Maternal Serum Levels of Vitamin D and Foetal Bone Developement

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Preface

I januar 2014 tok jeg kontakt med min veileder, Dr Åshild Bjørnerem. Jeg ønsket å skrive oppgave om noe som omhandlet kvinnehelse, men har lite erfaring med oppgaveskriving på vitenskapelig bakgrunn, og ønsket å ha fokus på skriveprosessen.

Jeg og Åshild gjennomførte et par møter, diskuterte mulige prosjekter og problemstillinger, og ble enige om å gjennomføre et prosjekt basert på et eksisterende datasett. Jeg er takknemlig for det forarbeidet som her er gjort i forskningssamarbeidet som Åshild har hatt med Braidy Davies, Susan Walker, Xiaofang Wang og Ego Seeman ved Universitetet i Melbourne, Australia, hvor prosjektet er designet og data samlet inn. Prosjektbeskrivelsen består av deler av en protokoll som tilhører eksisterende datasett, slik at mitt arbeid i den sammenheng var å sette meg inn i hva som var gjort, og å få taket på hva vi skulle undersøke gjennom å lese protokollen og ulike artikler innen dette tema. I juli 2014 brukte jeg 4 uker på å registrere og systematisere 3D fostermålinger fra ultralydbilder og inn i et Excel-ark. På denne måten ble jeg kjent med data settet.

I tidsrommet mars-april 2015, startet jeg å skrive introduksjon parallelt med å lese artikler om emnet, og utarbeide litteratur tabeller for å skaffe meg oversikt over tema. Jeg var tidlig i perioden på kurs i Endnote samt SPSS. I samme periode skrev jeg metode, som i stor grad eksisterte fra protokollen, men som måtte tilpasses de dataene vi har benyttet oss av. Prosessen med å sette seg inn i litteratur, samt skrive introduksjon har vært langsom og krevende, men svært lærerik. Jeg startet med resultater, diskusjon og konklusjon i mai 2015, og har således vært litt forsinket i forhold til hva som var planlagt i forkant. Det er mye tilgjengelig litteratur som omhandler vitamin D, fostervekst og beinhelse, slik at mye tid har gått til litteratursøk og lesing.

Jeg har i hele prosessen hatt tett kontakt og mange møter med veileder. Vi har hatt lange faglige diskusjoner, og jeg har fått mange innspill til forbedringer underveis. Veileder har gjort de statistiske analyser med meg til stede, samt bidratt for å heve nivået på metode og resultater. Hun har tatt seg god tid til å forklare og trene meg i vitenskapelig tenkemåte, statistiske analyser og kritisk fortolkning av resultater. Det har vært et fruktbart samarbeid som jeg har hatt stort utbytte av, og veileder har viet meg mye tid og tålmodighet.

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Abstract

Background: Rickets disease is a well-known example of the importance of vitamin D for optimal bone health during growth. Pregnant woman often have vitamin D deficiency. However, it is not clear whether maternal vitamin D influence their offspring's bone-health, because results from different studies are conflicting. Therefore we wanted to study whether low maternal serum levels of 25-hydroxyvitamin D (25(OH)D) is associated with reduced foetal bone size and altered shape.

Methods: In this prospective cohort study, 401 healthy, pregnant women aged 20-43 years were recruited in 2008-2009 at Mercy Hospital for Woman, Melbourne, Australia and 72 of them had serum levels of 25(OH)D measured at 28 weeks gestation. Foetal femur length (FL), distal femoral metaphysal cross sectional area (CSA), splaying index (distal CSA/FL) and femur volume (FV) were measured using 2D and 3D ultrasound at 20 and 30 weeks gestation. Associations between maternal 25(OH)D and z-scores of the foetal femur measurements were tested using Pearson's correlation and linear regression analysis, and the proportion of explained variance was estimated from R^2.

Results: At 20 weeks gestation, there was no significant correlation between maternal 25(OH)D and foetal femur z-scores (all $p \geq 0.10$). At 30 weeks gestation, there was an inverse correlation of maternal 25(OH)D with foetal FL and FV, but not with other femoral z-scores. Each SD lower 25(OH)D, was associated with 0.41 higher FL z-score ($p < 0.001$) and 0.27 higher FV z-score ($p = 0.030$). These associations remained after adjustment for maternal body mass index, height, smoking, parity and ethnicity. Maternal 25(OH)D explained 16.5% of the variance in FL and 7.3% of the variance in FV.

Conclusion: The inverse associations of 25(OH)D with FL and FV are surprising and challenging to explain. More research is needed to clarify the influence of maternal vitamin D on foetal bone development.

Introduction

The importance of vitamin D for optimal bone health is well known (1-4). Severe vitamin D deficiency in children causes rickets disease, with reduced mineralization and softening of the growing bone (4, 5). In adulthood vitamin D is important for maintenance of optimal bone-health because it is essential for calcium homeostasis (5). Moreover studies have shown associations between low levels of vitamin D, falls, fractures, and osteoporosis in adults (2, 3, 6). Further, both children born small, and children with poor childhood growth are reported to have higher risk for hip fracture later in life (7). This suggests that intrauterine growth may be associated with peak bone mass in young age, and also bone-health later in life.

