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Maternal 25-hydroxyvitamin D level and fetal bone growth assessed by ultrasound: a systematic review

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KEYWORDS: fetal bone formation; fetal growth; intrauterine growth restriction; ultrasound; vitamin D

ABSTRACT

Objectives *To assess systematically the role of maternal vitamin D levels in fetal bone growth.*

Methods *PubMed, EMBASE and Cochrane databases were searched using the search words [Vitamin D] in combination with [fetal, fetus, intrauterine, or prenatal AND growth, development, bone, femur, or humerus]; [crown–rump length]; or [ultrasonography, prenatal]. Criteria for inclusion in this systematic review were data on maternal serum 25-hydroxyvitamin D (25(OH)D) during pregnancy and measurement of fetal growth by ultrasound.*

Results *We identified 750 publications initially, from which five observational studies were selected for inclusion in the final review. The parameters studied were humerus length (HL) and femur length (FL) and their Z-scores, femoral volume, femoral distal metaphyseal cross-sectional area (CSA), femoral proximal metaphyseal diameter (PMD), femoral mid-shaft diameter and crown–rump length. In one study, 25(OH)D was associated directly with FL; in another study 25(OH)D only correlated with FL and HL Z-scores when calcium intake was insufficient. Two studies found no association between 25(OH)D and FL, but detected a direct association with femoral PMD, and an inverse relation with femoral distal metaphyseal CSA, respectively.*

Conclusions *Observational studies investigating the role of maternal vitamin D levels in fetal bone growth are sparse. Their evidence suggests that low maternal 25(OH)D levels may affect fetal bone growth under certain circumstances, especially in cases of simultaneous low calcium intake. Further studies are necessary.*

INTRODUCTION

While the importance of vitamin D in bone mineralization and growth in children is well known, only some[1–11], not all[12–21], studies have demonstrated associations between maternal serum 25-hydroxyvitamin D (25(OH)D) in pregnancy and the anthropometrics of the newborn. Positive associations have been shown between maternal 25(OH)D and birth weight[1–3,7,9–11], birth length[1–3,7] and birth head circumference[1,2,7], while inverse associations with anterior fontanel size have been found[1,8]. The risk of being born small-for-gestational age was increased in women with low 25(OH)D in some[3–6], but not all[13], studies. Furthermore, a U-shaped association between vitamin D levels and fetal size was found in white, but not in black, women[4]. Among randomized controlled trials (RCTs) on vitamin D supplementation during pregnancy, a study from India[7] showed longer birth length in the supplemented group, while no such association was found in the UK[8] or in Bangladesh[21]. Another five RCTs did not report on birth length[12–14,22,23]. Correlations between 25(OH)D and direct measurements of fetal growth have been investigated only rarely.

During pregnancy, vitamin D insufficiency (25(OH)D < 50 nmol/L) is frequent, with a prevalence of 16–98% in cohorts of pregnant women[24–33]. This large span may be due to seasonal variations and differences in latitude, supplementation policy, culture and ethnicity[29,34]. In studies involving primarily Caucasians, the prevalence is 16–77%[24,25,27,28,30,31,35], in African-Americans it is 68–96%[25,29], in Hispanics 31–81%[25,29] and in Arab women 46–100%[26,32,33].

Vitamin D, either absorbed from the diet or generated in the skin after ultraviolet B exposure, undergoes hepatic 25-hydroxylation to 25(OH)D, which reflects the vitamin D pool of the total body. A small fraction of 25(OH)D is 1-hydroxylated, primarily in the kidneys,

