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Maternal 25-Hydroxyvitamin D and Parathyroid Hormone Concentrations and Offspring Birth Size

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Context: There is inconsistent evidence that maternal 25-hydroxyvitamin D [25-(OH)D] deficiency may impair fetal growth.

Objective: The objective of the study was to examine the relationship between maternal 25-(OH)D and PTH concentrations at less than 16 and 28 wk gestation and offspring birth size.

Design: This was an observational study.

Setting: The study was set at a hospital antenatal clinic.

Participants: Women with singleton pregnancies, before 16 wk gestation, participated.

Interventions: No interventions were used.

Main Outcome Measure: Knee-heel length at birth was the main outcome measure.

Results: Altogether 374 of 475 (79%) women completed this study. We found no evident relationship between birth size measures and maternal 25-(OH)D or PTH at recruitment (~11 wk). Gestation length was 0.7 wk (95% confidence interval −1.3, −0.1) shorter and knee-heel length was 4.3 mm smaller (−7.3, −1.3) in infants of 27 mothers with low 25-(OH)D (<28 nmol/liter) at 28–32 wk *vs.* babies whose mothers had higher concentrations. This latter difference was reduced to −2.7 mm (−5.4, −0.1) after adjustment for gestation length, suggesting some of the apparent growth deficit is explained by shorter gestation. There was no evidence that other birth measures were affected. Maternal PTH concentration at 28–32 wk was positively related to knee-heel length, birth weight, and mid-upper arm and calf circumferences. These associations were independent of 25-(OH)D concentration.

Conclusions: Low maternal 25-(OH)D in late pregnancy is associated with reduced intrauterine long bone growth and slightly shorter gestation. The long-term consequences for linear growth and health require follow-up. The positive relationship between maternal PTH and measures of infant size may relate to increased mineral demands by larger babies, but warrants further investigation. ***(J Clin Endocrinol Metab* 91: 906–912, 2006)**

DATA FROM RANDOMIZED controlled trials of oral vitamin D supplementation during pregnancy, in women with low 25-hydroxyvitamin D (25-(OH)D) concentration, provide somewhat inconsistent results regarding effects on offspring birth size (1–6).

In a small study of 30 25-(OH)D-deficient women, offspring crown-heel length was negatively related to maternal PTH concentration at delivery (7).

In an observational study in a largely Caucasian and 25-(OH)D-sufficient population at 38.2 degrees south in Geelong, Australia, we examined the relationship between maternal 25-(OH)D and offspring size at birth and at 1 yr of age. There are no data describing the nature of this relationship (*e.g.* linear *vs.* threshold), so a randomized trial at this stage, in a "low risk" group, could not be justified. Our primary hypothesis was that offspring of mothers with 25-(OH)D concentration less than 28 nmol/liter at 28–32 wk gestation (lower limit of normal range for our laboratory) would have smaller knee-heel length at 1 yr of age. We report here on our secondary hypotheses, regarding relationships between maternal blood 25-(OH)D and PTH concentrations and offspring birth size.

Subjects and Methods

This study was approved by the Barwon Health Research and Ethics Advisory Committee and procedures were in accordance with the Helsinki Declaration. Women were recruited in the antenatal clinic at Geelong Hospital before 16 completed weeks gestation, calculated from date of last menstrual period. Exclusion criteria were multiple pregnancy, maternal disease or disability likely to affect pregnancy outcome or calcium metabolism, dark skin or concealing clothing [because such women are at known high risk of deficiency and need to be screened and usually treated (8)], and intention to move from the area in the foreseeable future.

After obtaining written informed consent, we collected socio-demographic data and data regarding health and obstetric history, maternal smoking at recruitment, and details of medications and nutritional supplements. We also asked women to complete questionnaires regarding calcium intake and sun exposure, but these data will be reported separately. Maternal weight, height, and head circumference were recorded. Venous blood samples were taken into serum gel and EDTA tubes and centrifuged within 2 h, then stored at −70 C until analysis. At 28–32 wk gestation, questionnaires were readministered and further blood samples were taken. This time point was chosen because prior data (1–6) suggested that 25-(OH)D concentrations in the third trimester

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Abbreviations: 95% CI, 95% Confidence interval; 1,25-$(OH)_2D$, 1,25 dihydroxyvitamin D; 25-(OH)D, 25-hydroxyvitamin D; IQR, interquartile range.