The main sources of vitamin D is sunlight exposure of the skin and dietary intake of fatty fish or supplements as cod liver oil (3). Vitamin D from the skin or diet is converted in the liver to the inactive form 25-hydroxyvitamin D [25(OH)D] and further converted in the kidney to the active form 1,25-dihyroxyvitamin D [$1,25(OH)_2D$]. The $1,25(OH)_2D$ is not easy available for measurement because of low serum concentration and short half-life. Even despite of vitamin D deficiency, the levels of $1,25(OH)_2D$ can be normal or elevated, making it inappropriate for assessment of vitamin D status (3). The main circulating form, 25(OH)D, is therefore commonly used for clinical assessment of the vitamin D status.

The most important role of Vitamin D is to maintain stable serum levels of calcium within the physiological range (5). This is processed through binding of $1,25(OH)_2D$ to nuclear vitamin D receptors in the small intestine, which induces the expression of epithelial calcium channels, so the calcium absorption increases (3, 5).

Pregnant woman often have vitamin D deficiency, which is reports in 12.1% - 28% of pregnant women when defined as a 25(OH)D below 25 -30 nmol/L) (8, 9). It is not clear whether maternal serum levels of vitamin D will influence their offspring's bone-health, because results from different studies are conflicting (10-14). One study reported a weak association between low maternal serum 25(OH)D in late pregnancy and reduced whole-body and lumbar–spine bone-mineral content (BMC) in offspring at nine years of

age (10). Another study added to this evidence, as they reported that maternal serum levels of vitamin D was closely related to the offspring bone development (11). This study detected no association of maternal 25(OH)D with femur length (FL) but a greater distal femoral metaphyseal cross-sectional area (CSA) and a higher splaying index(distal CSA/FL) in 19 and 34 weeks gestation (r ranging from 0.10-0.17, and p ranging from 0.001 to 0.05). These findings of wider foetal bone were interpret as a sign of intrauterine rickets in foetuses of mothers with low serum levels of 25(OH)D (< 25nmol/L) in late pregnancy. Another study looking at the effect of maternal vitamin D on foetal bone found a positive correlation between maternal serum levels of 25(OH)D and 3D femoral volume (FV) in 34 weeks gestation ($r = 0.147$, $p = 0.006$) (15).

In contrast to these findings, a study published the same year reported an association of higher maternal serum levels of 25(OH)D with larger distal tibial CSA and higher BMC of the full-term newborn offspring (12). Another study reported an association between low maternal serum levels of vitamin D in late pregnancy and reduced intrauterine growth, by using knee-heel length in newborn offspring as a measurement of intrauterine growth of long bones (16). Moreover, a large study of 3960 mother-child-pairs reported no association between maternal serum levels 25(OH)D and offspring BMC at 9-10 years of age (13). A review on maternal vitamin D deficiency and its implications on the foetus and new-born skeletal health concluded that low maternal serum vitamin D levels during pregnancy could not cause abnormal foetal skeletal parameters, as mothers provide the foetuses with adequate calcium for normal intrauterine bone development despite low maternal serum levels of 25(OH)D (14).

Based on these reports, it is not clear whether there is an association between maternal vitamin D and foetal bone development. A clearer understanding of this matter is important, as the long-term health benefit could be substantial, if sufficient maternal 25(OH)D levels lead to improvement of bone-health in the offspring. We therefore wanted to revisit this issue, and test the hypothesis that low maternal serum levels of 25(OH)D is associated with shorter femur length, greater distal femoral metaphyseal CSA, higher splaying index, and smaller femoral volume as a measure of reduced bone size and altered shape.

Method

This is a prospective cohort study which was conducted with baseline between July 2008 and June 2009, and 401 healthy, women aged 20-43 years of age with a normal, singleton pregnancy were recruited at their 20 weeks routine ultrasound scan at the Imaging Department at The Mercy Hospital for Women, Melbourne, Australia. After exclusion of 60 women who had preterm birth, complications in pregnancy, moved or were too busy to come for further follow-up, we had valid measurements on 341 mother-offspring pairs at 20 weeks gestation and 338 at 30 weeks gestation, and 318 of them had 3D measurements performed. Gestation was determined based on the last menstrual period unless the first ultrasound measurements (crown rump length (CRL) before 12 weeks or biparietal diameter (BPD) for 12-20 weeks) differed more than 7 days, gestational age was then based on assessment by ultrasound. We excluded foetuses that had major malformation detected by ultrasound scan, or a preterm delivery before 37 weeks gestation. All participants gave written, informed consent when entering the study. Mercy Health & Aged Care Human Research Ethics committee approved the study.

FL is a routine measurement for assessment of gestational age and growth with high reproducibility. Foetal size and shape was monitored using 2D ultrasound scan of FL, and 3D ultrasound scan of the FV at two occasions; at 20 (range 17-22) and 30 (range 27-34) weeks gestation. Measurements were undertaken by two experienced ultrasonographers using a Phillips IU22, Phillips HDI-5000 or a Phillips HDI-300 ultrasound machine, and used the 3D ultrasound equipment (Voluson 730 Expert GE, with RAB 4.0-8.0 MHz 3D probe). The foetal FV was semi-automatically calculated using the VOCAL computer package from GE (Fig. 1). In brief, scanning of foetal femur using a high-resolution, real time 2D ultrasound scanner was made in the traditional plane. The 3D transducer was rotated to make the femur horizontal then fixed in this plane as the basis and the cursor moved along the axis of the femur from one end of the diaphysis to the other, slice-by-slice every 3 mm. The integration of FV was calculated automatically by the 3D ultrasound scanner as the cursor moved forward and the area was enclosed. In this study we used measurements of foetal FL and distal femoral metaphyseal CSA, and calculated FV and splaying index as distal CSA/FL. All

measurements were repeated twice, for 2D measurements, the most accurate assessment was used, and for 3D measurements, the average of the two measurements was used in our analysis. In a similar study the coefficient of variance (CV) in FL was 0.6 and 0.4% and in femur distal cross-sectional area (CSA) 4.4 and 3.2 %, respectively, at 20 and 30 weeks gestation (11).

