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Maternal vitamin D in pregnancy and offspring bone measures in childhood: The Vitamin D in Pregnancy study

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ABSTRACT

Previously we have demonstrated an association between maternal serum 25-hydroxyvitamin D (25(OH)D) during pregnancy and knee-heel length in offspring at birth. However, it is unknown whether maternal serum 25(OH)D is associated with bone measures in childhood. Thus, we aimed to examine associations between 25(OH)D at two stages of pregnancy and offspring bone measures at 11 years.

Women were recruited from a single antenatal clinic in Victoria, Australia before 16 weeks gestation and provided two serum samples to determine 25(OH)D status at recruitment and 28–32 weeks gestation. Children and their mothers were followed up at 11 years of age. Children undertook dual energy X-ray absorptiometry scans at the lumbar spine and total body.

Maternal 25(OH)D at recruitment (before 16 weeks gestation) was positively associated with the children's bone mineral content and density in boys, but not girls. In boys, a 10 nmol/L (4 ng/mL) increase in maternal 25(OH)D was associated with a median 0.5 g (95% CI 0.1,0.8) and 0.009 g/cm^2 (95% CI 0.001,0.017) increase in bone mineral content and density at the spine, respectively, and a median 0.006 g/cm^2 (95% CI 0.001,0.011) increase in at the total body. There was no sustained associations with 25(OH)D at the later timepoint (28–32 weeks) with any outcome.

At age 11 years, maternal 25(OH)D levels during early pregnancy, but not late were positively associated with bone measures in boys, but not girls.

1. Introduction

It is increasingly accepted that there are developmental origins of chronic disease in adulthood [1]. Osteoporosis is no exception and indeed the risk of developing osteoporosis in later life and consequent fracture may be programmed by early life exposures [2,3].

Vitamin D, measured as the circulating level of 25(OH)D, is a key substrate for the production of 1,25 dihydroxyvitamin D, a secosteroid hormone which plays an important role in bone growth and mineralisation [4]. A substantial number of Australian women, including those of child-bearing age, have low serum 25(OH)D levels [5]. Maternal insufficiency is a cause for concern not only for the mothers, but also because it exposes offspring to insufficiency in potentially critical early phases of development [6]. Optimising vitamin D levels may thus be an affordable modifiable factor to target during pregnancy to enhance offspring bone development.

Previously we have reported that maternal serum 25(OH)D level at 28–32 weeks gestation, but not at an earlier stage of gestation, was associated with a reduced knee-heel length, a proxy measure of long bone development in utero, in the offspring at birth [7]. Other prospective studies have explored associations between maternal 25(OH)D and offspring bone measures in early life [8]. In one study, 25(OH)D levels were associated with changes in foetal bone morphology as early as 19 weeks gestation [9]. Positive associations between maternal 25(OH)D levels and offspring bone mineral content (BMC) in childhood [10] and young adulthood [11] have previously been described,

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although null [12] and inverse associations in childhood have also been reported [13].

An apparent difference in studies is the timing of the 25(OH)D measurements. To our knowledge, no study to-date has collected maternal vitamin D levels at two defined time points in each individual and examined whether temporality of vitamin D status plays an important role in this association. We thus aimed to determine whether vitamin D status during early and later gestation was associated with bone measures in offspring aged approximately 11 years.

2. Materials and methods

2.1. Participants

Mothers were recruited as part of a prospective longitudinal study based in Geelong, a regional city in south-eastern Australia [7]. Mothers with a singleton pregnancy were recruited before 16 weeks gestation from the primary public maternity hospital servicing the region, University Hospital Geelong (formerly Geelong Hospital) between 2003 and 2004. From 475 women recruited, 402 infants were born to mothers who had at least one blood sample taken during pregnancy and provided some measurements at time of birth. Two hundred and eight mother-child pairs were assessed at follow-up during the period 2013 to 2016, when the children were aged ~11 years. The study approval was obtained from the Barwon Health Human Research Ethics Committee. Informed consent was obtained at each phase from the mothers and optional assent was provided by offspring at the current follow-up.