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affected offspring growth, but also for practical reasons, because all women then had routine venipuncture (for glucose challenge test).

The birth weights of the babies were measured by obstetric staff on regularly calibrated scales. Other infant measurements were undertaken by a trained nurse, between 12–72 h of age. Knee-heel length was measured with a hand-held BK5 infant knemometer (Force Technology, Brondby, Denmark). Instrument software calculated the mean of 10 sequential readings and generated a printed report of all readings and the calculated mean. We also measured crown-heel length to the nearest millimeter using an Ellard newborn lengthboard (Ellard Instrumentation Ltd., Seattle, WA); head, mid-upper arm, and maximal calf circumference were measured using a plastic encircling tape (Child Growth Foundation, London, UK); and triceps, subscapular, and suprailiac skin folds were measured using Holtain calipers (Holtain, Crymych, UK).

Laboratory assays

Assays were undertaken in batches after women had delivered, in fully accredited laboratories at The Royal Children's Hospital, Melbourne, that participate in national and international quality assurance schemes. 25-(OH)D was measured by RIA (Immunodiagnostic Systems, Tyne and Wear, UK), an assay measuring 100% of 25-(OH)D_3, with reportedly 75% cross-reactivity with 25-(OH)D_2. The coefficient of variation was 10.2% at 30 nmol/liter and 10.1% at 100 nmol/liter. Intact PTH was measured by chemiluminescent enzyme-labeled immunometric assay using the Immulite 2000 autoanalyser (Diagnostic Products Corporation, Los Angeles, CA). Between run imprecision is 7.8% at 5.2 pmol/liter and 8% at 37.7 pmol/liter; laboratory reference range for healthy adults is 1.3–6.8 pmol/liter. Total calcium and albumin were measured using the Vitros 250 autoanalyzer (Ortho-Clinical Diagnostics, Rochester, NY).

Sample size calculation and statistical analyses

Prior data (9) suggested that, overall, 6.2% [95% confidence interval (95% CI) 4.2, 8.2] would have 25-(OH)D concentration less than 28 nmol/liter. Using a conservative 4.2% estimate (lower end of the confidence interval), we calculated that with 360 subjects we would have 80% power (with two-sided $\alpha = 0.05$) to detect a 0.75 SD difference in knee-heel length at birth and in birth weight between offspring of women with 25-(OH)D concentration less than 28 nmol/liter and those equal to or greater than 28 nmol/liter at 28–32 wk gestation, using a two-sample *t* test.

From previous experience we estimated that 20% would abort, move from the area, withdraw from the study, or fail to have one or both study bloods taken, so we aimed to recruit 360/0.8 women: 450 in total. However, some women attended off-site blood sampling facilities and forgot to take the study request slip. We took steps to deal with this but also increased total recruitment to 475 women.

We used *t* test or χ^2 test to compare characteristics of women who completed the study *vs.* those who did not, and measurements of infants whose mothers had low *vs.* higher 25-(OH)D concentration. The latter comparisons were examined to identify potential confounding effects using multiple linear regression.

25-(OH)D and PTH measures were skewed, as expected, so we used logarithmic values in analyses and tabulated geometric means. Log_2 values of PTH and 25-(OH)D were used in regression models, so that regression coefficients represented the estimated effect of doubling these blood values. For paired comparisons of 25-(OH)D, PTH, and calcium concentrations between early and later pregnancy, we used a linear mixed model to allow for potential confounding by season of measurement. Season was represented in these models by way of a sinusoidal curve based on month. Finally, we used a locally weighted smooth regression technique ("lowess") to explore for potentially nonlinear association between the primary infant size measure, knee-heel length, and maternal log 25-(OH)D concentration.

Results

Subject characteristics

Between April 2002 and September 2003 we recruited 475 women from approximately 2470 women registering for antenatal care at this hospital. Barriers to recruitment were failure to fulfill inclusion criteria, refusal, and logistic barriers to recruiting more than two to three women per clinic. Median [interquartile range (IQR)] gestation at recruitment was 11 (9, 13) wk. Altogether, 45 women left the study prenatally; 13 aborted, six moved away, and 26 withdrew. Of the 430 who remained in the study, 51 did not provide a blood sample at 28–32 wk. A further four women decided to withdraw from the study after delivery, and one neonate was transferred to a tertiary hospital, so five babies were not measured. Thus 374 women (79% of those recruited) provided infant measures at birth and a blood sample at 28–32 wk gestation, and 359 (96%) of these provided a blood sample at recruitment.