Maternal height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg by an electronic scale wearing light clothes without shoes. A questionnaire addressing parity, ethnicity and maternal smoking was filled in. Maternal serum levels of 25(OH)D was measured at 28 weeks gestation using an equilibrium radioimmunoassay after extraction with acetonitrile (Instar, Stillwater, MN, USA), with intra- and interassay CV of 6 and 15%.

Statistical analyses

All outcome variables were checked for normality. Royston models were fitted to the 2D and 3D measurements of foetal bone size and shape to create z-scores, which is accounting for the duration of gestation (17). Here we present the means with standard deviations (SD) and range. We used t-tests to compare differences between group means with standard errors of the mean (SEM) as e.g. for the difference between nulliparous vs. parous women, and Caucasian vs. those of other ethnicity of origin. The associations between maternal body mass index (BMI) and 25(OH)D as exposures (x) and foetal bone size and shape (FL, distal femur CSA, splaying index (distal CSA/FL) and FV as endpoints (y), were tested using Pearson's correlations and linear regressions models, adjusted for BMI, parity (nulliparous vs. parous), ethnicity (Caucasian vs. those other ethnicity), maternal height and smoking, which are factors well known to be associated with foetal growth. The proportion of the variance in foetal FL and FV explained by 25(OH)D and other covariates was estimated from R^2, before and after inclusion of covariates. To evaluate whether the association between 25(OH)D and foetal femur traits was modified by BMI, we included interaction terms between 25(OH)D and BMI. Data was analysed using SAS Software package, v9.3 (SAS Institute Inc., Cary, NC, USA) and $p < 0.05$ is considered significant.

Results

Characteristics of the mothers and offspring are shown in Table 1. The mean (SD) maternal age, height and weight were 31.2 years (4.4), 164.4 cm (6.7) and 76.9 kg (15.5). Of a total of 336 women who provided information about their parity, 154 (45.8%) were nulliparous, and of the 182 (54.2%) parous women, 121, 48, 6, 5, 1, and 1 of them had given birth to 1, 2, 3, 4, 5 and 6 children, respectively. Of a total of 331 women who provided information about their ethnicity, 265 (77.3%) were of Caucasian origin, and of the 78 (22.7%) women of other ethnicity, 15 were Indian, 27 were from other Asian countries, 15 were Arabs from the Middle East states, 2 were Africans, 2 were Maori from New Zealand, 2 were of mixed ethnicity with parents from Asian and Caucasian, and 1 was of mixed Maori and Caucasian ethnicity of origin.

A total of 72 mothers had mean (SD) 25(OH)D serum levels of 71.8 nmol/L (17.7), seven had 25(OH)D below 50 nmol/L, none had levels below 25 nmol/L. At 20 weeks gestation, the foetal FL was 3.16 cm (0.24), distal metaphyseal CSA was 0.35 cm^2 (0.11), splaying index was 0.11 cm^2/cm (0.03) and FV was 1.01 cm^3 (0.29). At 30 weeks gestation FL, CSA, splaying index and FV were 5.78 cm (0.34), 0.67 cm^2 (0.23), 0.12 cm^2/cm (0.04) and 4.08 cm^3 (1.01), respectively.

All maternal blood samples were collected between May and September during the winter season. There was no significant variation in 25(OH)D by date of measurement ($r = -0.02$, $p = 0.854$), as shown in Fig. 2. However, 25(OH)D levels correlated inversely with maternal BMI ($r = -0.34$, $p = 0.004$, Fig 2). Maternal BMI was not significantly correlated with foetal FL or FV at 20 weeks gestation, but with FL ($r = 0.17$, $p = 0.002$) and marginally with FV ($r = 0.11$, $p = 0.053$) at 30 weeks gestation (Fig. 3). Nulliparous had higher mean (SEM) 25(OH)D [76.5 (3.4) vs. 68.4 nmol/L (2.4), $p = 0.051$], and lower BMI [27.7 (0.4) vs. 29.0 kg/cm^2 (0.4), $p = 0.020$] than parous women. Moreover, Caucasian had marginally higher 25(OH)D [73.5 (2.1) vs. 64.8 nmol/L (6.1), $p = 0.099$], and higher BMI [28.7 (0.3) vs. 27.4 (0.6), $p = 0.062$] than those of other ethnicity.

At 20 weeks gestation, there were no significant correlation between maternal 25(OH)D and foetal FL, CSA, splaying index or FV (all $p \geq 0.10$) as shown for raw-data in Fig. 4 and z-scores in Table 2. However, at 30 weeks gestation, 25(OH)D correlated inversely with foetal FL and FV, and each SD lower 25(OH)D was associated with 0.41 higher FL z-score ($p < 0.001$) and 0.25 higher FV z-score ($p = 0.043$). Each SD lower 25(OH)D correlated with 0.31 higher FL z-score ($p = 0.023$) in women of Caucasian origin, and correlated marginally with 0.51 higher FL z-score ($p = 0.060$) in those of other ethnicity.