2.2. Vitamin D assay

Maternal serum samples were taken at recruitment and at 28–32 weeks of pregnancy. Venous samples were centrifuged within 2 h and stored at −70 °C until they were analysed. Assays were undertaken in batches by radioimmunoassay (Immunodiagnostic Systems, Tyne and Wear, UK); this assay reports a 75% cross-reactivity with 25(OH)D_2 and measures 100% of 25(OH)D_3. At 30 nmol/L the co-efficient of variation (CV) was 10.2%, and similarly 10.1% at 100 nmol/L. All testing was performed at the Royal Children's Hospital, Melbourne, which participates in both national and international quality assurance schemes.

2.3. Offspring DXA measures

At the 11 year follow-up, lumbar spine and whole body scans were performed for the offspring (GE Lunar Prodigy, Madison, USA); the scans were analysed using paediatric software (GE Encore v. 13) by the same operator (NKH). From scans, BMC, areal bone mineral density (BMD) and total lean and fat mass measures were ascertained. Equipment-specific phantoms were used to perform daily calibration of the densitometer. Further estimations of volumetric density, bone mineral apparent density (BMAD), were calculated using the methods of Katzman et al. [14]. In accordance with the International Society for Clinical Densitometry (ISCD) paediatric guidelines, the regions of interest included lumbar spine (L2-L4) and total body less head (TBLH) [15].

2.4. Other offspring measures

Offspring were measured soon after birth (0-72 h) by trained personnel using standardised techniques to collect weight (± 0.1 g), and knee-heel length (± 0.1 mm). At the current follow-up children were weighed on electronic scales (± 0.1 kg) with minimal clothing and shoes removed. Height was measured using a stadiometer (± 0.1 cm; Harpenden). Children's pubertal stage was collected by self-report and categorised as Tanner stage 1–5; groups were subsequently collapsed into a binary low/high variable (1–2 = low and ≥3 = high) [16,17].

2.5. Maternal anthropometry and measurements

Mothers were assessed when recruited and at the 11 year follow-up. Lifestyle and socio-demographic factors during pregnancy such as smoking status and education were completed by self-reported questionnaire at recruitment. Maternal height (± 0.1 cm) was measured at recruitment and at the 11 year follow-up. For these analyses we used maternal questionnaire data from recruitment and anthropometry from follow-up; however, if data were missing for the appropriate visit, the alternate values were used (height n = 2, education and parity n = 2).

2.6. Statistical analysis

Characteristics of responders were compared to those of non-responders. Mann-Whitney U tests were used for non-parametric continuous variables across two groups or Kruskal-Wallis when there were three or more groups. t-Tests were used for normally-distributed continuous variables and chi-square tests used to compare categorical variables.

Outcomes, exposure and other covariates are presented as median (interquartile range) or mean (± standard deviation). Median and quartile regressions (0.1, 0.25, 0.5, 0.75 and 0.90) were performed to examine the relationship between maternal vitamin D (exposure of interest) and offspring bone outcomes using L1-norm estimation. Maternal vitamin D was entered into regression models separately as both a continuous and categorical covariate. For the 25(OH)D categorical variable a cut-off point of 28 nmol/L (~11 ng/mL) which was used in the original analyses as used in previous results from this cohort at birth [7] (n = 12 women serum 25(OH)D < 28 nmol/L at both recruitment and n = 12 at 28–32 weeks), and also a higher data driven 75.9 nmol/L (~30 ng/mL). A bootstrap technique was used to calculate simultaneous estimation of parameter standard errors and p-values (i.e. simultaneous estimation of 10th, 25th, 50th, 75th and 90th percentile regression models) to avoid type-I error inflation due to implementing multiple regression models. Quantile regression model was used instead of conventional linear regression model (i.e. least squares regression) because L1-norm estimation is robust against outliers and influential data, it does not assume a particular parametric distribution for the response, nor does it assume a constant variance for the response, unlike least squares regression. Models were built by using backwards elimination variable selection technique and tested for both offspring and maternal factors. Maternal height, smoking and education, and children's sex, height, fat mass, lean mass, birth knee-heel length and pubertal stage were all tested in models predicting bone outcomes. The final variables were child lean mass, fat mass, height, pubertal status and sex. Sex interactions were observed between 25(OH)D at recruitment and some bone outcomes; thus, for the purpose of these analyses, data were stratified by sex. All analyses were performed using Stata (version SE 15.0).