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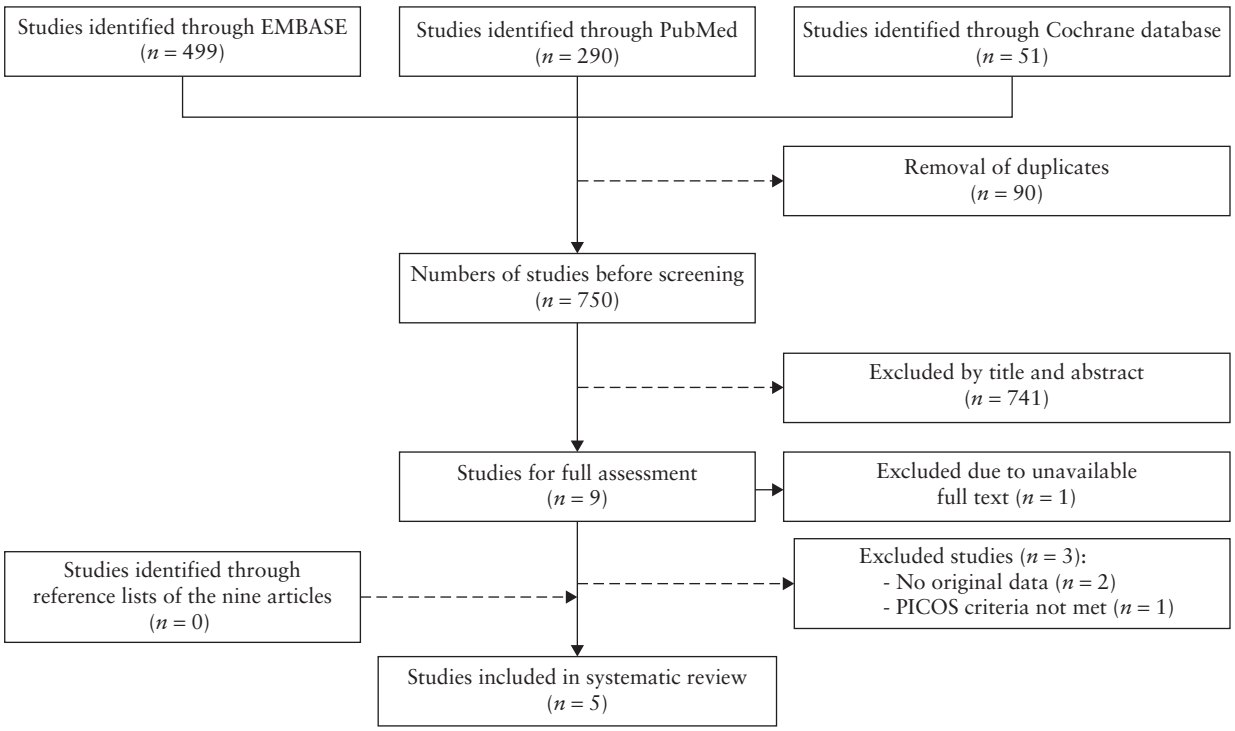

Figure 1 Flowchart showing selection of studies for inclusion in this systematic review assessing the role of maternal vitamin D levels in fetal bone growth. PICOS, participants, interventions, comparators, outcomes and study design.

to 1,25-dihydroxy vitamin D ($1,25(OH)_2D$). During pregnancy, 25(OH)D crosses the placenta easily, whereas the metabolically active $1,25(OH)_2D$ crosses only in low concentrations. However, extrarenal 1-hydroxylation of 25(OH)D to $1,25(OH)_2D$ takes place in the decidua and placenta, supplying both mother and fetus[36].

Given the high prevalence of hypovitaminosis D in pregnancy and how this can affect availability in the fetus, the identification of a link between maternal vitamin D insufficiency and fetal bone mineralization and growth may have considerable impact worldwide. We conducted a systematic review of the evidence of an association between maternal 25(OH)D levels and fetal growth, based on the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) guidelines[37].

METHODS

Search strategy

We performed a systematic search on PubMed, EMBASE and Cochrane databases, using [Vitamin D] as the main search word combined with the secondary search terms [fetal, fetus, intrauterine or prenatal AND growth, development, bone, femur or humerus], [crown–rump length], or [ultrasonography, prenatal]. The search was limited to human studies in English, with no date limitation, up to 1 September 2013.