After adjustment for maternal BMI, each SD (17.7 nmol/L) lower maternal 25(OH)D was associated with 0.38 higher FL z-score ($p = 0.003$, Table 3). The proportion of variance in FL explained by 25(OH)D alone was 16.5%, and 17.0% after 25(OH)D was combined with BMI. After further adjustment for parity, ethnicity, maternal height and smoking, the association between 25(OH)D and FL remained similarly (STB -0.40, $p = 0.003$), and the proportion of variance in FL explained by this full model was 26.0%. The proportion of variance in FV explained by 25(OH)D alone was 7.3%, increased to 13.6% in a similar full model, and the association of 25(OH)D with FV remained the same in this model (STB -0.35, $p = 0.016$). There was no significant interaction between 25(OH)D and BMI in the model of FL ($p = 0.372$) or model of FV ($p = 0.178$).

Discussion

We report an association of lower maternal serum levels of 25(OH)D with longer foetal femur and larger femur volume at 30 weeks gestation. These associations remained significant even after adjustment for maternal BMI, height, parity, ethnicity and smoking. At 20 weeks gestation there were a similar pattern of inverse correlation of maternal serum vitamin D with FL and FV, but without reaching significance at this stage of the pregnancy. In contrast to what we hypothesized, we found no association of low maternal serum 25(OH)D with wider distal metaphyseal area or higher splaying index. The maternal 25(OH)D explained 16.5% of the variance in FL and 7.3% of the variance in FV.

Mahon et al. reported lower maternal serum levels of 25(OH)D to be associated with greater distal metaphyseal area and higher splaying index of the distal femur at 34 weeks gestation and also as early as at 19 weeks gestation (11). In contrast the study by Viljakainen et al. reported larger cross sectional area of the tibia, and higher bone mass in the newborn of mothers with serum levels of 25(OH)D above the median compared with those with levels below the median (12). There was a weak association of lower maternal vitamin D with higher birth weight z-score, but no significant association of maternal Vitamin D with birth length z-score. However, Morley et al. reported that women with low 25(OH)D (below 28nmol/L) in late pregnancy gave birth to children with shorter knee-heel-length as newborn than those with higher levels of 25(OH)D in late pregnancy (16). This difference remained significant after correcting for various maternal factors, including BMI, but the difference was reduced from 4.3 mm shorter knee-heel-length to 2.7 mm shorter after adjustment for gestational age. However, their results were not significant when looking at the strength of the association between vitamin D and offspring knee-heel-length at birth in linear regression analysis, indicating that it might be a threshold effect rather than a linear association between 25(OH)D and knee-heel-length.

All the above reports suggest a connection between maternal serum vitamin D levels and foetal skeletal dimensions, but these associations are not clear or conclusive based on those studies, as discussed in a review by Kovacs (14). He concluded that newborn are reported to have a normal skeleton despite severe maternal vitamin D deficiency,

based on results from both animal and human data. Furthermore the author emphasize that rickets may rather develop after birth, because the intestinal function will then be dependent on $1,25(OH)_2D$ for sufficient calcium absorption. His view is supported by a results from a large prospective cohort study by Lawlor et al. published in The Lancet in 2013, which tested the association of maternal 25(OH)D during pregnancy with bone-outcomes in almost 4000 offspring at 9 years of age (13). They could not find any association between maternal vitamin D levels during gestation and the offspring BMC.

To the best of our knowledge, no previous study has reported an inverse relationship between maternal serum vitamin D and foetal femur parameters, and we do not know of any biological reason for these results. As results in previous studies are conflicting, it could be that maternal serum levels of 25(OH)D does not affect foetal skeletal dimensions as suggested by Kovacs (13). Another possibility is that our results could be random findings because of the small sample size. However, the p-values were low, which signal highly significant results, and moreover the results in our data were similar for both raw-data (Figure 4) and z-scores for bone size and shape (Table 2).

The inverse relationship between BMI and vitamin D is well known (18, 19). Therefore we considered whether the inverse association of maternal vitamin D with FL and FV could be explained by maternal BMI. However, maternal BMI contributed to explain only a small proportion of variance in FL and FV and results remained independent of BMI. There may be other potential confounding factors related to low vitamin D or high BMI, such as substances derived from maternal fat deposits affecting foetal growth directly or from altering placental functions (20, 21).

The strength of our study is that we derived z-scores from a large sample-size and we accounted for a range of potential confounding factors in the analyses. One limitation in this study is the small sample size with vitamin D measurements available. We only had one single measurement of maternal serum 25(OH)D and no umbilical cord 25(OH)D. The intra-observer variability between the two sonographers has not been tested.

Conclusion

The inverse association of Vitamin D and foetal bone size and shape in this study are unexpected and challenging to explain. As there are opposing results in numerous studies there is a need of a large and thorough follow-up study, to approach the question of whether maternal serum levels of vitamin D influence on foetal bone development in the beginning of life and also later in life. There is an ongoing randomised, double-blind, placebo-controlled trial of the effect on vitamin D supplementation in pregnancy, the MAVIDOS study where whole body bone mass will be measured in newborn offspring with follow-up until four years of age (22). Results from that study are expected to make a contribution to increase our understanding on this issue.

