Pregnancy in dark winters: implications for fetal bone growth?

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Objective: To prospectively examine the prevalence of hypovitaminosis D in pregnancy and to correlate maternal and fetal vitamin D to fetal anthropometry.

Design: A prospective cohort study.

Setting: Tertiary referral maternity hospital.

Patient(s): Sixty pregnant women.

Intervention(s): Serum 25-hydroxyvitamin D (25OHD) was measured in early pregnancy, at 28 weeks, and in cord blood at delivery.

Main Outcome Measure(s): The prevalence of hypovitaminosis D and the relationship between fetal growth and serum 25OHD concentrations.

Result(s): Two subgroups were analyzed to examine results in the context of seasonal variation in 25OHD: a winter and a summer cohort. Fetal anthropometry was assessed at 20 and 34 weeks, and at delivery the neonatal anthropology was recorded. There was a high prevalence of hypovitaminosis D ranging from 33% to 97%, with a marked seasonal variation. Fetal 25OHD concentrations correlated with all biometry at 20 weeks. In the winter cohort, a correlation was found between early pregnancy 25OHD and femur length at 20 weeks, and between 28-week 25OHD and femur length at 34 weeks. Infant length was shorter in those with early pregnancy 25OHD less than the median (52.1 vs. 53.6 cm).

Conclusion(s): The high prevalence of maternal hypovitaminosis D during winter months in northern latitudes may have detrimental effects on fetal skeletal growth. (Fertil Steril 2013;99:206–11. © 2013 by American Society for Reproductive Medicine.)

Key Words: Fetal growth, fetal femur length, pregnancy, vitamin D

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Vitamin D is required for normal calcium homeostasis and bone mineralization, and vitamin D deficiency leads to rickets in childhood or osteomalacia in later adult life (1, 2). Hypovitaminosis D in pregnancy has been linked to a wide variety of additional adverse outcomes, including preeclampsia, low birthweight, gestational diabetes, and an increased predisposition to autoimmune disease in later life (3), but the evidence is inconsistent and inconclusive as regards causality (4). The developing fetus entirely depends on the maternal pool of calcium; as such, there are growing concerns about the implications of hypovitaminosis D during pregnancy (5, 6). Despite the increasing awareness in the medical literature, it appears that the public health message to date is inadequate, with many studies reporting a high prevalence of hypovitaminosis D in pregnant populations (7, 8). This is possibly compounded by the lack of consensus in the literature regarding recommendations for antenatal vitamin D supplementation (9). Risk factors for vitamin D deficiency include dark, pigmented skin, regular use of sunscreen, maternal obesity, and living in high-latitude regions, especially during winter or spring months (3). In northern countries at latitudes above 42° north, endogenous production of vitamin D essentially ceases from November until March (10, 11).

Accurate assessment of the prevalence of hypovitaminosis D is limited in many studies to date by the heterogeneity of populations studied, the effects of seasonal variation, and the reliance on a single assessment of 25OHD at just one time point in pregnancy (12, 13). Our objective was to clarify...
the prevalence of hypovitaminosis D in pregnancy by assessing 25OHD concentrations in early pregnancy, at 28 weeks’ gestation and in fetal blood from the umbilical cord at delivery in two cohorts of healthy Caucasian women pregnant at opposite times of the year to account for seasonal variation. We also sought to assess the possible implications of vitamin D deficiency for fetal growth, in particular fetal bone.

MATERIALS AND METHODS

This was a prospective cohort study of 60 mother and infant pairs at the National Maternity Hospital, Dublin, Ireland, with institutional ethics approval and written maternal consent. At our institution, routine screening for vitamin D deficiency is not performed. All women were Caucasian and were recruited to the study at first antenatal consultation. Women were excluded if they had an underlying medical condition, if they were younger than 18 years of age, or if they were unable to give full informed consent.