3. Results

3.1. Sample characteristics

Of the original 402 mother-child pairs, 208 (51.7%) participated in the 11 year follow-up. Of these, 195 had DXA measurements for both the spine and TBLH, and 183 had complete DXA measures and maternal vitamin D measurements at both recruitment (before 16 weeks) and 28–32 weeks gestation. A further two older children were excluded from analyses as their bone measures were outliers (+5.9 SD and +4.6 SD).

Women who were included in final analyses had similar characteristics to non-responders and those not included in the current analyses (Table 1); except that, women included in the current analyses were older than those who did not return. There were no significant differences in any offspring birth measures.

Table 1
Characteristics and differences between the current sample (n = 181) and those excluded or lost to follow-up since birth (n = 221).

Characteristics	Included in analyses	Not included in analyses	p
Maternal			
Age at delivery (years)	30.3 (4.2)	29.2 (5.1)	0.02
Height (cm)	165.8 (6.7)	166.4 (7.4)	0.82
Weight (kg)	73.6 (60.4–79.4)	71.4 (61.3–82.9)	0.56
Smoked during pregnancy n (% yes)	34 (19.0)	38 (17.5)	0.70
Completed high school (% yes)	112 (61.9)	119 (54.3)	0.15
Parity			0.53
First child	65 (36.7)	92 (42.2)	
1–2	97 (54.8)	108 (49.5)	
3 or more	15 (8.5)	18 (8.3)	
25(OH)D recruitment (nmol/L)	56.1 (42.2–73.2)	54.8 (39.7–67.2)	0.13
25(OH)D 28–32 weeks (nmol/L)	56.8 (44.2–74.7)	55.9 (39.9–84.4)	0.85
Offspring			
Birth weight (kg)	3.51 (0.6)	3.57 (0.5)	0.30
Knee-heel length (mm)	121.1 (7.4)	120.3 (8.1)	0.33
Gestation length (wks)	40.0 (39–41)	40.0 (39–40)	0.78

Data presented as mean (± SD) or median (IQR).

In this analysis, mothers had a mean age of 30.3 years at time of delivery and a median serum vitamin D level of 56.1 (42.2–73.2) nmol/L at recruitment and 56.8 (44.2–74.7) nmol/L 28–32 weeks. Maternal 25(OH)D levels varied by season at both time points ($p < 0.001$) (Supplementary Table 1), with levels highest in summer and lowest in winter. No associations were detected between 25(OH)D levels and maternal height, parity, smoking status or education at either time point (data not shown).

Table 2 shows the characteristics of the offspring at current follow-up. There were 93 (51.4%) boys and 88 (48.6%) girls; no sex differences were observed in terms of age, height, weight or TBLH bone measures. However, girls had a greater median BMD and BMAD at the spine, which appeared to be driven by a tendency for a higher median BMC. No differences were detected in continuous 25(OH)D levels for mothers of boys vs girls at either visit. However, there were less mothers of girls above the higher 75.2 nmol/L cut-off at 28–32 weeks ($p = 0.02$). Boys had a longer knee-heel length at birth, and there was a tendency for boys to have a higher birthweight.