Fig. 1. Measurements of 2D foetal femur length (FL), and 3D distal metaphyseal cross sectional area (CSA) and femur volume (FV)

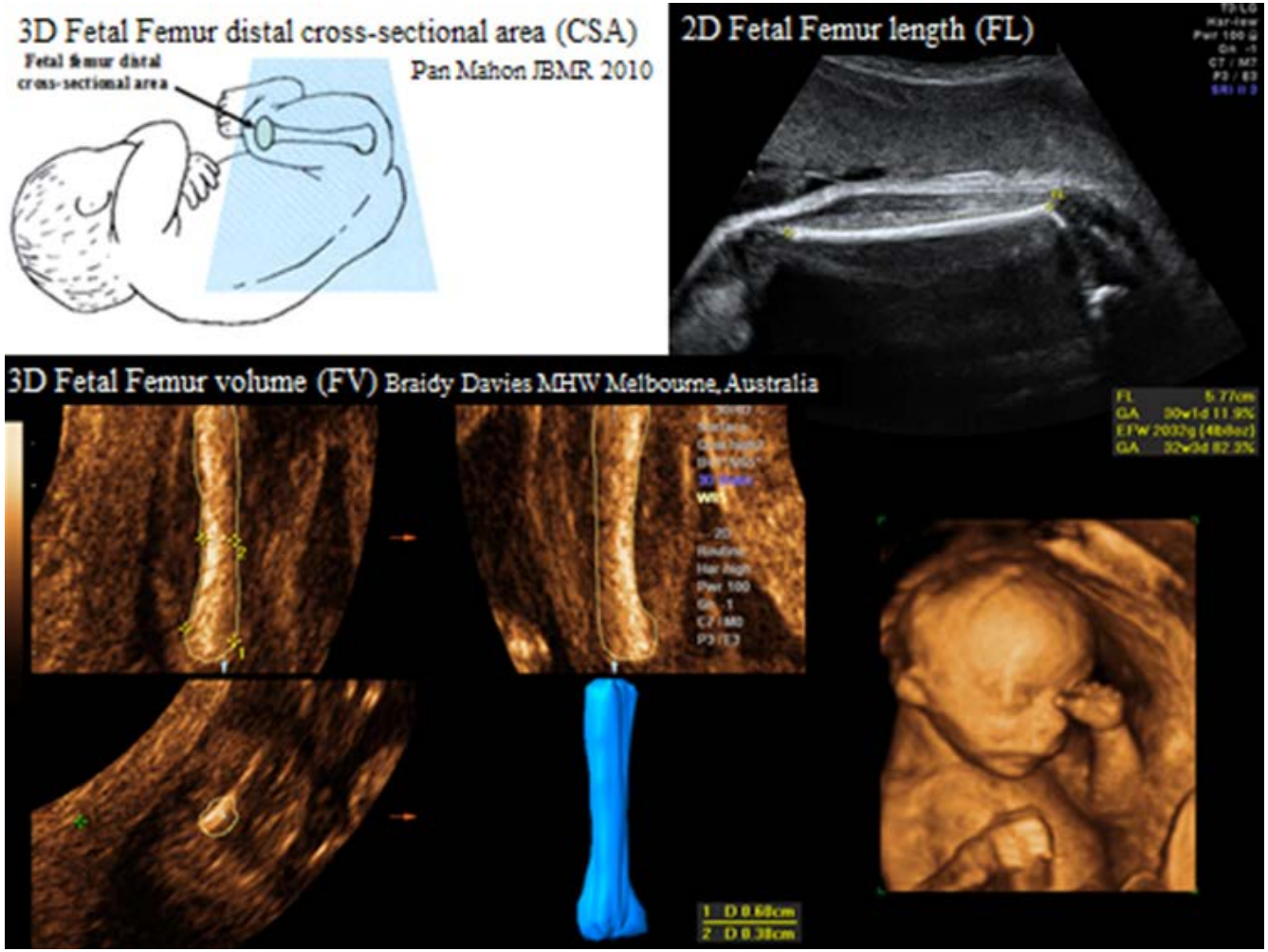
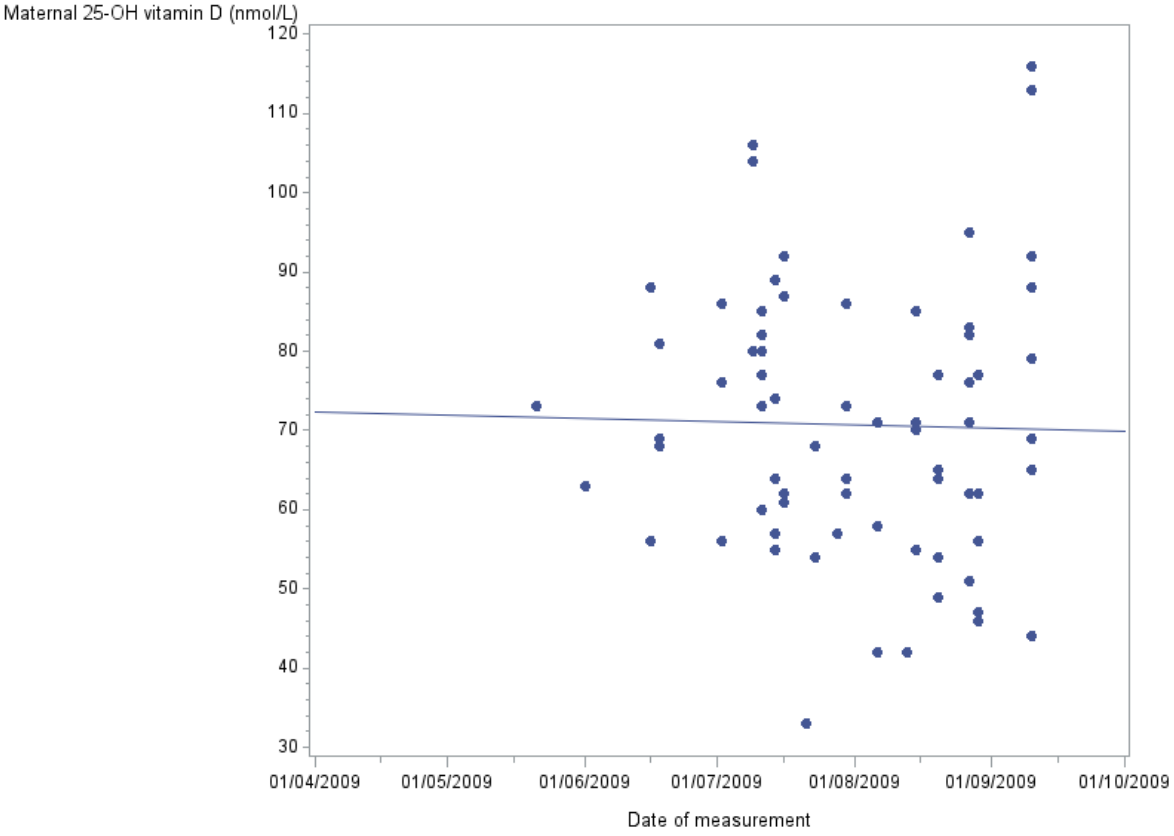


