COMMENTARY

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Vitamin D Insufficiency and Skeletal Development In Utero

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Over the last 10 years, our perception of what constitutes normal vitamin D status has undergone a substantial revision. Prior to this, *suboptimal* vitamin D was defined at a very basic level by the presence or absence of associated bone disease (i.e., rickets in children and osteomalacia in adults). As a consequence, *vitamin D deficiency* was determined by serum concentrations of 25-hydroxyvitamin D (25-OHD) of less than 25 nM (10 ng/mL), and anything higher was "normal." However, this has changed with the observation that several parameters of calcium homeostasis continue to correlate with serum levels of 25-OHD up to concentrations as high as approximately 80 nM (32 ng/mL).[1,2] The implication is that optimal vitamin D status is achieved only at 25-OHD concentrations above this; anything less is suboptimal or "insufficient." Based on these revised parameters, it has been concluded that vitamin D insufficiency is a global phenomenon, with an estimated 1 billion people worldwide having suboptimal levels of 25-OHD.[3] Some groups appear to be at greater risk of vitamin D insufficiency than others, notably pregnant women.[4–8] In a study carried out in Pittsburgh, PA, Bodnar and colleagues showed that 74% to 95% of pregnant black women and 46% to 62% of pregnant white women were vitamin D insufficient.[5] Notably, during early pregnancy, almost 45% of the African-American mothers had 25-OHD levels that were less than 37.5 nM.[5] A key question arising from these epidemiologic data concerns the physiologic impact of vitamin D insufficiency during pregnancy. In the current issue of the Journal, Mahon and colleagues have addressed this through a prospective longitudinal study of pregnant women in which they have characterized the impact of maternal vitamin D status on in utero measures of fetal skeletal development.[9]

The precise definition of what constitutes vitamin D insufficiency versus vitamin D deficiency is still subject to some debate. In some instances, *vitamin D deficiency* is defined as a serum concentration of 25-OHD of less than 50 nM, whereas *vitamin D sufficiency* refers to a 25-OHD level of greater than 75 nM.[10] As a result, serum concentrations of 25-OHD of between these values correspond to the aforementioned vitamin D insufficiency. In the study of 424 pregnant women described by Mahon and colleagues,[9] the authors have defined *sufficiency* as being 25-OHD concentrations greater than 70 nM based on National Diet and Nutrition Survey data from the United Kingdom. *Vitamin D deficiency* was defined as being less than 25 nM 25-OHD, and interestingly, the authors then subdivided intervening serum concentrations of 25-OHD into two groups: "borderline" (50 to 70 nM) and "insufficient" (25 to 50 nM), providing an additional perspective on the physiologic impact of maternal vitamin D status.

High-resolution 3D ultrasound (3DUS) analysis of the pregnant women showed that suboptimal vitamin D status is associated with increased femur metaphyseal cross-sectional area and femur splaying index at 19 and 34 weeks of gestation. These changes contrasted with the measurement of femur length, which showed no variability across the different categories of vitamin D status. The authors have shown previously that children born to mothers with vitamin D deficiency (<25 nM 25-OHD) or insufficiency (<50 nM 25-OHD) during pregnancy exhibit deficits in bone mineral content at 9 years of age.[11] However, the 3DUS study presented here is the first of its kind to describe changes in skeletal morphology in utero that are related to maternal vitamin D status. The splaying and associated metaphyseal widening documented in this study are analogous to the radiographic characteristic of the femoral and tibial bowing that occurs with rickets. In the case of the latter, changes in metaphyseal morphology occur as a consequence of gravitational compression of "soft" undermineralized bone. By contrast, the in utero observations described in the current study occur despite a low-gravity environment. The underlying basis for this remains unclear and will be the focus of future studies.

The data presented by Mahon and colleagues remain observational, and causality cannot be assumed automatically. Nevertheless, they are provocative on several levels given current interest in the clinical impact of vitamin D insufficiency. Significantly, the authors demonstrated differences in skeletal development associated with vitamin D status as early as week 19 of gestation. This is coincident with the well-documented rise in maternal levels of the active form of vitamin D,

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1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], that occurs early in gestation, thereby facilitating enhanced intestinal uptake of calcium in the mother as compensation for the increased fetal demand for calcium as pregnancy progresses.[12] Enhanced conversion of 25-OHD to 1,25-$(OH)_2$D in the setting of pregnancy is thought to be due primarily to activity of the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase) in maternal kidneys. Renal activity of this enzyme is defined principally by the stimulatory effects of parathyroid hormone (PTH) in response to decreased serum calcium levels. The authors of the current study measured only circulating levels of maternal 25-OHD and not 1,25-$(OH)_2$D. Nevertheless, it seems unlikely that the variations in vitamin D status they describe will have a major impact on maternal synthesis of 1,25-$(OH)_2$D, questioning the involvement of such a mechanism in mediating fetal responses to vitamin D.