3.2. Maternal 25(OH)D as a continuous variable and offspring bone mineral measures

In univariate analyses there was a trend for 25(OH)D at recruitment to explain 2.0% variance in spine BMD ($r = 0.14$, $p = 0.06$) and BMC (r = 0.14, p = 0.06). When the sample was further stratified by sex, in boys maternal 25(OH)D was associated with 5.8% variance in spine BMD ($r = 0.24$, $p = 0.02$) and 4.8% variance of BMC ($r = 0.22$, $p = 0.03$). Furthermore, in boys maternal 25(OH)D at recruitment was associated with 4.4% variance in TBLH BMD ($r = 0.21$, $p = 0.046$) but not with TBLH BMC ($r = 0.14$, $p = 0.18$). There was no univariate associations between maternal 25(OH)D at recruitment at the spine or TBLH in girls. There was no univariate associations between maternal 25(OH)D at 28–32 weeks with spine or TBLH BMC or BMD in pooled data, nor in either sex (all $p > 0.05$).

In adjusted sex-pooled models, there was no significant association with maternal 25(OH)D at either time point and offspring bone (Fig. 1), however there was a negative trend for TBLH BMD and BMC with 25(OH)D at 28–32 weeks. This was completely attenuated after further adjustment for season with TBLH BMD (β −0.002 95% CI −0.006, 0.001) and TBLH BMC (β −2.57 95% CI −7.55, 2.40). There was also a positive trend between 25(OH)D at recruitment and spine BMC but this was further attenuated after adjustment for season (β 0.34 95% CI −0.09,0.77). A sex interaction with maternal 25(OH)D at recruitment was observed, predicting some bone outcomes, thus data were subsequently stratified by sex. In models adjusted for offspring height, lean mass, fat mass and pubertal stage, maternal 25(OH)D at recruitment was positively associated with both BMC and BMD at the spine and BMD of the TBLH, in boys but not girls (Table 3).

The relationships between maternal 25(OH)D at recruitment and all bone outcomes were apparent in boys, but not in girls. For boys, in adjusted models for the spine, 10 nmol/L (4 ng/mL) increments in maternal 25(OH)D were associated with 0.5 g (95% CI 0.12,0.78) increases in BMC and 0.009 g/cm^2 (95% CI 0.0008,0.016) increases in BMD. For TBLH, every 10 nmol/L increase was associated with a 0.006 g/cm^2 (95% CI 0.001, 0.011) in BMD and a non-significant 7.5 g (95% CI −3.5,18.6) increase in BMC. The addition of season of serum collection and maternal 25(OH)D at 28–32 weeks to all models did not alter the magnitude nor significance of associations with maternal 25(OH)D at recruitment, except for the relationship with TBLH BMC which reached significance (β 15.4, 95% CI 3.1,27.6). In addition to median regression, simultaneous quantile regression was performed

Table 2
Sex differences in offspring clinical measures.

Offspring	Boys n = 93	Girls n = 88	p for difference
Birthweight (kg)	3.6 (0.6)	3.4 (0.6)	0.06
Birth knee-heel (mm)	123.6 (118.7–124.8)	119.4 (115.3–124.7)	0.005
Age (years)	10.9 (10.7–11.5)	10.9 (10.7–11.3)	0.32
Gestation length (wks)	40 (38–41)	40 (38–42)	0.74
Height (cm)	148.6 (7.9)	148.3 (6.6)	0.98
Weight (kg)	39.6 (35.0–47.4)	40.0 (35.1–48.7)	0.83
Tanner Stage n (% low)	80 (86.0)	75 (85.2)	0.88
TBLH BMC (g)	1063.8 (937.0–1231.7)	1089.0 (931.0–1320.4)	0.63
TBLH BMD (g/cm^2)	0.820 (0.777–0.870)	0.822 (0.778–0.868)	0.99
TBLH area (cm^2)	1329.7 (1177.7–1456.4)	1328.8 (1208.5–1509.8)	0.55
TBLH BMAD	0.092 (0.088–0.097)	0.091 (0.088–0.096)	0.16
Spine BMC (g)	23.6 (19.9–27.3)	24.7 (21.8–28.5)	0.06
Spine BMD (g/cm^2)	0.794 (0.705–0.854)	0.851 (0.784–0.907)	< 0.001
Spine area (cm^2)	29.9 (27.6–32.8)	29.4 (27.1–32.1)	0.20
Spine BMAD (g/cm^3)	0.143 (0.133–0.154)	0.156 (0.147–0.166)	< 0.001
Mothers 25(OH)D < 28 nmol/L recruitment	6 (6.5)	6 (6.8)	0.92
Mothers 25(OH)D < 75.9.nmol/L recruitment	6 (6.5)	6 (6.8)	0.92
Mothers 25(OH)D < 28 nmol/L 28 = 32 weeks	74 (79.6)	72 (81.8)	0.70
Mothers 25(OH)D < 75.9 nmol/L 28 = 32 weeks	77 (82.8)	60 (68.2)	0.02

