Prenatal and infant predictors of bone health: the influence of vitamin D$^1$–$^4$

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ABSTRACT
Vitamin D is essential for the health of pregnant women and their infants. Vitamin D insufficiency and deficiency during pregnancy are reflected in lower maternal weight gain and biochemical evidence of disturbed skeletal homeostasis in the infant, with, in extreme situations, reduced bone mineralization, radiologically evident rickets, and fractures. Populations at risk for vitamin D deficiency are those for which, for environmental, cultural, or medical reasons, exposure to sunlight is poor and the dietary intake of vitamin D is low. The infants born in such populations have low vitamin D stores and may receive little additional vitamin D if they are breast-fed without supplements for long periods. In the short term, lack of vitamin D supplementation in infancy leads to biochemical disturbances, reduced bone mineralization, slower growth, and eventual alterations in bone shape and increased risk of fracture, the hallmarks of rickets. In the longer term, lack of vitamin D supplementation may result in reduced bone size and mass during childhood and an increased risk of type 1 diabetes mellitus. Clear recommendations are needed regarding the intake of vitamin D during pregnancy and infancy. Such recommendations should be based on functional outcomes, rather than biochemical measurements, so that the medical problems resulting from the lack of this essential nutrient can be overcome. 

KEY WORDS
Pregnancy, rickets, growth, bone

INTRODUCTION
A definition of bone health should include elements reflecting both the final structure and purpose of the whole skeleton and the processes regulating bone growth, differentiation, and cellular activity. The final purpose of bone is to provide anchorage for muscles, protection for internal organs, and a repository for bone marrow and minerals. We know that, among adults, fractures are more common among individuals with thinner smaller bones for whom bone geometric features and internal architecture are abnormal and bone cell activity is unbalanced, so that either too much bone is resorbed or too little bone is formed. This article focuses on what we know about the role of vitamin D in pregnancy and early infancy in determining later outcomes, specifically its effects on bone growth and bone mass.

VITAMIN D IN THE LAST TRIMESTER OF PREGNANCY
The definitions of vitamin D deficiency, insufficiency and sufficiency are based currently on biochemical measurements of 25-hydroxyvitamin D concentrations, particularly in relation to the associated changes in parathyroid hormone concentrations. A typical serum 25-hydroxyvitamin D concentration for the borderline between deficiency and insufficiency is 12 nmol/L (5 ng/mL); there is much greater debate regarding the borderline between insufficiency and sufficiency, with values ranging from 20 nmol/L (8.3 ng/mL) to 80 nmol/L (33 ng/mL) being suggested. It is unusual for individuals displaying radiologic features of vitamin D deficiency-induced osteomalacia or rickets to have “sufficient” serum 25-hydroxyvitamin D concentrations, unless they have experienced a sudden recent increase in their exposure to sunlight or oral vitamin D supplementation (reviewed in ref 1).

MALNUTRITION
It was previously well reported that maternal osteomalacia can be associated with infantile rickets (2). Affected infants have radiologic manifestations including cupping, splaying, and fraying of the metaphyses, together with cortical thinning. The case reports generally highlighted this problem as occurring among individuals who are refugees or displaced persons or, alternatively, recent immigrants to temperate climates from sunnier parts of the world. However, individual case reports indicated other causes of infantile rickets, including the repeated use of phosphate enemas during pregnancy (3), maternal renal insufficiency (4), and maternal malabsorption (5) and preeclampsia (6). These latter examples might reasonably be expected to affect the supply of both vitamin D and minerals to the fetus.

VITAMIN D DEFICIENCY WITHOUT MALNUTRITION
It was reported that serum 25-hydroxyvitamin D concentrations decrease during pregnancy (7, 8); debate regarding absolute values continues, with reports of similar or lower values among...
pregnant women, compared with nonpregnant women (7, 9). The greatest decline is observed during the third trimester (8), and 25-hydroxyvitamin D concentrations are lower in cord blood than in maternal blood (typically by 25–30% of the maternal value) (10). There are correlations between maternal and cord blood 25-hydroxyvitamin D concentrations and those of its breakdown product 24,25-dihydroxyvitamin D, which suggests passive diffusion of both compounds across the placenta (11).