Eligibility

Our research question was established on the basis of the 'participants, interventions, comparators, outcomes and study design' (PICOS) approach in accordance with the PRISMA guidelines[37]. Studies were eligible for final inclusion if they were RCTs or observational studies reporting original data, researching the association between fetal growth assessed by ultrasound and vitamin D measurement and/or vitamin D intake in populations of healthy women with fetuses without known severe medical conditions. If any inclusion criterion was not met, the study was excluded.

Study selection

Of 840 papers identified, 90 were duplicates (Figure 1). Of the remaining 750 studies, 741 were excluded after screening by title and/or abstract for eligibility based on the selected PICOS inclusion criteria. At least two of four reviewers were involved in all stages of literature assessment. Of the resulting nine papers, eight were available in full-text and screened by all four reviewers, and the reference lists of these eight papers were screened for additional relevant citations, but none was identified. Three papers were excluded after full-text assessment, leaving five papers to be included for review. The quality of each of these remaining five studies was evaluated by all four reviewers using a predefined checklist based on other reports[38,39], focusing on the risk of selection and attrition bias, exposure (vitamin D level expressed as 25(OH)D), outcome assessment, confounding factors and analysis. Studies were rated overall as having low, fair or good quality. Discrepancies were resolved between the four investigators.

Table 1 Characteristics of studies included in this systematic review assessing the role of maternal vitamin D levels in fetal bone growth

Characteristic	*Mahon (2010)*[40]	*Fernandez-Alonso (2011)*[41]	*Ioannou (2012)*[42]	*Young (2012)*[43]	*Walsh (2013)*[44]
Design	Observational	Observational	Observational	Observational	Observational
Population size	424	498	357	171	60
Location (latitude)	Southampton, UK (51°N)	Almeria, Spain (36°N)	Southampton, UK (51°N)	Baltimore, US (37°N) Rochester, US (43°N)	Dublin, Ireland (53°N)
Age (mean or median)	31.7 years	30 years	31.6 years	17.1 years	31.7 years
BMI (mean or median)	23.9 kg/m^2	23.9 kg/m^2	23.7 kg/m^2	24.7 kg/m^2	26.6 kg/m^2
Maternal weight gain (mean)	N/A	N/A	N/A	16.8 kg	N/A
Alcohol intake* (% of participants)	N/A	N/A	25.2%	N/A	N/A
Smoking (% of participants)	14%	17.9%	13.6%	10%	3.33%
Ethnicity	Caucasian: 97.4%	Caucasian: 83.5%; Arabic: 9.6%	N/A	African American: 66.7%; White: 33.3%	Caucasian: 100%
Parity (% nulliparous)	59.4%	52.4%	59.1%	91.8%	N/A
Vitamin D intake (mean)	N/A	N/A	N/A	5.4 µg/day	2.8 µg/day
Calcium intake (mean)	N/A	N/A	N/A	917 mg/day	N/A
25(OH)D measurement method	Diasorin RIA	Roche Modular E 170 analyzer	Diasorin RIA	Diasorin RIA	Immunodiagnostic Systems
25(OH)D (mean or median)	61 nmol/L (34 wks)	68.4 nmol/L (11–14 wks)	63 nmol/L (34 wks)	54.7 nmol/L (At delivery)	45.6 nmol/L (14 wks); 54.4 nmol/L (28 wks); 31.8 nmol/L (cord blood)
GA at fetal growth exam	19, 34 wks	11–14 wks	19, 34 wks	34 wks	20, 34 wks
Fetal growth parameters	FL, FDM-CSA, femoral splaying index	CRL	FL, PMD, MSD, FV	Femur and humerus *Z*-score	HC, BPD, AC, EFW

Only the first author of each study is given. *Alcohol comsumption of ≥ 4 units/week. 25(OH)D, 25-hydroxyvitamin D; AC, abdominal circumference; BPD, biparietal diameter; CRL, crown–rump length; EFW, estimated fetal weight; FDM-CSA, femoral distal metaphyseal cross-sectional area; FL, femur length; FV, femoral volume; HC, head circumference; MSD, femoral midshaft diameter; N/A, not available; PMD, femoral proximal diameter; RIA, radioimmunoassay; wks, gestational weeks.