Characteristics of the 374 women whose data were included in analyses, *vs.* women with excluded data, are shown in Table 1. There was little apparent difference between them. Five women were from South East Asia and all others were of Caucasian origin. Data from three non-Caucasians were used in analyses. At recruitment 75 women reported taking a multivitamin preparation; 58 remembered the brand name, and 25 (40%) of these were taking a preparation containing vitamin D (24 D_3 and one D_2). At 28–32 wk, three women reported taking a calcium preparation containing vitamin D_3 and 102 reported taking a multivitamin preparation. Supplement composition was known for 81 women; 32 (40%) contained vitamin D_3 (none D_2).

Altogether 179 of 374 (48%) babies were male. Mean (SD) birth weight was 3.54 (0.52) kg and median (IQR) gestation length was 40 (39, 40) wk. Fourteen (3.8%) infants were born before 36 wk gestation; one baby did not have length measured and four did not have knemometry.

Postnatal measurements were done at a median (IQR) age of 2 (2, 3) d and mean (SD) knee-heel length was 121.7 (7.8) mm. The neonatal knee-heel length measurement of one child was clearly implausible and was excluded from analyses, but her other measurements were included.

Maternal 25-(OH)D, PTH, calcium, and albumin concentrations

25-(OH)D concentration was less than 28 nmol/liter in 27 of 374 (7.2%) women at 28–32 wk and 23 of 359 (6.4%) at recruitment. As expected, geometric mean 25-(OH)D concentration varied by month and was lower in winter (May to October) than in summer (November to April; Table 2). There

TABLE 1. Characteristics of women whose data were included in analyses, *vs.* those whose data were excluded (aborted, moved away, withdrew, or did not provide a blood sample at 28–32 wk or infant birth measures)

	Excluded	Included	*P* for difference[a]
n	101	374	
Mean (SD) age in years	28.9 (5.5)	29.3 (4.7)	0.5
Mean (SD) height in cm	166 (7)	166 (7)	0.8
% (n) With university education	15 (15)	17 (65)	0.5
% (n) First child	29 (29)	30 (113)	0.8
% (n) Australian born	89 (90)	93 (346)	0.3

[a] *t* test for comparison of means, χ^2 for proportions.

TABLE 2. Maternal 25-(OH)D, PTH, and total calcium and albumin concentrations by season and stage of pregnancy

	Winter[a]	Summer[b]	For difference	
			95% CI	*P* value
At recruitment				
n	232	127		
% (n) With 25-(OH)D <28 nmol/liter	9.4 (22)	0.8 (1)	4.4, 12.4	0.001
Geometric mean 25-(OH)D, in nmol/liter	49.2	62.6	−1.6, −1.4[c]	<0.001
Geometric mean PTH, in pmol/liter	1.45	1.44	−1.2, 1.2[c]	0.9
Mean calcium in nmol/liter	2.23	2.27	−0.07, −0.003	0.03
Mean albumin in g/liter	35.9	36.8	−1.96, 0.09	0.07
At 28–32 wk gestation				
n	210	164		
% (n) With 25-(OH)D <28 nmol/liter	10.0 (21)	3.7 (6)	1.4, 11.3	0.02
Geometric mean 25-(OH)D, in nmol/liter	48.3	68.9	−1.4, −1.2[c]	<0.001
Geometric mean PTH, in pmol/liter	1.68	1.46	−1.0, 1.4[c]	0.1
Mean calcium in nmol/liter	2.14	2.16	−0.6, 0.2	0.3
Mean albumin in g/liter	30.8	30.6	−0.7, 1.0	0.7

[a] May to October.
[b] November to April.
[c] 95% CI for ratio.

was a modest increase in 25-(OH)D concentrations between early and late pregnancy (geometric mean ratio 1.06, 95% CI 1.02, 1.10, $P = 0.004$) after adjustment for seasonal variation.