We recently investigated cord blood 25-hydroxyvitamin D concentrations among a cohort of women who underwent cesarean delivery during the spring of 2003, in Sheffield, United Kingdom. The purpose of the study was to determine whether variations in vitamin D status that occurred “naturally” among pregnant women, primarily of white background, were associated with subsequent infant growth. Ethical permission for the study was granted by the South Sheffield Research Ethics Committee, and informed consent was obtained in all cases. Data were collected from the notes on maternal illness during pregnancy, ethnicity, and whether vitamin D supplementation occurred at any stage during the pregnancy. All of the infants were born between the beginning of February and the end of May 2003. On the basis of the observations of Maxwell et al (12), we aimed to recruit 110 women, targeting a 0.5-standard deviation score difference in the length of the infants at age 6 mo in relation to endogenous variations in vitamin D status among the mothers. Blood was obtained from the cord in 108 cases, left to clot for 20 min, and then centrifuged for 10 min at 3000 rpm. Serum was stored at −20 °C before batch analysis of 25-hydroxyvitamin D concentrations with a competitive radioimmunoassay, after solvent extraction (IDS, Tyne and Wear, United Kingdom). Anthropometric measurements were performed at birth and at 3 and 6 mo of age, with recording of head circumference to the nearest 1 mm (paper tape measure), length to the nearest 1 mm (Rollametre; Childhood Growth Foundation, London, United Kingdom), and weight to the nearest 10 g (Seca 724 electronic balance scales, calibrated daily; Seca Ltd., Birmingham, United Kingdom) at each time point. Assessment of vitamin D intake from formula, supplements, and solid foods was made at the later time points. The mode of feeding (breast, formula, or mixed) was recorded.

The 25-hydroxyvitamin D concentrations in cord blood are presented in Figure 1. We found that >70% of babies had cord blood 25-hydroxyvitamin D concentrations of <20 nmol/L. Fifteen of the 108 mothers were nonwhite. Figure 2 presents a comparison of the 25-hydroxyvitamin D concentrations for the nonwhite and white mothers. Although values for the infants of nonwhite mothers were 3.2 nmol/L lower, on average, than were those for the infants of white mothers (P = 0.0017 for difference), the median value for infants of white mothers was still <20 nmol/L. Twenty-five percent of mothers took a vitamin D supplement, and the difference in the 25-hydroxyvitamin D concentrations in cord blood for those who had and those who had not received vitamin D supplements during pregnancy was 4.1 nmol/L. Despite supplementation, the median value for the supplement-treated group was <20 nmol/L.

In follow-up assessments, we were unable to determine a consistent association between cord blood 25-hydroxyvitamin D concentrations and either weight or length at 3 or 6 mo postnatal age. There was a significant association with head circumference z scores (age-adjusted; reference data from the Childhood Growth Foundation for infants in the United Kingdom (23)), which remained after adjustment for confounding factors; given the number of tests performed, however, this should be regarded as a possible rather than a definite association.

**VITAMIN D SUPPLEMENTATION DURING PREGNANCY**

Prospective studies of vitamin D supplementation have been performed with populations at risk of vitamin D deficiency. Marya et al (13) studied 120 multiparous Indian women in Rohtak, India. Seventy-five women received no supplemental vitamin D or calcium, 25 received 1200 IU of vitamin D and 375 mg of calcium per day during the third trimester, and 20 received 2 doses of 600 000 IU of vitamin D orally at months 7 and 8 of pregnancy.

There were significant decreases in maternal alkaline phosphatase and cord blood alkaline phosphatase concentrations among subjects who received the vitamin D supplementation.
The decrease was greatest in the cord blood alkaline phosphatase concentration for the subjects who received 600,000 units at months 7 and 8. There were small increases in maternal and infant serum phosphate concentrations among the vitamin D supplement-treated infants. Birth weights were, on average, 3.14 ± 0.45 kg for the infants of the 20 mothers who received 600,000 IU of vitamin D twice, 2.89 ± 0.32 kg for the infants of the mothers who had received both vitamin D and calcium, and 2.73 ± 0.36 kg for the control group. There was no indication of the method through which women were assigned to the groups or of their vitamin D status before supplementation.