Results presented as median (IQR), mean (SD) or n (%).
BMC: bone mineral content BMD: bone mineral density BMAD: Bone mineral apparent density.

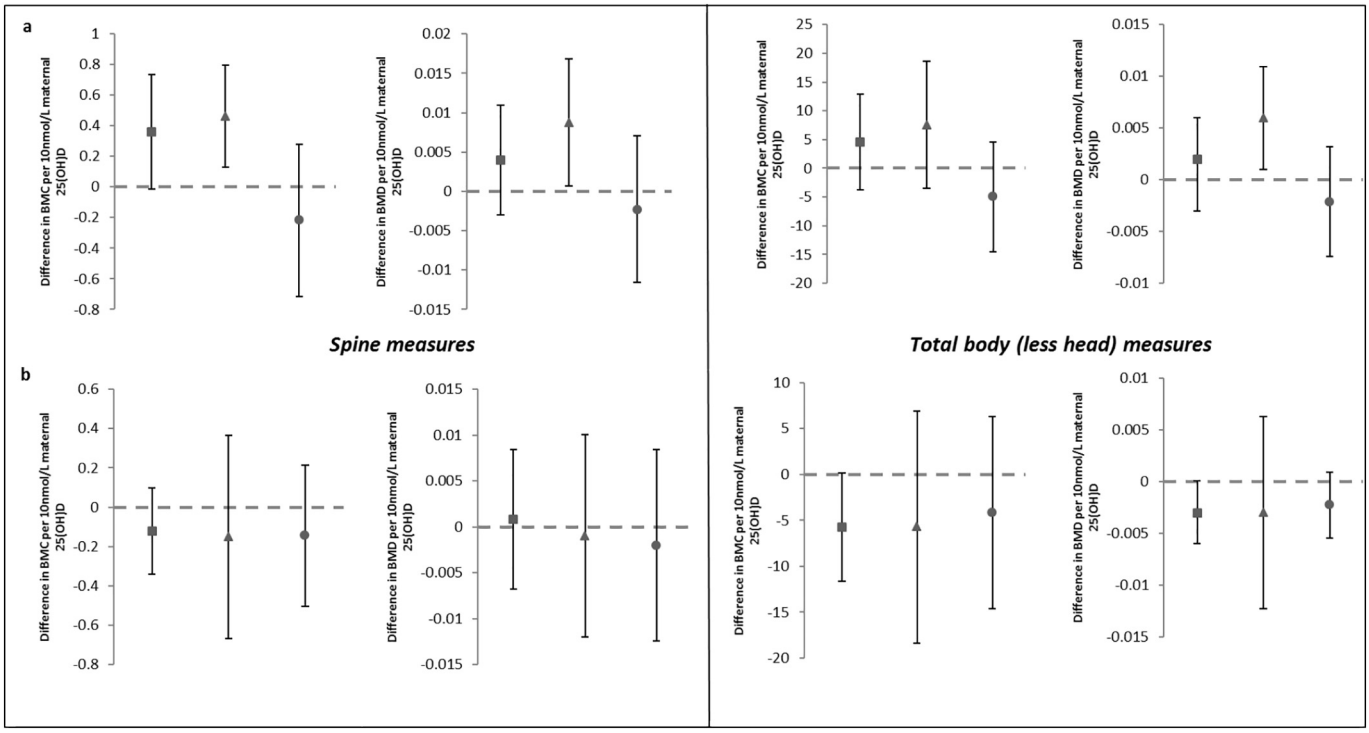

Fig. 1. Differences in bone measure per 10 nmol/L maternal 25(OH)D at recruitment as measured by dual energy X-ray absorptiometry (presented at beta co-efficient and 95% confidence intervals).
Top
a) 25(OH)D at Recruitment.
Bottom
b) 25(OH)D at 28–32 weeks
Pooled sample ■
Boys ▲
Girls ●
Models adjusted for offspring Tanner stage, lean mass, fat mass and current height (and sex for pooled sample).
BMC: bone mineral content (g) BMD: bone mineral density (g/cm^2).

Table 3
Expected differences in offspring of mothers with serum hydroxyvitamin D 25(OH)D at recruitment above different cut-off points presented as beta co-efficient and 95% CI.

Bone measure	Boys[a]	Girls[a]
Vitamin D recruitment (using 28 nmol/L cut-off)		
Spine BMC (g)	5.01 (1.66, 8.36)	1.30 (−2.67, 5.26)
Spine BMD (g/cm^2)	0.076 (0.004, 0.148)	−0.002 (−0.085, 0.061)
TBLH BMC (g)	128.68 (−29.19, 286.55)	−43.32 (−166.49,81.85)
TBLH BMD (g/cm^2)	0.052 (0.006, 0.100)	−0.002 (−0.048, 0.043)
Vitamin D recruitment (using 75.9 nmol/L cut-off)		
Spine BMC (g)	2.49 (0.29, 4.70)	−0.22 (−4.24, 3.81)
Spine BMD (g/cm^2)	0.061 (0.011, 0.110)	−0.031 (−0.094,0.033)
TBLH BMC (g)	89.28 (36.16, 142.40)	13.90 (−45.20, 73.01)
TBLH BMD (g/cm^2)	0.046 (0.20, 0.072)	0.001 (−0.022, 0.025)

BMC: bone mineral content; BMD: bone mineral density.
[a] Models adjusted for child height, lean mass, fat mass and Tanner stage.

(Supplementary Table 1). In each of the outcomes, the relationship was significant at the 0.1, 0.25 and 0.5 quantiles (with the exception of the 0.5 quantile for TBLH BMC), but not in the higher quantiles, indicating that the relationship was more prominent in those with lower bone measures. There were no associations detected between maternal 25(OH)D at 28–32 weeks and bone outcomes for boys (Table 3).