Synthesis of 1,25-$(OH)_2$D also occurs in the decidual and trophoblastic cells of the placenta.[13,14] This may contribute to circulating levels of the hormone in pregnant women but may equally be more important for localized actions of vitamin D such as immune responses to infection.[15] Notably, in contrast to the kidneys, activity of 1α-hydroxylase in placental cells is not subject to regulation by PTH. Instead, placental synthesis of 1,25-$(OH)_2$D is more akin to that described for cells such as macrophages, where the capacity for extrarenal 1α-hydroxylase activity depends primarily on the availability of substrate for the enzyme, namely, 25-OHD. In this setting, the variations in vitamin D status described in the current article by Mahon and colleagues may lead to concomitant changes in placental synthesis of 1,25-$(OH)_2$D, but it is unclear whether this will have any significant effect on fetal development and/or function. Clearly, this is likely to be a focal point for future research, but another possibility is that effects of maternal 25-OHD are mediated via extrarenal synthesis of 1,25-$(OH)_2$D within the fetal skeleton itself. It has been recognized for many years that 25-OHD can cross the placenta[16] and that chondrocytes are an extrarenal source of 1α-hydroxylase activity.[17] The significance of this with respect to skeletal development in the fetus has been underlined by recent characterization of mouse models in which the gene for 1α-hydroxylase (*Cyp27b1*) was either knocked out or overexpressed in chondrocytes.[18] In this study, loss of chondrocyte 1α-hydroxylase activity was sufficient to increase the width of the hypertrophic zone of the mouse growth plate at day 15.5 of a conventional 21-day gestation. By contrast, chondrocyte-specific *Cyp27b1* transgenic mice had reduced width of the hypertrophic zone in embryonic growth plates. The authors hypothesize that local conversion of 25-OHD to 1,25-$(OH)_2$D acts to regulate osteoclast invasion via changes in vascular endothelial growth factor signaling. Loss or gain of function within this mechanism thus would lead to dysregulation of the cartilaginous matrix at the chondroosseous junction and concomitant alterations in bone size. While it is difficult to draw immediate parallels between this study and the work of Mahon and colleagues, it is nevertheless tempting to speculate that extrarenal metabolism of 25-OHD plays a key role in mediating the effects of vitamin D status in utero.

An intriguing question raised by Mahon and colleagues in their article concerns the possible impact of vitamin D–associated changes in skeletal morphology in utero on bone and joint disease in adult life. Previous studies have supported a link between dietary and environmental factors during pregnancy, childhood growth, and risk of osteoporotic fracture in adult life.[19] Thus in future studies it will be interesting to determine the extent to which the alterations in 3DUS parameters measured in the current article continue into adult life. In this respect, it is noteworthy that analysis of the chondrocyte-specific *Cyp27b1*-knockout and *Cyp27b1*-overexpressing mice did not reveal any persistence of fetal bone phenotype beyond the immediate neonatal period,[18] suggesting that other factors, such as endocrine maintenance of calcium and phosphate balance (the two mineral components of the hydroxyapatite bone matrix), are more important in defining postnatal bone development.

By demonstrating a clear phenotypic consequence of impaired maternal vitamin D status, Mahon and colleagues have added to the growing body of evidence supporting improved strategies for vitamin D supplementation during pregnancy. In common with other association studies that have linked vitamin D status with physiologic or disease parameters, prospective clinical trials are required to define a more causal role for vitamin D. For pregnant women in particular, this is complicated by the need for studies to fully define the dosage and timing of supplementation regimes that will safely ensure optimal serum levels of 25-OHD. In the current study, metaphyseal cross-sectional area and splaying data showed the greatest difference when comparing vitamin D–deficient ($<$25 nM 25-OHD) versus vitamin D–sufficient ($>$75 nM 25-OHD) mothers. It will be interesting in the future to explore more closely the potential differences between vitamin D–sufficient mothers and more common status groups such as vitamin D insufficiency. In this respect, the authors' use of a ''borderline'' sufficiency/insufficiency grouping remains contentious given the broad acceptance of *vitamin D insufficiency* as a general term for suboptimal vitamin D status.[3,10] Irrespective of the categorization of vitamin D status, perhaps the most noteworthy observation is that the authors were able to link vitamin D status with relatively early changes in skeletal phenotype. Although this was based on a single measurement of serum 25-OHD levels in the pregnant women, the overarching conclusion is that any strategies to tackle maternal vitamin D insufficiency need to be initiated at an early stage of pregnancy. Given the evidence linking vitamin insufficiency with adverse events in pregnancy, such as preeclampsia,[20] it is possible that such strategies will have benefits above and beyond the developmental changes documented in the current issue of the Journal.

Disclosures

The authors have no conflicts of interest to declare.

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