39.6,41.1)	231	40.4 (39.6,41.1)	0.99
Duration of breast feeding, median (IQR), months	190	4 (0, 9)	210	6 (1, 11)	0.01
Use of vitamin D supplementation, n (%)	210	79 (37.6)	223	103 (46.2)	0.07
Milk intake, median (IQR), pints per day	212	0.5 (0.26,0.73)	225	0.5 (0.3, 0.7)	0.99
Physical activity, median (IQR), minutes per week	179	30 (9, 60)	202	30 (0, 60)	0.61
Height, mean (SD), cm	210	123.6 (5.8)	224	123.8 (5.8)	0.68
Height z-score, mean (SD)	210	0.44 (1.04)	224	0.45 (1.04)	0.88
Weight, mean (SD), kg	210	24.7 (4.4)	224	24.7 (4.2)	0.94

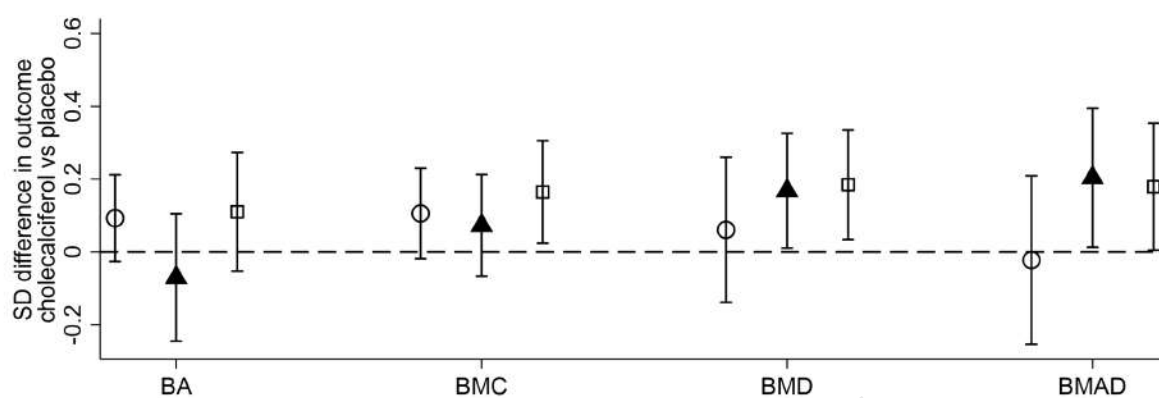
Weight z-score, mean (SD)	210	0.35 (1.05)	224	0.35 (1.01)	0.95
BMI, mean (SD), kg/m ²	210	16.1 (2.0)	224	16.0 (1.8)	0.80
BMI z-score, mean (SD)	210	0.13 (1.09)	224	0.12 (0.98)	0.90
Whole-body-less-head					
BA, mean (SD) cm ²	216	949.23 (61.18)	231	954.41 (65.92)	0.39
BMC, mean (SD), g	216	558.60 (78.92)	231	570.40 (76.84)	0.11
BMD, mean (SD), g/cm ²	216	0.586 (0.053)	231	0.596 (0.048)	0.05
BMAD, mean (SD), g/cm ³	216	0.0190 (0.0014)	231	0.0193 (0.0013)	0.04
Lean mass, mean (SD), g	216	14255 (2257)	230	14515 (2154)	0.21
Fat mass, median (IQR), g	216	5931 (4938,7536)	230	5830 (4819,7360)	0.39
Lumbar Spine					
BA, mean (SD) cm ²	216	30.06 (4.66)	231	30.14 (4.31)	0.85
BMC, mean (SD), g	215	19.51 (3.89)	230	19.84 (3.68)	0.37
BMD, mean (SD), g/cm ²	215	0.647 (0.057)	230	0.656 (0.059)	0.12