For girls, there were no associations observed between maternal 25(OH)D at recruitment, nor 28–32 weeks gestation and bone outcomes in any model (Table 3). All associations remained unchanged after the addition of season of serum sample to all models.

Maternal serum 25(OH)D and estimations of volumetric BMD.

There were no sex interactions for 25(OH)D in models predicting BMAD. In adjusted models, there was a non-significant positive trend for maternal vitamin D at recruitment and spine BMAD (β 0.001 95% CI −0.001,0.003). However, the relationship with TBLH BMAD was not significant (β 0.0002, 95% CI −0.0002,0.0008).

3.3. Maternal 25(OH)D as a binary exposure and offspring bone

When 25(OH)D was examined with the original cut point of 28 nmol/L, in adjusted models (offspring height, lean mass, fat mass and pubertal stage) there were significant associations in boys, but not girls (Table 3). In boys of mothers who had a serum 25(OH)D level > 28 nmol/L at recruitment, mean spine BMC and BMD and TBLH BMD were higher. There was no relationship in either sex with 25(OH)D at 28–32 weeks gestation (data not shown). To explore the potential of a higher cut-off point bone outcomes were examined in deciles of 25(OH)D. In unadjusted data, the relationship with 25(OH)D at recruitment appeared to be strongest in deciles 9 and 10 thus 25(OH)D was expressed as a binary variable accordingly (cut-off point 75.9 nmol/L). Using this cut-off point, maternal 25(OH)D levels in the highest two deciles were associated with higher spine and TBLH BMD and BMC, in boys but not girls (Table 3). There were no associations using the higher cut-point with 25(OH)D at 28–32 weeks gestation, with the exception of TBLH BMD in boys (β −0.04 95% CI −0.08, −0.003), however this was not independent of season (β 0.03

95% CI −0.05,0.02).