Maxwell et al. undertook a double-blind, controlled study of supplemental vitamin D given in the last trimester of pregnancy to Asian women living in London. The average serum 25-hydroxyvitamin D concentration at booking was 20 nmol/L, and the women received either placebo or 1000 units of vitamin D per day during the third trimester. The infants were examined at birth and at 3-mo intervals to 1 y of age.

Maternal well-being appeared to be enhanced among subjects who received the supplement, inasmuch as there was increased weight gain (63 g/d, compared with 46 g/d), increased retinal-binding protein and thyroid-binding prealbumin concentrations, and a reduction in serum alkaline phosphatase activity measured in blood obtained near the time of delivery. Serum alkaline phosphatase activity was reduced among the infants of the treated women, compared with the infants of control subjects, and the rate of intrauterine growth retardation was reduced from 29% to 15%. There was no difference in birth length or birth weight, but an increase in the size of the fontanelle was noted among the infants of the control mothers.

Weights remained approximately equal between the groups at 3 mo and began to diverge from 6 mo onward, such that by 12 mo the infants of control mothers weighed 8.98 ± 0.62 kg, compared with 9.39 ± 0.66 kg for the treated group (14). The incremental increase in weight during the 12-mo period was 5.92 ± 0.92 kg for the infants of control mothers and 6.39 ± 0.78 kg for the infants of treated mothers. A similar pattern was observed for length, with divergence from 6 mo onward and a difference of 1.2 cm at the age of 1 y (76.2 ± 1.9 cm for infants of treated mothers, compared with 74.6 ± 1.7 cm).

Supplementation studies were also undertaken with white populations. Delvin et al. assessed the effects of 1000 IU/d vitamin D, compared with placebo, among 40 women from 6 mo of gestation onward. All of the deliveries were in June; all infants were singletons who were breast-fed, without postnatal vitamin D administration. There were no significant changes in maternal whole-blood or ionized calcium, immunoreactive parathyroid hormone, or 1,25-dihydroxyvitamin D concentrations between the groups. 25-Hydroxyvitamin D concentrations increased after 45 and 90 d of supplementation (65 nmol/L, compared with 30 nmol/L at term). Among the infants of control mothers, the 25-hydroxyvitamin D concentration in cord blood was lower (17 nmol/L, compared with 45 nmol/L) and that of 1,25-dihydroxyvitamin D was higher (147 pmol/L, compared with 95 pmol/L). There was no significant difference between the infants with respect to total or ionized calcium, magnesium, or immunoreactive parathyroid hormone concentrations.

The infants were studied again at the age of 4 d and, among infants of control mothers, the total calcium concentration was lower (2.1 mmol/L, compared with 2.3 mmol/L), the ionized calcium concentration was lower (0.98 mmol/L, compared with 1.25 mmol/L), the 25-hydroxyvitamin D concentration was lower (12 nmol/L, compared with 33 nmol/L), and the 1,25-dihydroxyvitamin D concentration was lower (140 nmol/L, compared with 225 nmol/L). Although immunoreactive parathyroid hormone concentrations were higher among control mothers and their infants at all times, there was no significant difference between the groups of either mothers or infants.

**INTERPRETATION**

In combination, these observations suggest that, among infants of mothers who have good vitamin D stores or who receive adequate vitamin D through the effects of sunshine or diet during pregnancy, fetal growth is normal, as is fetal mineral accretion. In addition, neonatal mineral homeostasis is better maintained and postnatal growth is normal, possibly irrespective of postnatal vitamin D supplementation. In cases of poor maternal vitamin D stores, however, fetal growth may be retarded, mineral accretion may be reduced, neonatal hypocalcaemia is more common, and postnatal linear growth and weight gain may be reduced.

**POSTNATAL VITAMIN D AMONG TERM INFANTS**

It has been known for many years that lack of vitamin D supplementation leads to slower growth in infancy (16). However, the longer-term effects on bone growth and mass have been more difficult to determine. A retrospective study by Zamora et al. involved 149 prepubertal girls at the time of their enrollment into a calcium supplementation study. Birth records and questionnaires were sent to parents and their pediatricians, and 127 responded. Of the 21 subjects excluded from this study, 17 were formula-fed, 5 were preterm, 3 were habitual milk-avoiders, and for 3 there was no reliable information on vitamin D supplementation. Of the remaining 106, 91 had received vitamin D supplements from birth to the age of 6 mo.