There was minimal evidence of seasonal variation in PTH concentration, and no evidence that mean PTH differed between the two time points in gestation ($P = 0.3$). Calcium concentrations were not skewed and were analyzed in the raw scale; there was evidence of seasonal variation; mean concentration dropped by 0.091 nmol/liter (95% CI 0.065, 0.116, $P < 0.001$) from the first to third trimesters. At 28–32 wk, geometric mean 25-(OH)D concentration was higher in 32 women taking a supplement containing vitamin D than in 321 taking either no supplement or a supplement containing no vitamin D; 75.4 *vs.* 54.7 nmol/liter, $P < 0.001$ by Mann-Whitney *U* test.

There was no evidence of a relationship between serum calcium and $\log_2$ maternal 25-(OH)D concentrations, adjusting for albumin concentration, at recruitment or at 28 wk ($P = 0.7$ and $P = 0.5$ respectively). Likewise, there was no such evidence for $\log_2$ PTH ($P = 0.6$ and $P = 0.5$ respectively). There was a weak but statistically clear inverse association between $\log_2$ maternal 25-(OH)D and $\log_2$ PTH: Pearson coefficient of correlation (r) for $\log_2$ PTH *vs.* $\log_2$ 25-(OH)D was −0.18 ($P < 0.001$) at recruitment and −0.15 ($P = 0.003$) at 28–32 wk (Fig. 1).

Maternal 25-(OH)D and gestation length

Babies of mothers in the low 25-(OH)D group at 28–32 wk (<28 nmol/liter) had a 0.7-wk shorter mean gestation length than babies of mothers with 25-(OH)D equal to or greater than 28 nmol/liter (Table 3). Adjustment in a linear regression model for factors that might confound this association (*e.g.* maternal age, height, whether a smoker, whether this was the first pregnancy, or season) made little difference. This association was also not altered by inclusion in the model of $\log_2$ PTH, total calcium and albumin concentrations, from the same blood sampling. When we considered maternal 25-(OH)D concentration as a continuous variable (Table 4), a unit increase in $\log_2$ 25-(OH)D (equivalent to doubling of 25-(OH)D) was associated with a 0.3-wk increase in gestation length (95% CI 0.04, 0.5) and was likewise unaffected by adjustment as above.

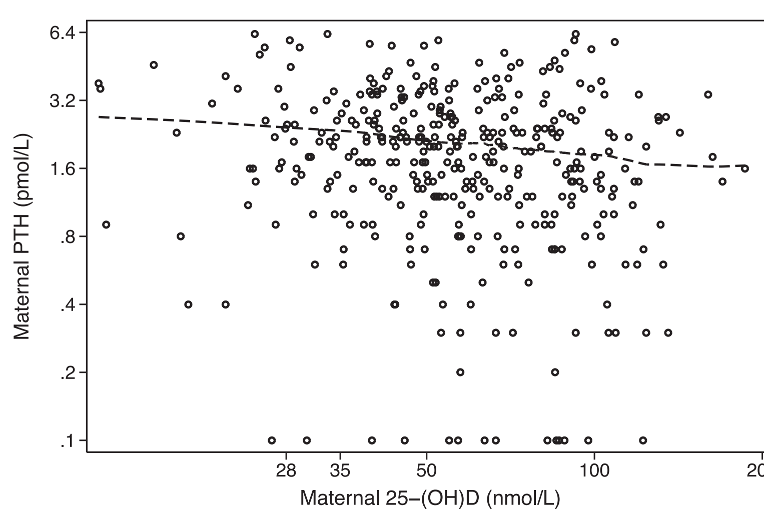

FIG. 1. Scatter plot of maternal PTH concentration *vs.* maternal 25-(OH)D concentration (both in log scale) at 28–32 wk gestation, with lowess curve superimposed.

TABLE 3. Mean (SD) gestation length and infant birth size according to maternal 25-(OH)D status at 28–32 wk