Two specific cohorts were recruited. The first group (winter cohort) consisted of 30 women who were recruited in early pregnancy in September/October and delivered in March/April. A further 30 women were recruited in March/April who delivered in September/October (summer cohort). All women had 25OHD measured in early pregnancy (mean 14.3 ± 2.6 weeks), at 28 weeks’ gestation and in fetal blood from the umbilical cord at delivery. At 20 weeks’ gestation (range: 19 + 1 to 21 + 5), a routine fetal anomaly ultrasound was performed, and fetal biometry including biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) was recorded. Fetal biometry was assessed again ultrasonographically at 34 weeks’ gestation (range: 33 + 4 to 34 + 5 weeks).

Serum 25OHD concentrations were measured by competitive radioimmunoassay (Immunodiagnostic Systems Limited). The coefficients of variation (CV) for the 25OHD assay are as follows: interassay CV at a concentration of 29 nmol/L was 6.2% and at a concentration of 106 nmol/L was 7.7%; intra-assay CV at a concentration of 29 nmol/L was 3.0% and at a concentration of 74 nmol/L was 2.7%. To ensure a high standard of analysis, we participate in the Vitamin D External Quality Assessment Scheme (14).

At delivery infant birthweight, the length and head circumference were recorded. Serum 25OHD concentrations of above 50 nmol/L were classified as sufficient, and concentrations of <30 nmol/L were considered at high risk of deficiency as per the recent Institute of Medicine (IOM) report (4).

Dietary vitamin D intake was assessed using a 3-day food diary, which was completed 1 to 2 weeks after the first antenatal consultation. Women were asked to record their usual food and beverage intake over 3 consecutive days, including a weekend day. Dietary data were entered and analyzed using Weighed Intake Software Program (WISP) (Tinuviel Software), which uses food composition data from the Food Standards Agency (15).

Data were assessed for normality using Shapiro-Wilk and P-P plots. Bivariate correlations were assessed using Pearson’s correlation coefficient for normally distributed data and Spearman’s rho for nonparametric data. Further analysis was performed by comparing outcomes for those patients above and below the median for 25OHD concentrations, namely 41.0 nmol/L in early pregnancy, 45.7 nmol/L at 28 weeks, and 30.8 nmol/L in cord blood. Comparison of means within groups of patients was accomplished with the independent samples t-test. P < .05 was considered statistically significant. Statistical analysis was performed using SPSS Windows’s version 18.0 (SPSS, Inc.). Sample size calculation based on a significance level set of 5% and power set at 90% suggested that 26 in each group would be required to detect 1 standard deviation difference in 25OHD between the two groups.

RESULTS

Vitamin D Status and Season

The baseline characteristics of the cohort are shown in Table 1. Within the total study population, the mean 25OHD concentration was 45.7 ± 22.6 nmol/L in early pregnancy, 54.4 ± 33.4 nmol/L at 28 weeks’ gestation, and 31.8 ± 12.6 nmol/L in fetal blood at delivery. All pregnancies resulted in healthy term deliveries ranging from 37 + 1 to 42 + 1 weeks’ gestation.

A marked seasonal variation in 25OHD was observed (Tables 1 and 2). In women in the summer cohort whose early pregnancy sample was taken in March/April, the prevalence...
of vitamin D sufficiency was only 10%, and the prevalence of those at high risk of deficiency was 50%. In the winter cohort whose early pregnancy samples were drawn in September/October, the prevalence of sufficiency was 67%, and only 7% were at high risk of vitamin D deficiency (P < .05). As the pregnancies progressed, the seasonal effect was maintained, such that by 28 weeks’ gestation (December/January for the winter cohort, and June/July for the summer cohort) just 30% of the winter cohort had sufficient 25OHD concentrations compared with over 53% of the summer cohort (P < .05). In the winter cohort who delivered in March/April, the fetal sample showed that only 3% were sufficient and 47% were at high risk of deficiency (25OHD < 30 nmol/L). Though 25OHD concentrations were statistically significantly higher at 28 weeks in the summer cohort who delivered in September/October than at 28 weeks in the winter cohort, only 13% of the summer cohort were sufficient, and 43% were at high risk of deficiency at the time of delivery.

The mean dietary intake of vitamin D as assessed by the 3-day food diary in early pregnancy and including intake from both diet and supplements was low, at just 2.78 ± 1.8 μg for the total cohort. There was no statistically significant difference in dietary vitamin D intake between the summer and winter cohorts (3.03 ± 2.1 μg vs. 2.52 ± 1.5 μg, P = .3).