4. Discussion

To our knowledge, this study is the first to report differential relationships between maternal 25(OH)D at two stages of pregnancy and bone outcomes in offspring. Our results suggest that maternal 25(OH)D levels during earlier pregnancy may have a greater effect on offspring bone measures at age 11 years, in boys. There have been no studies describing childhood bone measures directly and risk of osteoporotic fracture later in life; however, BMD has been shown to track in childhood [18] and thus will be a major determinant of peak bone mass [19]. Given that peak bone mass plays a substantial role in the risk of developing osteoporosis in later life [20], these findings are of potential clinical significance.

The reported associations between maternal 25(OH)D and offspring bone measures concur with findings from previous cohort studies. The Princess Anne Hospital Cohort was the first to describe this relationship during childhood, in their seminal study published a decade ago [10]. The Raine cohort recently demonstrated the longevity of this relationship in offspring aged 20 years [11]. However, it must be noted that findings from the Avon Longitudinal Study of Parents and Children (ALSPAC) study contradicted these and found no association between 25(OH)D at any stage of pregnancy and offspring bone measures at age 9 years [12]. The reasons for the differences in these findings are unclear; however, heterogeneity in sample characteristics, study design and unaccounted confounding may explain some of the discrepancies. A notable difference between these studies was the timing at which the vitamin D levels were taken. Princess Anne Hospital and Raine cohorts had defined time points that differed substantially, at 34 and 18 weeks, respectively, whereas ALSPAC had samples from varying stages of pregnancy and predicted third trimester values. The later time point in our current study somewhat aligns with that of the Princess Anne Hospital Cohort whereas the first time point is closer, albeit somewhat earlier than that of the Raine study. Our overall findings more strongly concur with that of the Raine study, given that it was the first and not the second, later time point that was most consistently associated with offspring measures. Given that both our study and the RAINE study are Australian, differences in seasonal variations of 25(OH)D levels corresponding to geographical differences may play a part in these discrepancies. However, the variation in season observed in the study by Javaid et al. showed a comparable variation of maternal 25(OH)D levels by season (~35 (Winter) vs 36 (Spring) vs 76 (Summer) vs 52 (Autumn) nmol/L) [10].

From a developmental perspective, it is biologically plausible that vitamin D may have effects on foetal development in both early and late pregnancy. Long bone growth accelerates in the second trimester; however, up to 80% of mineral accrual occurs during the third trimester [21]. Our early time point coincides with the end of the first trimester. By week eight of pregnancy a cartilaginous scaffold is completely formed and in weeks eight to twelve of gestation, the primary ossification centres form both in the long bones and vertebrae [22]. Thus, early pregnancy may be an appropriate time to assess maternal 25(OH)D levels. A recently published randomised control trial (RCT) which investigated the influence on bone measures of the offspring due to vitamin D supplementation during pregnancy reported an overall null association. However, the authors also reported a seasonal interaction between treatment groups and season of birth [23]. Winter-born babies were shown to have higher bone mass in the supplement group vs the control group. From this the authors concluded that preventing a nadir in vitamin D levels during late gestation resulted in increased neonate bone accrual, supporting the original findings from the Princess Anne Hospital cohort. However, using this same logic it would be expected that an association would have also been seen in babies born in spring, which was not observed. This relationship may be further elucidated in the planned follow-up during childhood.

The findings that maternal 25(OH)D in the earlier stages of pregnancy, but not the later, has an effect on offspring bone are in contrast with earlier findings from this cohort at birth [7]. Previous results revealed that 25(OH)D levels > 28 nmol/L at 28–32 weeks, but not recruitment, were associated with a longer knee-heel length. However, this association was largely attenuated by gestation length and thus reduced overall growth as a result of shorter gestation may have explained this relationship rather than reduced bone development per se. In the current analysis, gestation length was not associated with any offspring bone measure (data not shown).