BMAD, mean	214	0.254 (0.028)	231	0.258 (0.029)	0.10
(SD), g/cm ³					

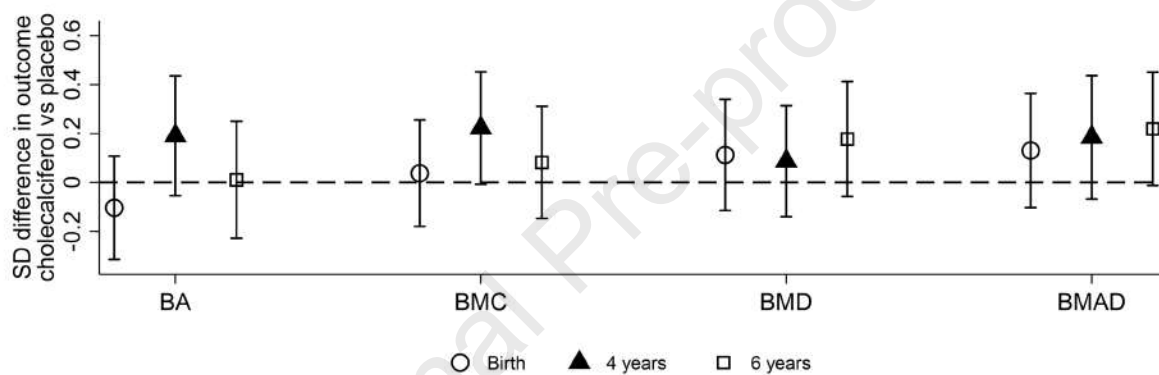
* p was obtained from t test, Wilcoxon rank sum test or Chi² test for normally distributed variables (displayed as mean (SD)), non-normally distributed variables (displayed as median (interquartile range) and categorical variables (displayed as n (%)), respectively.