Within the cohort, 37 women were taking prenatal vitamin supplementation, which included vitamin D. The mean gestation at commencement of supplements was 7.4 ± 6.5 weeks and at discontinuation 35.9 ± 8.3 weeks. The mean 25OHD concentration of those taking prenatal supplements was statistically significantly higher in early pregnancy compared with those receiving no supplementation (51.6 ± 23.7 nmol/L vs. 37.1 ± 17.5 nmol/L, P = .01); however, no statistically significant difference persisted at 28 weeks’ gestation (52.8 ± 24.2 nmol/L vs. 59.3 ± 46.4 nmol/L, P = .5) or in fetal blood at delivery (33.7 ± 13.8 nmol/L vs. 29.8 ± 9.3 nmol/L, P = .2).

### Vitamin D Status and Fetal Biometry

The correlation of fetal biometry with both maternal and fetal 25OHD concentrations is shown in Table 3. Overall, fetal 25OHD concentrations correlated with fetal biometry at 20 weeks’ gestation (HC r = 0.39, P = .002; BPD r = 0.34, P = .008; AC r = 0.34, P = .009; and FL r = 0.35, P = .008). In the winter cohort, a positive correlation was found between maternal early pregnancy 25OHD and fetal femur length at 20 weeks’ gestation (r = 0.34, P = .07). Additionally, a positive correlation was noted between both maternal 28-week 25OHD (P = .02) and fetal 25OHD (P = .009) and fetal femur length at 34 weeks’ gestation. No statistically significant difference was seen in the fetal biometry at 20 or at 34 weeks between those women taking and those not taking prenatal supplements. No statistically significant association was observed between maternal 25OHD and fetal biometry at any time point.

### Tables

#### Table 2

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Cohort</th>
<th>25OHD categories</th>
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<tr>
<td></td>
<td></td>
<td>≥ 50 nmol/L</td>
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<tr>
<td>First trimester</td>
<td>Winter (September/October)</td>
<td>20/30 (66.7%)</td>
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<tr>
<td>28-wk gestation</td>
<td>Summer (March/April)</td>
<td>3/30 (10%)</td>
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<tr>
<td>Summer (December/January)</td>
<td>9/30 (30%)</td>
<td>11/30 (36.7%)</td>
</tr>
<tr>
<td>Cord blood</td>
<td>Winter (March/April)</td>
<td>1/30 (3.3%)</td>
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<tr>
<td>Summer (September/October)</td>
<td>4/30 (13.3%)</td>
<td>13/30 (43.3%)</td>
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#### Table 3

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<thead>
<tr>
<th>25OHD groups</th>
<th>20-wk gestation</th>
<th>34-wk gestation</th>
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<tr>
<td></td>
<td>HC</td>
<td>BPD</td>
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<tr>
<td>Overall (n = 60)</td>
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<td></td>
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<tr>
<td>Early pregnancy</td>
<td>0.02</td>
<td>−0.05</td>
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<tr>
<td>28 wk</td>
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<td>0.2</td>
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<tr>
<td>Cord blood</td>
<td>0.39a</td>
<td>0.34a</td>
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<tr>
<td>Summer cohort</td>
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<tr>
<td>Early pregnancy</td>
<td>0.04</td>
<td>−0.05</td>
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<tr>
<td>28 wk</td>
<td>0.2</td>
<td>0.01</td>
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<tr>
<td>Cord blood</td>
<td>0.43a</td>
<td>0.32</td>
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<tr>
<td>Winter cohort</td>
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<tr>
<td>Early pregnancy</td>
<td>0.19</td>
<td>0.2</td>
</tr>
<tr>
<td>28 wk</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Cord blood</td>
<td>0.39a</td>
<td>0.31</td>
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Note: AC = abdominal circumference; BPD = biparietal diameter; EFW = estimated fetal weight; FL = femur length; HC = head circumference.

* P < .05.

noted in either cohort between maternal and fetal 25OHD and infant birthweight, length, or head circumference.