We found that there were stronger associations using a cut-point of 28 nmol/L rather than a using the higher cut-off point. However, we acknowledge our small sample size with levels below this level ($n = 12$ at each time point) limits the interpretation of these findings. Certainly, the findings from the MAVIDOS trial post-hoc analyses suggest that supplementation in those who were below 30 nmol/L was beneficial to offspring bone measures in infancy [23]. There has been much debate what constitutes true deficiency; however, RCTs to establish ideal levels are problematic, given the ethical implications of running trials in populations with levels below 30 nmol/L. Our findings suggest that there was still a positive effect of 25(OH)D at levels > 75.9 nmol/L in early pregnancy. Given our findings, we are unable to conclusively provide an ideal cut-off point in pregnancy.

There was no association with 25(OH)D at either time point in the in the sex-pooled data; however, there were significant sex interactions, whereby association with maternal 25(OH)D in early pregnancy was associated with bone measures in boys, but not girls. The combined effect of negative (albeit non-significant) coefficients for girls and positive coefficients for boys may have pulled the coefficients for the pooled data closer to the null. The reasons for the distinct sex differences remain unclear. This sexually dimorphic phenomenon has been described in several foetal developmental outcomes [24]. It remains unclear in this cohort whether this effect will be sustained into adulthood, or if it is transient and an artefact of the children's current developmental stages. One may assume the latter on the basis of previous literature. For example, the Raine study examined similar associations and found no sex interactions [11]. The ALSPAC study identified sex interactions [12], however, reported that they were completely attenuated after adjustments for offspring characteristics, unlike the observed interactions within this cohort. The velocity of bone accrual during late childhood and early adolescence remains similar in girls and boys until approximately age 11 years [25]. In the following year, velocity in girls increases whilst boys plateau until approximately age 13 years. Given that the age range of our cohort was 10–12 years, this may explain some of the discrepancies between sexes. However, we did attempt to address this issue by controlling for pubertal stages in the regression modelling but acknowledge that Tanner staging was assessed by self-report, so it is likely that some residual confounding remained.

There are limitations to these findings in this observational study. Although we have detailed data on both maternal and offspring characteristics, we cannot exclude the possibility of residual confounding. Several shared causal pathways such as genetics and lifestyle factors are possible that might influence both offspring bone measures and maternal vitamin D during pregnancy. Though results were adjusted for potential confounding we acknowledge that there may be unrecognised confounding impacting the observed associations. Although we have long-term follow-up, attrition within the cohort was considerable, although lower than in comparable cohorts [10,12]. Although this may bias our results, few differences in characteristics were observed between those who did and did not return for the 11 year follow up. Finally, there are some measurement issues with the use of DXA in children because of poorer precision in smaller subjects; however, DXA remains the ISCD preferred method for assessing paediatric BMC and areal BMD [14]. It should also be noted that the presented analyses were not adjusted for multiple comparisons given the small sample size, and thus should be interpreted within these constraints.

Despite these caveats, our findings support those from several other studies which suggest a potential association between maternal 25(OH) D levels during pregnancy and the bone health of the offspring. Future RCT data collected beyond infancy in MAVIDOS will help further elucidate this association. Furthermore, well designed RCTs in pregnancy could test the temporality of the association. Given that peak bone mass plays an important role in the risk of developing osteoporosis in later life, women should be aware of the importance of vitamin D from early pregnancy, in order to optimise offspring bone mineral measures. Future work should be considered to clarify optimal levels in pregnancy.

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Declaration of interests

NKH, SB-O, MM, SMH and JAP have nothing to declare. JDW received in-kind support from Swisse Wellness and is currently receiving grant support from the NHMRC both for for an unrelated project studying vitamin D and health in young women. JAP is currently recieving funding from the NHMRC for unrelated projects.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.04.013.

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