	Maternal 25-(OH)D concentration at 28–32 wk		Difference	95% CI for difference	Adjusted difference[a]	95% CI for difference
	<28 nmol/liter	≥28 nmol/liter				
n	27	347				
Gestation length in weeks	38.7 (1.5)	39.5 (1.9)	−0.7	−1.3, −0.1	−0.8	−1.4, 0.2
Knee-heel length in mm	117.7 (8.2)	122.0 (8.9)[b]	−4.3	−7.3, −1.3	−4.5	−7.5, −1.5
Crown-heel length in cm	49.8 (2.7)	50.4 (2.4)	−0.6	−1.5, 0.3	−0.6	−1.5, 0.3
Birth weight in grams	3397 (57)	3555 (52)	−157	−361, 47	−153	−348, 42
Head circumference in cm	34.5 (1.5)	34.7 (1.5)[c]	−0.2	−0.8, 0.4	−0.2	−0.8, 0.3
Subscapular skin fold in mm	6.7 (2.0)	6.3 (1.5)[d]	0.5	−0.1, 1.1	0.5	−0.1, 1.1
Triceps skin fold in mm	6.4 (1.4)	6.4 (1.7)[d]	0	−0.7, 0.7	0.2	−0.8, 0.5
Suprailiac skin fold in mm	7.0 (2.1)	6.9 (1.8)[d]	0.17	−0.5, 0.9	0.1	−0.6, 0.8
Mid-upper arm circumference in cm	10.6 (1.2)	11.0 (1.0)[d]	−0.4	−0.8, −0.03	−0.4	−0.8, −0.04
Calf circumference in cm	10.9 (1.0)	11.4 (1.0)[d]	−0.5	−0.9, −0.1	−0.5	−0.9, −0.1

[a] From linear regression models, adjusting for infant sex, maternal height, whether first child, whether the mother smoked in pregnancy, and season when blood sample was taken.
[b] One excluded and three missing measurements.
[c] Three missing measurements.
[d] Five missing measurements.

Maternal 25-(OH)D and infant birth size

Mean birth weight was higher in boys than girls (by 107 g, 95% CI 2, 213) and increased with gestation length. All measures of infant birth size were positively related to maternal height, and weight and BMI at recruitment. First children were smaller than later ones [knee-heel length differed by −1.9 cm (95% CI −3.6, −0.2), weight by −132 g (−247, −18), and crown-heel length by −0.6 cm (−1.1, 0.05)]. Analyses revealed no evidence of differences in birth size with further increases in parity. Offspring of 67 (18%) women who smoked during pregnancy were, on average, 340 g lighter (95% CI 207, 474) than others. Winter-born babies had smaller knee-heel length than babies with summer births [−2.1 mm (−3.8, −0.5)], but evidence for associations between season of birth and birth weight and crown-heel length was minimal (data not shown).

There was no evidence of association between maternal 25-(OH)D concentration at recruitment (median 11 wk) and any birth measure (data not shown).

However, in Table 3 we show that babies of mothers in the low 25-(OH)D group at 28–32 wk had lower mean knee-heel length (by 4.3 mm) than babies whose mothers had higher concentrations, as well as reduced mid-upper arm and calf circumferences. There was little difference in any other birth measure. Associations were little changed after adjustment for potentially confounding factors (infant sex, maternal height, weight or BMI, whether first child, whether the mother smoked in pregnancy). We considered the possibility that some correlate of season at 28–30 wk gestation other than 25-(OH)D might be involved. However, further adjustment for season when the blood sample was taken did not materially alter these associations (Table 3), and neither did separate adjustment for season of birth, although summer (*vs.* winter) birth was independently positively related to knee-heel length [by 1.9 mm (0.3, 3.6)].

However, after adjustment for gestation length alone, the estimated difference in knee-heel length between mothers with low *vs.* high 25-(OH)D at 28–32 wk was reduced to −2.7 mm (−5.4, 0.1), and associations with mid-upper arm and calf circumferences were substantially weakened.

We considered that the association between knee-heel length and maternal 25-(OH)D at 28–32 wk might be mediated via changes in maternal PTH or calcium concentration, so we included $\log_2$ PTH, total calcium, and albumin in the models, as well as gestation length. This made little difference; the estimated difference in knee-heel length was −3.2 mm (95% CI −5.9, −0.5).