Fig. 2. Maternal 25(OH)D by date of measurments (upper panel, r = - 0.02, p = 0.854) and by maternal body mass index (BMI) (lower panel, r = - 0.34, p = 0.004)



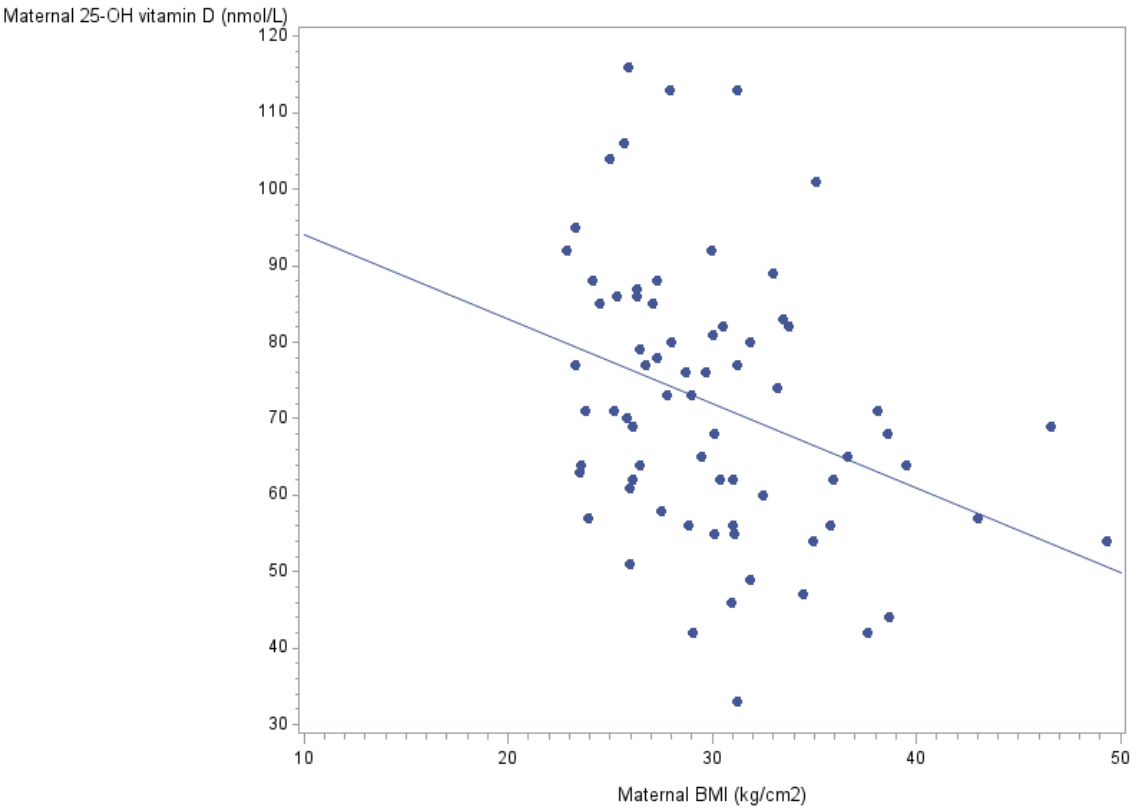


Fig. 3. Fetal femur length and femur volume at 20 and 30 weeks gestation by maternal body mass index (BMI)