A comparison of the fetal and neonatal anthropometry of those with serum 25OHD concentrations above and below the median for the cohort at each time point is shown in Table 4. Fetal biometry at 20 weeks’ gestation was statistically significantly greater for those with cord blood 25OHD above rather than below the median concentration. Additionally, the mean infant length at birth was statistically significantly shorter in those with a 25OHD concentration less than the median in early pregnancy (52.1 vs. 53.6 cm, \( P < .05 \)).

**DISCUSSION**

Our findings in a cohort of healthy pregnant women have demonstrated a high prevalence of hypovitaminosis D in pregnancy, despite over half of the cohort (37 women) reporting taking prenatal supplementation. Just 23 women (38%) in early pregnancy and 25 (42%) at 28 weeks’ gestation had sufficient vitamin D concentrations (25OHD \( \geq 50 \) nmol/L). The prevalence of high risk for vitamin D deficiency was particularly high in fetal blood at delivery, with just five cases (8%) having 25OHD \( \geq 50 \) nmol/L. Maternal 25OHD concentrations correlated with fetal 25OHD as assessed by cord blood at delivery, reflecting the fact that the developing fetus is entirely dependent on maternal 25OHD concentrations.

The effect of seasonal variation in 25OHD concentrations is highlighted once again by our findings. Concentrations of 25OHD were lowest at the beginning of spring and highest in mid-summer. Our cohort of women was Caucasian and living in Dublin, which is located at latitude 53° north. Exposure to sunlight is essential for the conversion of provitamin D3 to previtamin D3, and in countries at latitudes above 42°, skin production of vitamin D declines at the end of the summer and ceases during winter months (10).

Our winter cohort had the majority of their antenatal course during a time when exposure to effective sunlight was absent. In these pregnancies, we identified a positive correlation between 25OHD concentrations and fetal femur length at both 20 and 34 weeks’ gestation. Though no statistically significant direct correlation between 25OHD and neonatal anthropometry was identified, the mean infant length at birth was statistically significantly shorter in those with an early pregnancy 25OHD concentration less than the median in early pregnancy. Additionally all fetal biometry at 20 weeks’ gestation was statistically significantly greater for those with cord blood 25OHD above rather than below the median concentration for the cohort.

Our findings clearly demonstrate that the lack of exposure to sunlight in northern countries has a significant effect on vitamin D status that is not adequately compensated for by dietary intake or routine prenatal self-supplementation. This may have potential implications for fetal bone growth. Indeed, a number of investigators have identified similar associations between vitamin D status and fetal bone growth. Mahon et al. (5) speculated that maternal vitamin D insufficiency influenced femoral development from as early as 19 weeks’ gestation, and recommended that measures to improve maternal vitamin D status should be instituted in

<table>
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<td><strong>Comparison of fetal and neonatal anthropometry of those with 25-hydroxyvitamin D (25OHD) concentrations above and below the median for the cohort at each time point.</strong></td>
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<tr>
<td><strong>Cohort</strong></td>
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<td><strong>Maternal</strong></td>
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<td><strong>Cord blood</strong></td>
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Note: Median and standard deviation of fetal biometry at 20 and at 34 weeks’ gestation for those above and below the median 25OHD concentrations for the cohort. AC = abdominal circumference; BPD = biparietal diameter; EFW = estimated fetal weight; FL = femur length; HC = head circumference. \( P < .05 \).
early pregnancy. Viljakainen et al. (6), after studying a cohort of 125 women, suggested that maternal vitamin D status affects bone mineral accrual during the intrauterine period and affects bone size, despite the mean total intake of the mothers in their cohort meeting current recommendations.

Our study does have limitations worthy of consideration. Our cohort consisted of 60 women; however, our inclusion criteria were strict, including only Caucasian women living in Dublin. Our recruitment specifically aimed to assess the prevalence of hypovitaminosis D in the context of known seasonal variation in 25OHD concentrations. In addition, we do not have any information on maternal 25OHD at delivery. The assessment of maternal 25OHD concentrations in both early and later pregnancy as well as fetal 25OHD in cord blood and the addition of ultrasonographic data greatly strengthen our findings. The assessment of fetal biometry at both 20 and 34 weeks’ gestation is essential to assess any potential implications of vitamin D depletion on intrauterine growth; our results would suggest that there might indeed be a link that is worthy of further interrogation.