We then investigated the strength of linear association between maternal $\log_2$ 25-(OH)D concentration at 28–32 wk and infant birth size, and found no evidence of associations

TABLE 4. Association of gestation length and infant birth size with $\log_2$ maternal 25-(OH)D concentration at 28–32 wk

Infant measurement	Regression coefficient[a]	95% Confidence interval	Adjusted[b] regression coefficient	95% Confidence interval
Gestation length in weeks	0.3	0.04, 0.5	0.3	0.07, 0.6
Knee-heel length in mm	0.6	−0.6, 1.8	0.8	−0.5, 2.0
Crown-heel length in cm	0.3	−0.08, 0.6	0.3	−0.1, 0.6
Birth weight in grams	40	−39, 119	31	−51, 112
Head circumference in cm	−0.02	−0.2, 0.2	−0.05	−0.3, 0.2
Subscapular skin fold thickness in mm	−0.2	−0.4, −0.02	−0.2	−0.4, −0.06
Triceps skin fold thickness in mm	−0.3	−0.5, −0.02	−0.1	−0.4, 0.1
Suprailiac skin fold thickness in cm	−0.06	−0.4, 0.1	−0.06	−0.4, 0.2
Mid-upper arm circumference in cm	0.08	−0.07, 0.2	0.1	−0.06, 0.3
Calf circumference in mm	0.05	−0.1, 0.2	0	−0.2, 0.2

[a] Regression coefficient represents estimated change in measurement per doubling of maternal 25-(OH)D concentration.
[b] Regression coefficient adjusted for infant sex, maternal height, whether first child, whether the mother smoked in pregnancy, and season when blood sample was taken.

with knee-heel length, crown-heel length, or birth weight, before or after adjustment for potentially confounding factors. (Table 4) Consistent with the finding above of lower knee-heel length in babies born to mothers with 25-(OH)D concentrations less than 28 nmol/liter, exploratory analysis using a nonparametric smooth regression suggested the possibility of a positive linear association when 25-(OH)D concentration was less than 30–40 nmol/liter, but no association at higher concentrations (Fig. 2).

Log_2 25-(OH)D was weakly negatively related to both subscapular and triceps skin fold thicknesses, but the latter association was weakened after adjustment for potentially confounding factors and for season of blood sampling (Table 4).

Maternal PTH and infant birth size

There was no evidence of an association between maternal PTH concentration at recruitment and any measure of infant birth size (data not shown).

Maternal $\log_2$ PTH was not associated with gestation length but was strongly positively associated with infant knee-heel length, and weakly associated with birth weight and both mid-upper arm and calf circumferences (Table 5). Adjustment for potentially confounding factors as above (infant sex, maternal height, whether first pregnancy, whether mother smoked, and season of blood sampling) did not materially alter associations. Further adjustment for gestation length did not change the results. Adjusted associations are shown in Table 5. These were not altered by adjustment for $\log_2$ 25-(OH)D, calcium and albumin concentrations, or by separate adjustment for season of birth.

We next investigated the possibility that the relationship between $\log_2$ maternal PTH at 28–32 wk and infant knee-heel length differed according to whether the mother was vitamin D deficient, because Brunvand *et al.* (7) found a negative relationship between maternal PTH at delivery and crown-heel length among 30 vitamin D-deficient women. Estimated change in knee-heel length per doubling of PTH was 1.5 mm (−0.5, 3.6), among the 27 women with 25-(OH)D less than 28 nmol/liter and 1.2 mm (95% CI 0.6, 1.8) in women with higher 25-(OH)D concentrations; P for interaction = 0.9. There was no evidence of an association between $\log_2$ PTH and crown-heel length in either group.

Discussion

Infants of mothers who were vitamin D deficient (<28 nmol/liter) at 28–32 wk gestation had 4.3 mm shorter knee-heel length than other babies. This difference was reduced to −2.7 mm after adjustment for gestation length, suggesting that some of the apparent deficit may be explained by the observed reduction in mean gestation length with low maternal 25-(OH)D. There was no association with maternal 25-(OH)D at recruitment (~11 wk), suggesting there is no constitutional reason why some women have both low 25-(OH)D and infants with smaller knee-heel length.

The biological basis for an association between maternal 25-(OH)D deficiency and fetal long bone growth is not immediately apparent. There is placental production of biologically active 1,25 dihydroxyvitamin D (1,25-$(OH)_2$D) from 25-(OH)D. We speculate that moderately severe maternal vitamin D deficiency (serum 25-(OH)D < 28 nmol/liter) would result in reduced fetal circulating 25-(OH)D and 1,25-$(OH)_2$D concentrations and decreased local generation of this metabolite in fetal bone. Osteoblasts have 1,25-$(OH)_2$D receptors and several osteoblast-specific genes are 1,25-$(OH)_2$D responsive. (10) Low 25-(OH)D concentrations in mother and, therefore, low 25-(OH)D and/or 1,25-$(OH)_2$D in the fetus may lead to reduced osteoblastic activity, affecting long bone growth. Alternatively, maternal 25-(OH)D deficiency may induce calcium stress, stimulating fetal PTH/PTHrP activity leading to contraction of the cortical bone envelope, manifest by reduced long bone growth (11).