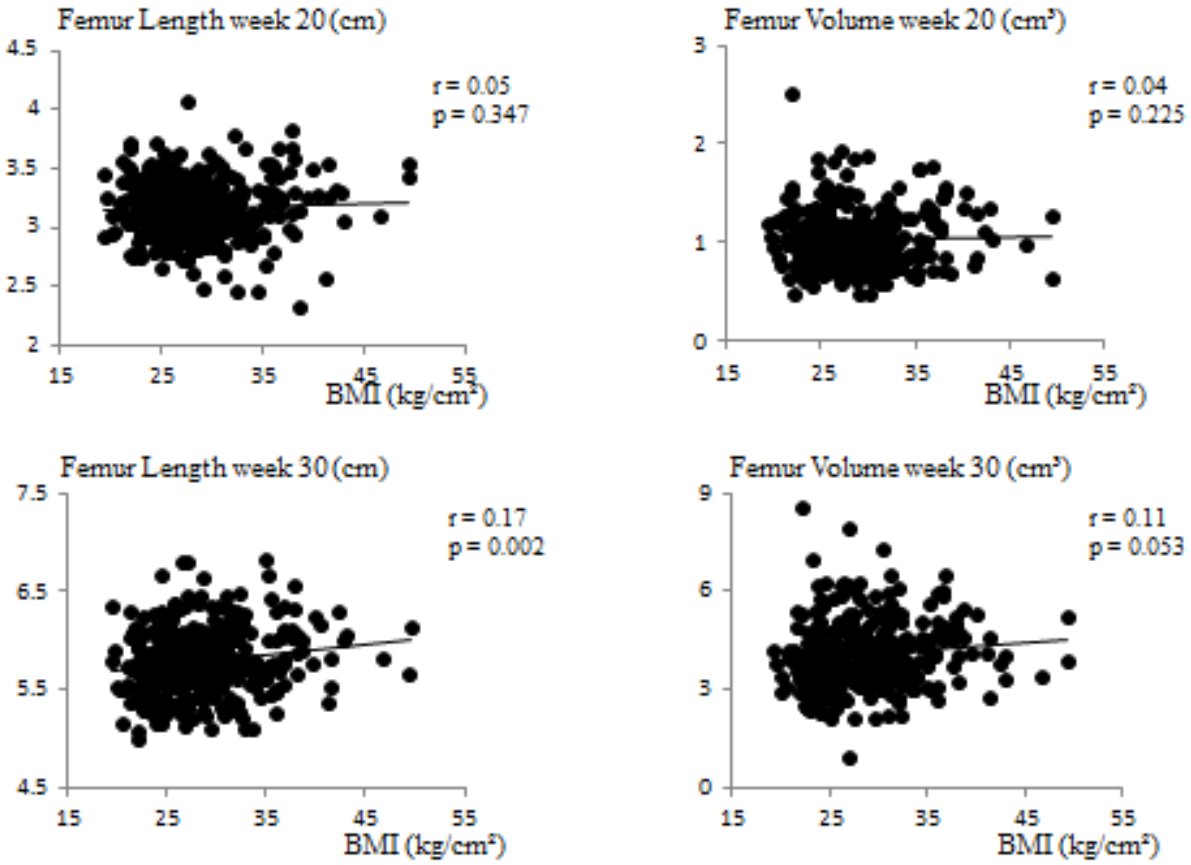


Fig. 4. Fetal femur length, distal metaphysal cross-sectional area (CSA), splaying index and femur volume at 20 and 30 weeks gestation by maternal levels of 25(OH) vitamin D