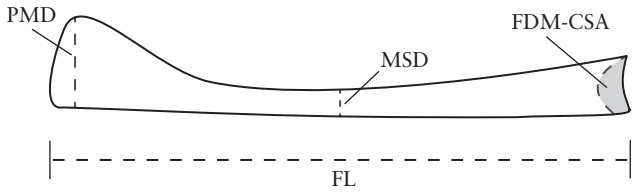

Figure 2 Sites for fetal femur measurement. FL, femur length; FDM-CSA, femoral distal metaphyseal cross-sectional area; MSD, femoral mid-shaft diameter; PMD, femoral proximal metaphyseal diameter.

RESULTS

Study characteristics

Our systematic search identified no RCTs and five observational studies on maternal 25(OH)D levels and fetal growth parameters in humans[40–44]. These studies were conducted in geographical locations with latitudes between 36°N and 53°N and differed in ethnic distribution (Table 1). The mean maternal age was 30–32 years in four studies[40–42,44], while one study[43] included only pregnant adolescents below 18 years of age. Between 52% and 59% of the women were nulliparous in three studies[40–42], one did not report parity[44] and in the study of adolescents 91.8% were nulliparous[43]. The percentage of smokers in each study ranged from 3.3% to 17.9%, while alcohol consumption was only assessed in one study[42]. Maternal body mass index was similar between studies. 25(OH)D measurements were performed using three different immunoassays (Table 1).

25(OH)D associations with fetal growth

Mahon *et al.*[40] measured femur length (FL) and femoral distal metaphyseal (FDM) cross-sectional area (CSA) (Figure 2), and calculated a femoral splaying index (FDM-CSA/FL-ratio). 25(OH)D correlated inversely with FDM-CSA and femoral splaying index (Table 2). This new ultrasound measurement was validated in 50 pregnancies in both weeks 19 and 34. Grouped according to selected cut-offs for vitamin D deficiency, insufficiency and sufficiency (25, 50 and 70 nmol/L, respectively), no significant associations with FDM-CSA and femoral splaying index were reported (data not shown). No associations with FL were observed.

Fernandez-Alonso *et al.*[41] found no association, on uni- and multivariable analysis, between 25(OH)D and crown–rump length (CRL) at first-trimester standard ultrasound examination in weeks 11 + 0 to 13 + 6; they did not, however, control for or define gestational age.

Ioannou *et al.*[42] measured 25(OH)D, femoral mid-shaft diameter (MSD), femoral proximal metaphyseal diameter (PMD) and FL at week 34, and calculated femoral volume (FV) from a defined equation. On univariate analysis, logFV and femoral PMD were positively associated with 25(OH)D, whereas FL and femoral MSD did not correlate with 25(OH)D (Table 2). In multivariable regression analysis, 25(OH)D was associated with femoral PMD only. The confounding variables that were included in the multivariable analysis in each study are listed in Table 3.

Young *et al.*[43] found on multivariable analysis that pregnant adolescents with 25(OH)D > 50 nmol/L *vs* < 50 nmol/L at delivery had higher fetal FL and humerus length (HL) *Z*-scores at 34 weeks (Table 2). An estimated calcium intake < 1050 mg/day was also associated with lower FL and HL *Z*-scores in adjusted analyses. Interaction analyses showed that the FL and HL *Z*-scores were only associated with 25(OH)D when calcium intake was below 1100 mg/day (calcium sufficiency threshold). On multivariable analysis, both FL and HL *Z*-scores were higher in woman with a sufficient calcium intake > 1100 mg/d and/or 25(OH)D > 50 mmol/L compared with the combined calcium/vitamin D insufficiency group (calcium intake < 1100 mg/d and 25(OH)D < 50 nmol/L).

Walsh *et al.*[44] found a positive correlation between 25(OH)D at 28 weeks and FL at 34 weeks in the winter pregnancy subgroup. Other statistically significant correlations related to measurements of fetal growth obtained prior to the sampling of 25(OH)D in the umbilical cord at delivery, e.g. a correlation between cord blood 25(OH)D levels and FL at 20 weeks gestation, and are therefore less suggestive of direct causality.