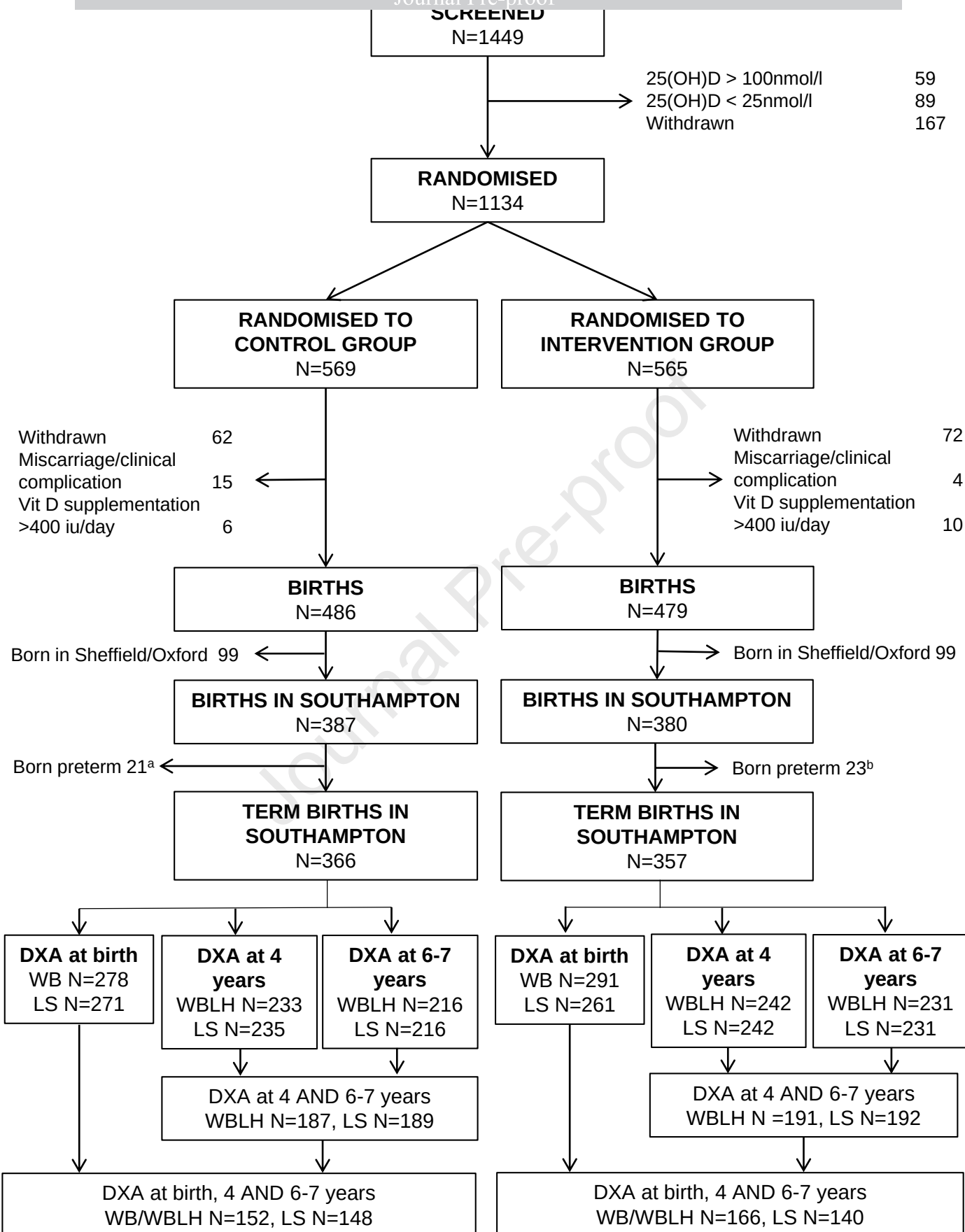


A Whole Body (Birth) / Whole-body-less-head (4 and 6-7 years)



B Lumbar Spine





Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nicholas C Harvey reports financial support was provided by Versus Arthritis. Cyrus Cooper reports financial support was provided by BUPA Health Foundation. Elizabeth Curtis reports financial support was provided by Wellcome Trust. Rebecca Moon reports financial support was provided by National Institute for Health and Care Research. Elizabeth Curtis reports financial support was provided by National Institute for Health and Care Research. Keith Godfrey reports financial support was provided by National Institute for Health and Care Research. Keith Godfrey reports financial support was provided by Alzheimer's Research UK. Cyrus Cooper reports equipment, drugs, or supplies was provided by Merck Serono GmbH. Rebecca Moon reports a relationship with Kyowa Kirin International PLC that includes: travel reimbursement. Elizabeth Curtis reports a relationship with Eli Lilly and Company that includes: travel reimbursement. Elizabeth Curtis reports a relationship with Pfizer Inc that includes: travel reimbursement. Elizabeth Curtis reports a relationship with Thornton and Ross Limited that includes: travel reimbursement. Elizabeth Curtis reports a relationship with UCB that includes: travel reimbursement. Keith Godfrey reports a relationship with Abbott that includes: speaking and lecture fees. Keith Godfrey reports a relationship with Nestec that includes: speaking and lecture fees. Keith Godfrey reports a relationship with Danone that includes: speaking and lecture fees. Keith Godfrey reports a relationship with BenevolentAI Bio Ltd that includes: speaking and lecture fees. M Kassim Javaid reports a relationship with UCB that includes: consulting or advisory and speaking and lecture fees. M Kassim Javaid reports a relationship with Amgen Inc that includes: consulting or advisory and speaking and lecture fees. M Kassim Javaid reports a relationship with Kyowa Kirin that includes: consulting or advisory and speaking and lecture fees. Cyrus Cooper reports a relationship with ABBH that includes: consulting or advisory. Cyrus Cooper reports a relationship with Amgen Inc that includes: consulting or advisory. Cyrus Cooper reports a relationship with Eli Lilly that includes: consulting or advisory. Cyrus Cooper reports a relationship with GlaxoSmithKline Inc that includes: consulting or advisory. Cyrus Cooper reports a relationship with Medtronic that includes: consulting or advisory. Cyrus Cooper reports a relationship with Merck that includes: consulting or advisory. Cyrus Cooper reports a relationship with Novartis that includes: consulting or advisory. Cyrus Cooper reports a relationship with Pfizer that includes: consulting or advisory. Cyrus Cooper reports a relationship with Roche that includes: consulting or advisory. Cyrus Cooper reports a relationship with Servier that includes: consulting or advisory. Cyrus Cooper reports a relationship with Takeda that includes: consulting or advisory. Nicholas Harvey reports a relationship with Alliance for Better Bone Health that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with Amgen Inc that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with MSD that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with Eli Lilly that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with Servier that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with Théramex that includes: consulting or advisory and speaking and lecture fees. Nicholas Harvey reports a relationship with Shire that includes: consulting or advisory and speaking

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