In contrast to evidence from randomized trials, (3, 4) we found no clear evidence of a relationship between low maternal 25-(OH)D and any other measure of birth size.

25-(OH)D deficiency (and lower PTH) was associated with smaller knee-heel length but not crown-heel length, suggesting influences on long bone growth, but not total linear growth. Accurately measured knee-heel length comprised a mean of 24% of crown-heel length, but the latter is more difficult to measure accurately because of high neonatal

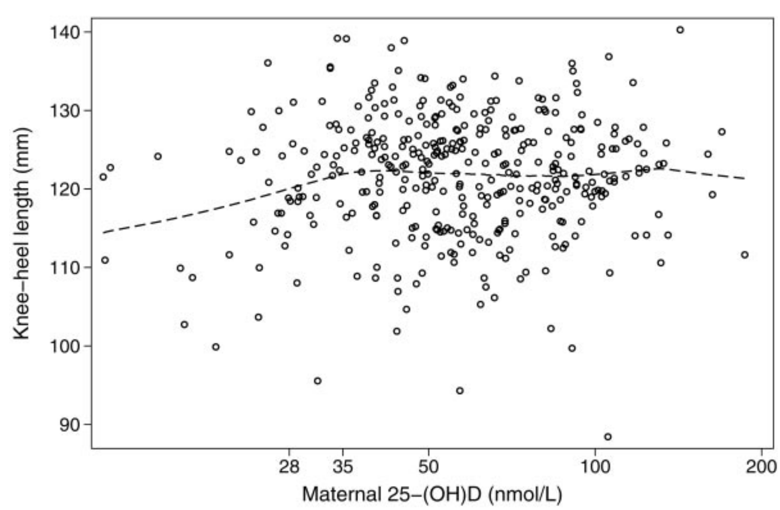

FIG. 2. Scatter plot of knee-heel length in infants at birth *vs.* (log scale) maternal 25-(OH)D concentration at 28–32 wk gestation, with lowess curve superimposed. The *smooth curve* suggests a possible association between knee-heel length and 25-(OH)D concentrations below about 40 nmol/liter.

TABLE 5. Association of gestation length and infant birth size with $\log_2$ maternal PTH concentration at 28–32 wk

Infant measurement	Regression coefficient[a]	95% Confidence interval	Adjusted[b] regression coefficient	95% Confidence interval
Gestation length in weeks	−0.002	−0.1, 0.1	−0.006	−0.1, 0.1
Knee-heel length in mm	1.2	0.6, 1.8	1.1	0.6, 1.7
Crown-heel length in cm	0.1	−0.06, 0.3	0.1	−0.06, 0.3
Birth weight in grams	46	4, 88	40	8, 73
Head circumference in cm	0.03	−0.1, 0.1	0.02	−0.1, 0.1
Subscapular skin fold thickness in mm	0.008	−0.1, 0.1	0	−0.1, 0.1
Triceps skin fold thickness in mm	0.1	0.01, 0.3	0.1	−0.02, 0.2
Suprailiac skin fold thickness in mm	−0.005	−0.2, 0.1	−0.02	−0.2, 0.1
Mid-upper arm circumference in cm	0.1	0.02, 0.2	0.1	0.03, 0.2
Calf circumference in cm	0.08	−0.001, 0.2	0.1	0.02, 0.2

[a] Regression coefficient represents estimated change in measurement per doubling of maternal PTH concentration.
[b] Regression coefficient adjusted for infant sex, maternal height, whether first child, whether the mother smoked in pregnancy, season when blood sample was taken, and (for all variables other than gestation length) gestation length.

flexor tone, so measurement error could have contributed to the weaker findings for crown-heel length.

We found no evidence of a linear relationship between maternal $\log_2$ 25-(OH)D at 28–32 wk and infant measures, but our data for knee-heel length were consistent with a linear relationship below a threshold value for sufficiency (Fig. 2).