Quality assessment

Only three of the five studies[40,42,43] were rated as being of fair quality overall (Table 3). One study[41] was rated low overall particularly because of the use of CRL as an estimate of fetal growth without definition of, or adjustment for, gestational age. In another study on the same cohort, 25(OH)D was higher at 12–13 weeks of gestation compared with before 12 weeks[45], indicative of confounding by gestational age. The fifth study[44] used univariate analysis only and also suffered from a lack of appropriate sample size calculation. None of the studies used the presently-considered gold standard LC-MS/MS method for the 25(OH)D analysis.

DISCUSSION

This systematic review revealed that the role of vitamin D in human fetal bone formation has not been studied adequately. There is currently no convincing evidence that maternal vitamin D status *per se* affects bone formation *in utero*. No RCTs and only five observational studies have addressed this matter, and among the latter only three were judged as being of fair quality. 25(OH)D was associated with FL and HL *Z*-scores in one study of fair quality, but only when calcium intake was low[43]. However, the 6-week reverse time relation between 25(OH)D determination and fetal scanning hampered the validity of this association. The two other papers of fair quality had large populations and showed no association with FL[40,42]. Instead, these two studies showed a direct association between 25(OH)D and femoral PMD[42], and an inverse one between 25(OH)D and both FDM-CSA and splaying index[40].

Table 2 Associations between 25-hydroxyvitamin D (25(OH)D) and fetal growth reported by studies included in this systematic review

			Univariate analysis		
Reference	*Determinant*	*Outcome*	r	P	*Multivariate analysis*
Mahon (2010)[40]	25(OH)D	FDM-CSA (19 wks)	$r = -0.16$	$P = 0.002$	Little change in effect
		FDM-CSA (34 wks)	$r = -0.10$	$P = 0.05$	
		Femoral splaying index (19 wks)	$r = -0.17$	$P = 0.001$	Little change in effect
		Femoral splaying index (34 wks)	$r = -0.11$	$P = 0.03$	
		FL (19 wks)	$r = 0.00$	N.S.	—
		FL (34 wks)	$r = 0.07$	N.S.	
Fernandez-Alonso (2011)[41]	25(OH)D	CRL (11–14 wks)	$r = 0.005$	N.S.	N.S.
Ioannou (2012)[42]	25(OH)D	logFV (34 wks)	$r = 0.147$	$P = 0.006$	N.S.
		FL (34 wks)	$r = 0.084$	N.S.	N.S.
		PMD (34 wks)	$r = 0.176$	$P = 0.001$	$P < 0.05$
		MSD (34 wks)	$r = 0.002$	N.S.	N.S.
	25(OH)D < 75 *vs* ≥ 75 nmol/L	logFV (34 wks)	0.67 *vs* 0.70 mL	$P = 0.006$	—
		FL (34 wks)	—	N.S.	—
		PMD (34 wks)	13.5 *vs* 14.1 mm	$P = 0.001$	—
		MSD (34 wks)	—	N.S.	—
	25(OH)D < 50 *vs* ≥ 50 nmol/L	logFV (34 wks)	Direct association	$P = 0.051$	—
		PMD (34 wks)	Direct association	$P = 0.021$	—
	25(OH)D < 25 *vs* ≥ 25 nmol/L	logFV (34 wks)	Direct association	$P = 0.043$	—
		PMD (34 wks)	Direct association	$P = 0.011$	—
Young (2012)[43]	Ca-intake < 1050 *vs* ≥ 1050 mg/d	Femur *Z*-score (34 wks)	−0.680 *vs* −0.319	$P = 0.020$	*Z*-score increase = 0.196
	Ca-intake < 1050 *vs* ≥ 1050 mg/d	Humerus *Z*-score (34 wks)	−0.337 *vs* 0.003	$P = 0.032$	*Z*-score increase = 0.180
	25(OH)D < 50 *vs* > 50 nmol/L	Femur *Z*-score (34 wks)	−0.746 *vs* −0.321	$P = 0.003$	*Z*-score increase = 0.225
	25(OH)D < 50 *vs* > 50 nmol/L	Humerus *Z*-score (34 wks)	−0.403 *vs* 0.012	$P = 0.006$	*Z*-score increase = 0.208
	Ca/D insufficient* *vs* sufficient†	Femur *Z*-score (34 wks)	Direct association	$P < 0.002$	Direct association
	Ca/D insufficient* *vs* sufficient†	Humerus *Z*-score (34 wks)	Direct association	$P = 0.003$	Direct association
Walsh (2013)[44]	25(OH)D (cord)	HC, BPD, AC, FL (20 wks)	$r = 0.39, 0.34, 0.34, 0.35$	$P < 0.05$	—
	25(OH)D (14, 28 wks)	HC, BPD, AC, FL (20 wks)	—	N.S.	—
	25(OH)D (14,28 wks and cord)	HC, BPD, AC, FL (34 wks)	—	N.S.	—
	25(OH)D (28 wks, winter cohort)	FL (34 wks, winter)	$r = 0.43$	$P = 0.02$	—
	25(OH)D (cord, winter cohort)	FL (34 wks, winter)	$r = 0.48$	$P = 0.009$	—
	25(OH)D (14, 28 wks, < median *vs* > median)	BPD, HC, AC, FL (20 and 34 wks)	—	N.S.	—
	25(OH)D (cord, < median *vs* > median)	FL (20 wks)	32.6 *vs* 35.5 mm	$P < 0.05$	—
		BPD, HC, AC (20 wks)	—	$P < 0.05$	
	25(OH)D (cord, < median *vs* > median)	BPD, HC, AC, FL (34 wks)	—	N.S.	—