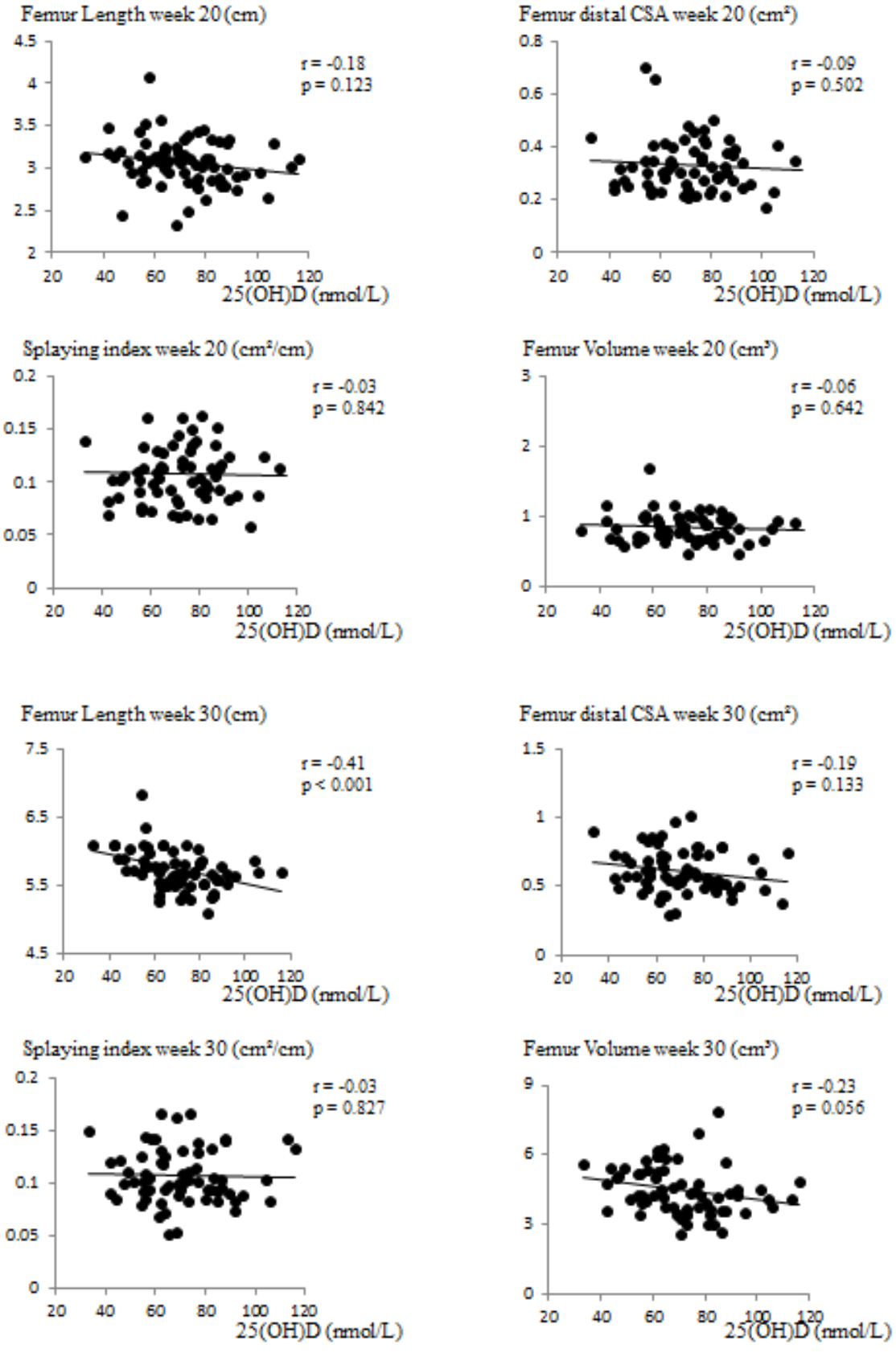


Table 1: Characteristics of the mothers and the offspring during gestation

Mothers	n	Mean (SD)	Range
Age (years)	343	31.2 (4.4)	20-43
Height (cm)	341	164.4 (6.7)	145-188
Weight (kg)	341	76.9 (15.5)	49-140
Body mass index (BMI, kg/cm²)	341	28.4 (5.2)	19-49
25(OH)Vitamin D (nmol/L)	72	71.8 (17.7)	33-116
Smoking during pregnancy (n, %)	337	28 (8.3)	
Nulliparous (n, %)	336	154 (45.8)	
Caucasian ethnicity (n, %)	343	265 (77.3)	
Age of the fetuses at 20 weeks scan (weeks)	341	19.9 (0.7)	17.3-22.9
Femur length (cm)	341	3.16 (0.24)	2.33-4.08
Distal femoral metaphysal cross-sectional area (cm²)	317	0.35 (0.11)	0.15-1.21
Distal femoral metaphysal largest diameter (cm)	318	0.87 (0.14)	0.53-1.51
Femoral splaying index (cm²/cm)	316	0.11 (0.03)	0.05-0.37
Femoral volume (cm³)	320	1.01 (0.29)	0.47-2.52
Age of the fetuses at 30 weeks scan (weeks)	338	30.5 (1.1)	27.1-34.0
Femur length (cm)	338	5.78 (0.34)	5.01-6.82
Distal femoral metaphysal cross-sectional area (cm²)	318	0.67 (0.23)	0.27-2.14
Distal femoral metaphysal largest diameter (cm)	318	1.22 (0.19)	0.85-1.89
Femoral splaying index (cm²/cm)	313	0.12 (0.04)	0.05-0.33
Femoral volume (cm³)	318	4.08 (1.01)	0.98-8.54

Table 2: Correlations between maternal 25-hydroxyvitamin D levels and fetal z-scores

	All n = 72	Caucasian n = 58	Other ethnicity n = 14
Fetal z-scores at 20 weeks gestation			
Femur length	-0.20 (0.100)	-0.16 (0.229)	-0.34 (0.239)
Distal femoral metaphysal cross-sectional area	-0.06 (0.634)	-0.06 (0.648)	0.22 (0.500)
Distal femoral metaphysal largest diameter	-0.07 (0.572)	-0.10 (0.461)	0.17 (0.599)
Femoral splaying index	-0-02 (0.869)	-0.03 (0.808)	0.35 (0.265)
Femoral volume	-0.02 (0.885)	-0.02 (0.905)	0.11 (0.744)
Fetal z-scores at 30 weeks gestation			
Femur length	**-0.41(<0.001)**	**-0.31 (0.023)**	**-0.51 (0.060)**
Distal femoral metaphysal cross-sectional area	-0.11 (0.387)	-0.07 (0.644)	-0.24 (0.438)
Distal femoral metaphysal largest diameter	-0.11 (0.370)	-0.08 (0.563)	-0.23 (0.459)
Femoral splaying index	-0.03 (0.826)	-0.01 (0.927)	-0.10 (0.746)
Femoral volume	**-0.27 (0.030)**	-0.21 (0.137)	-0.43 (0.144)

Numbers are Pearson correlation coefficients with p-values in bracket

Table 3: Associations of maternal 25(OH)D with fetal femur scores

	Femur Length week 30			Femur Volume week 30		
	STB	**p**	**R^2**	**STB**	**p**	**R^2**
Model 1	-0.41	< 0.001	0.165	-0.27	0.030	0.073
Model 2	-0.38	0.003	0.170	-0.33	0.015	0.093
Model 3	-0.43	< 0.001	0.215	-0.35	0.011	0.113
Model 4	-0.39	0.003	0.249	-0.35	0.016	0.114
Model 5	-0.40	0.003	0.260	-0.35	0.016	0.136

25(OH)D = 25-hydroxyvitamin D; STB = standardized estimates in linear regression unadjusted models (Model 1), additionally adjusted for maternal body mass index (Model 2), for parity (Model 3), for ethnicity (Model 4), and for maternal height and smoking (Model 5).

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