Birth weights were similar for those who did or did not receive vitamin D in early infancy (3.235 and 3.189 kg), as were birth length (49.5 and 50.3 cm), weight at 1 y (9.3 and 9.1 kg), and length at 1 y (74.2 cm for both groups). At the time when the bone densitometric measurements before entry into the calcium supplementation study were performed, the children were of similar ages (7.9 y for the vitamin D-treated group and 7.8 y for the non–vitamin D-treated group). However, weight was greater for those who had received vitamin supplements early in life (26.9 kg, compared with 25.9 kg) and height was greater (128 cm, compared with 127.2 cm). Calcium intakes were similar for the 2 groups (841 and 837 mg/d).

There were clear differences between the groups in areal bone mineral densities at the radial metaphysis, the femoral neck, and the femoral trochanter. Children who had received vitamin D supplements had higher values for each site of measurement. There was, however, no significant difference in areal bone mineral densities at the lumbar spine. Dual-energy X-ray absorptiometry instruments do not fully adjust for the third dimension as they convert volume to area. The differences between groups disappeared after adjustment for body size, which suggests that the effect of early vitamin D supplementation was to create larger bones, rather than denser bones.
VITAMIN D AMONG INFANTS BORN PREMATURELY

Backstrom et al (18) studied the effects of administering either 200 IU/kg per day up to a maximum of 400 IU or 960 IU/d to a group of 43 preterm infants < 33 wk of age. Of the 43 subjects, 39 completed the study. There were no differences between the groups with respect to weight, length, occipital frontal circumference, any measure of bone mineral content or bone mineral density in the radius, or any measured biochemical parameter reflecting bone metabolism.

Backstrom et al (19) also investigated the long-term outcomes of children 9 to 11 y of age who were born prematurely and assigned 500 or 1000 IU of vitamin D in a factorial study that also investigated the use of calcium and phosphorus supplements to breast milk. There was no apparent long-term benefit for any of the supplement (mineral and/or vitamin D)-treated groups at 9 to 11 y of age.

In the larger studies of Lucas and colleagues (20, 21), premature infants who were assigned during their period of hospitalization to receive a variety of different diets, without specific randomization to different levels of vitamin D supplementation, showed the following characteristics. Premature infants were smaller at the age of 8–12 y than were term infants (20). There was no difference in body size-adjusted bone mass (21). There was increased bone turnover among children who had been born prematurely, compared with those born at term (21). In the preterm infant studies, those who had received low-mineral substrate diets during the period of hospitalization exhibited higher bone turnover at the age of 11–12 y than did those who had received higher-mineral substrate diets (21).

POSTNATAL VITAMIN D AND DIABETES

Retrospective studies, particularly that of Hypponen et al (22), suggested that postnatal vitamin D supplementation is associated with a reduction in the incidence of type 1 diabetes mellitus. Hypponen et al (22) studied a birth cohort of 10 366 children who were monitored from 1966; 81 developed type 1 diabetes. The incidence of type 1 diabetes up to December 1997 was increased 8-fold among those who received no supplementation, compared with the recommended dose of 2000 IU/d up to age 1 y. The biological mechanisms through which vitamin D might influence the pathophysiologic development of diabetes are unclear, although pancreatic islet cells do have vitamin D receptors and vitamin D is thought to have immunomodulatory roles.

CONCLUSIONS

Prenatal vitamin D status appears to affect postnatal mineral homeostasis and may influence growth. Prenatal vitamin D status affects growth and is fundamental to mineral homeostasis; it may also affect subsequent bone mass. Clearly, more data on functional outcomes in infancy and childhood, possibly extending into young adulthood, are required for determination of the effects of administration of different amounts of vitamin D during pregnancy. At this time, it is not possible to provide a single universal guideline for vitamin D supplementation during pregnancy, although supplementation with 400-1000 IU/d during the last trimester for those most at risk of deficiency would likely be safe and might be of benefit.

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REFERENCES