Only the first author of each study is given. *Calcium intake < 1100 mg/day and 25(OH)D < 50 nmol/L. †Calcium intake > 1100 mg/day and/or 25(OH)D > 50 nmol/L. AC, abdominal circumference; BPD, biparietal diameter; CRL, crown–rump length; FDM-CSA, femoral distal metaphyseal cross-sectional area; wks, weeks of gestation; FL, femur length; FV, femoral volume; HC, head circumference; MSD, femoral midshaft diameter; N.S., not significant; PMD, femoral proximal diameter.

Table 3 Quality assessment of studies included in this systematic review assessing the role of maternal vitamin D levels in fetal bone growth

Reference	*Selection, attrition*	*Exposure*	*Outcome assessment*	*Confounders*	*Analysis*	*Overall*
Mahon (2010)[40]	Good (population cohort, low attrition)	Fair (Diasorin RIA)	Good (3D ultrasound, triplet determination in subset)	Good (age, height, weight, skinfold thickness, parity, smoking, season)	Low (no sample size calculation)	Fair
Fernandez-Alonso (2011)[41]	Fair (hospital recruitment)	Low (Roche E170 immunoassay 25OHD3)	Good (routine ultrasound)	Low (no details on multivariate analysis and confounders)	Low (no sample size calculation)	Low
Ioannou (2012)[42]	Good (population cohort, moderate attrition)	Fair (Diasorin RIA)	Good (3D ultrasound)	Good (age, height, weight, skinfold thickness, smoking, alcohol intake, gestational age)	Low (no sample size calculation)	Fair
Young (2012)[43]	Fair (hospital recruitment)	Low (Diasorin RIA, reverse time relation)	Good (routine ultrasound)	Good (height, race, weight gain, smoking, vitamin D intake, calcium intake)	Good (sample size calculated, interaction analysis)	Fair
Walsh (2013)[44]	Fair (hospital recruitment)	Fair (IDS RIA)	Good (routine ultrasound)	Low (univariate analysis only)	Low (sample size calculation not related to outcome)	Low

Only the first author of each study is given. 3D, three-dimensional; IDS, Immunodiagnostic Systems Limited; RIA, radioimmunoassay.