We found a positive association between maternal 25-(OH)D at 28–32 wk (but not PTH) and gestation length, independently of season. Population data from Japan demonstrated that an increased risk of preterm birth was associated with conception in May or June, a group likely to have lower 25-(OH)D concentration in the third trimester (12, 13).

Increased oily fish intake may reduce risk of preterm birth, (14) and people consuming more oily fish generally have higher 25-(OH)D concentrations (15). It is possible that maternal 25-(OH)D is acting partly as a marker for intake of n-3 fatty acids from fish oil. If this were the case, the relationship between vitamin D concentration and gestation length should be stronger in winter *vs.* summer, because 25-(OH)D status in winter correlates better with dietary intake (9). No evidence was found for this.

Vitamin D_3 is the form synthesized in response to UV light exposure, but vitamin D_2 (ergocalciferol) is added to foods such as margarine spreads and some milks in Australia (16). Both can be converted to 1,25-$(OH)_2$D. A potential weakness of our study is that the vitamin D assay we used reportedly measures only 75% of vitamin D_2, so that 25-(OH)D concentration may have been underestimated in some women. Almost all supplements contained vitamin D_3, and we found that women who took them had higher 25-(OH)D concentration, although this could possibly be confounded by differences in diet or sun exposure.

Total 25-(OH)D was measured, including that bound to vitamin D binding protein. It is reported that the latter rises in pregnancy, (17) so free 25-(OH)D would have been lower than measured concentrations. Nevertheless, 25-(OH)D concentration remains a useful indicator of vitamin D status in pregnancy.

Unexpectedly, we found a strong positive relationship between maternal PTH at 28–32 wk and knee-heel length, as well as weaker positive relationships with birth weight and both mid-upper arm and calf circumferences. Our data contrast with those of Brunvand *et al.* (7), who found a negative relationship between maternal PTH at delivery and crown-heel length among 30 vitamin D-deficient women. However, in both the study by Brunvand *et al.* and our study, the number of vitamin D-deficient women was small, and unlike Brunvand *et al.*, we measured PTH at 28–32 wk rather than at delivery. There may also have been unidentified differences between the two populations, for example in calcium intake.

Maternal PTH concentration was positively associated with birth weight and both mid-upper arm and calf circumferences, but not skin fold thicknesses, suggesting that babies of mothers with higher PTH had longer limbs with a greater proportion of lean tissue. This hypothesis would need to be tested formally using dual-energy x-ray absorptiometry (DEXA), in another cohort.

The associations of low maternal 25-(OH)D and PTH with knee-heel length were mutually independent, suggesting they acted through different mechanisms. This was consistent with our finding of a modest association between 25-(OH)D and PTH at 28–32 wk. It is known that 25-(OH)D crosses the placenta, whereas it is very unlikely that PTH, a large protein, does so (18). However, there is a dearth of data from human pregnancies.

There are a number of different ways in which PTH may be associated with birth size. PTH may be elevated in mothers of bigger babies because of increased mineral demands, so higher PTH may be a consequence and not a determinant of fetal size. We and others (*e.g.* Ref. 19) have shown that maternal total calcium concentration falls during gestation, as the fetus becomes larger. However, PTH-birth size relationships were not altered by adjustment for whether the mother took multivitamin or mineral supplements. Alternatively, PTH may affect fetal growth indirectly via an influence on 1,25-$(OH)_2$D production or on some aspect of placental development or function, or both birth size and maternal PTH may be independently related to a third unidentified factor, so that there is an apparent relationship between them.

Maternal calcium intake is not a focus of this paper and we do not present any data on this. Nevertheless we confirmed that the associations we report were independent of calculated maternal calcium intake.

Conclusions

We have shown that low maternal 25-(OH)D in late pregnancy is associated with decreased knee-heel length at birth, a measure of intrauterine long bone growth. Vitamin D deficiency among pregnant women is preventable and treatable, but is increasingly reported from around the world, especially among women with dark skin living at higher latitudes, and among women who rarely expose their skin to sunlight (8, 20–25). We confirmed that deficiency is also seen in Caucasian women (7% at 28–32 wk in this cohort), even at 38.2 degree south. Follow-up of this cohort will investigate the possibility that this has long-term consequences for linear growth.

Our unexpected finding of a negative relationship between gestation length and maternal 25-(OH)D concentration at 28–32 wk may warrant further investigation in the context of a randomized trial.

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