What is clear from our findings is the important seasonal effect on vitamin D status. For women living in Ireland, and those in countries of similarly high latitude during winter months, vitamin D supply depends entirely on oral intake from natural foods stuffs and fortified food. It is becoming increasingly evident that vitamin D is involved in a wide variety of biological processes, and that vitamin D deficiency may have a number of potential adverse consequences (3, 9). Vitamin D supplementation during pregnancy during winter is therefore essential.

The optimal dosage of vitamin D supplementation—and indeed an acceptable definition of vitamin D deficiency, particularly in pregnancy—remains an elusive and often debated topic. For our definition of vitamin D sufficiency, insufficiency, and deficiency in this study, we used the recent U.S. Institute of Medicine (IOM) of the National Academies report (4). The 2011 Endocrine Society Clinical Guideline, however, defined vitamin D deficiency as a 25OHD below 20 ng/mL (50 nmol/L) and vitamin D insufficiency as a 25OHD of 21–29 ng/mL (52.5–72.5) nmol/L (16). Using this definition in our cohort, only 5 (8.3%) women were sufficient 25OHD in early pregnancy, and 10 (16.7%) women were sufficient at 28 weeks; just one fetus had a cord blood 25OHD concentration of greater than 72.5 nmol/L. Regardless of the definition used, there is no doubt that vitamin D deficiency is prevalent, despite supplementation in many.

This high prevalence of vitamin D hypovitaminosis D requires clear guidance on vitamin D supplementation during pregnancy; yet again there is a distinct lack of consensus in the literature. In the United Kingdom and Ireland, there are no clear recommendations for routine antenatal supplementation with vitamin D. The National Institute for Health and Clinical Excellence (NICE) guideline for antenatal care in 2008 (17) recommended informing women of the importance of maintaining adequate vitamin D stores in pregnancy, particularly for those at greatest risk of vitamin D deficiency who may chose to take vitamin D at a rate of 400 IU daily, a stance that was endorsed by the Scientific Advisory Committee on Nutrition (18). To achieve sufficiency, defined as 50 nmol/L, the IOM recommends 400–600 IU/day, which it states can be obtained through dietary means and supplementation, and indeed they caution that higher intakes of vitamin D may be harmful. Contrary to this, a number of investigators have suggested that during pregnancy vitamin D supplementation at 400 IU/day has minimal effect on circulating 25OHD concentrations in either mother or baby (19, 20).

In contrast, the Endocrine Society recommends an intake of 1,500–2,000 IU/day to achieve a circulating 25OHD level of more than 75 nmol/L. Doses of up to 4,000 IU daily have even been advocated by some (21). In 2011, Hollis et al. (21) randomized pregnant women to receive 400, 2,000, or 4,000 IU of vitamin D3 daily. They found that supplementation with 4,000 IU daily was safe and most effective in achieving sufficiency. They defended the safety of such high doses by pointing out that pregnant women from different native tribes in Africa have been shown to have an average circulating 25OHD level of 150 nmol/L throughout pregnancy from the natural environment (22). In their randomized, controlled trial, pregnant women receiving 4,000 IU/day vitamin D3 attained an average circulating 25OHD level of 111 nmol/L, well below the 150 nmol/L in a natural environment, though further studies are required to establish safe upper limit in pregnancy.

Our findings indicate that in an otherwise healthy population, with a higher than 50% rate of supplementation, the prevalence of hypovitaminosis D is higher than expected. This may have implications for intrauterine growth, in particular bone growth. We would advocate that all women living in areas of limited sunshine should be regarded as being at high risk of vitamin D deficiency in winter and should be advised accordingly, even in the absence of other risk factors. Further work is clearly warranted to establish the optimal and importantly safest dose of vitamin D supplementation in pregnancy as well as the potential implications of inadequate concentrations on fetal and later childhood and adult bone growth.

REFERENCES