The fetal femur, humerus and other long bones are formed by endochondral ossification. Little bone mineralization occurs before the third trimester. At the 34th week, secondary ossification centers form true growth plates in the femur. Parathyroid hormone (PTH), PTH-related protein and mineral supply play major roles in fetal endochondral bone formation and mineralization.

Less rigorous evidence is available in the form of animal studies and human case reports. Animal data show essentially no effect on fetal bone as a result of vitamin D deficiency, 1-alpha hydroxylase knock-out or vitamin D receptor knock-out. Under such conditions, rickets develops only *after* birth, when calcium and phosphorus supplies switch from placental to vitamin D-dependent intestinal uptake[46]. In casuistic reports of stillborn human neonates of mothers with extreme vitamin D deficiency, neither signs of rickets nor abnormal centers of ossification were seen, and skeletal ash weight and mineral content were normal when compared with those in stillborn neonates of mothers with normal vitamin D status[46]. Moreover, neonates with vitamin D receptor mutations have normal skeletal findings at birth[46]. In case reports of skeletal abnormalities shortly after birth in neonates of mothers with severe vitamin D deficiency, low calcium uptake was suggested as a possible cause or could not be ruled out.

While vitamin D *per se* does not, therefore, seem to affect fetal bone development, low maternal calcium intake may limit fetal bone growth and mineralization[47]. Recommended calcium intake during pregnancy is 800–1100 mg/day depending on maternal age[48]. In an RCT in women with estimated calcium intake below 600 mg/day, supplementing in pregnancy with 2000 mg calcium/day increased whole-body bone mineral concentration in the neonate, as measured 2 days postnatally[49]. Also, fetal FL was increased in pregnant adolescent women with a higher estimated calcium intake compared with controls[50]. Adequate intake of calcium and vitamin D has furthermore been found to be associated with higher birth length[51]. Only one RCT, however, has demonstrated an association between maternal vitamin D intake and birth length[7], and in this study, in rural India in the 1980s, the estimated calcium intake was low (437 and 421 mg/day in supplemented and control groups). At birth, maternal and cord serum calcium were higher in the supplemented vitamin D group compared with controls, which may reflect increased calcium intestinal absorption in the vitamin D-supplemented mothers. In a semi-RCT, there was increased birth length in pregnant Indian women randomized to either one dose of 1500 μg vitamin D, or two doses of 3000 μg vitamin D, compared with a non-randomized control group, all three groups having a low estimated calcium intake of 620–660 mg/day[1]. While other observational studies[1–3,7] have shown associations between maternal 25(OH)D and birth length, they did not control for maternal calcium dietary intake. Drawing from all the available evidence, it seems that a low vitamin D intake or 25(OH)D value may correlate with fetal long

bone length, but only when maternal calcium intake is low, as found by Young *et al.*[43].

The increased distal femoral splaying in fetuses of mothers with low 25(OH)D, as found in the study of Mahon *et al.*[40], could also be an indication of early fetal rickets if the calcium intake of these mothers was low. Early rickets is seen primarily above and below the knee as widening (splaying) of the epiphyseal plate[52]. The other inconsistent findings on femur size could also reflect unreported variations in maternal calcium intake. However, no calcium intake data were given in these studies.

A strength of this review was that we employed a systematic approach, according to the PRISMA guidelines. Limitations include the lack of RCTs and the paucity of well-conducted observational studies. Furthermore, a recent study[53] highlights the importance of race in correlating total serum 25(OH)D with bone mineralization, thus limiting the external validity of studies conducted in predominantly Caucasian or African-American populations, or populations with unreported ethnicity.

In conclusion, possible associations between maternal vitamin D intake or 25(OH)D levels and fetal ultrasound bone data have so far been studied inadequately. Future RCTs or observational studies should include estimates of maternal calcium intake in addition to other potential confounders, such as maternal height, race, obesity, smoking, alcohol intake and paternal factors. Current evidence does not suggest that maternal 25(OH)D status should be taken into account in estimates of fetal bone measurements.

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