Vitamin D Deficiency in Development: How Much Is Enough, and How Much Is Too Much?

The consequences of maternal vitamin D deficiency for the health of the newborn has received increasing attention over the past decade, stimulating a significant increase in the number of studies examining the effects of maternal vitamin D deficiency on various pregnancy, neonatal, and childhood outcomes. Pregnant and lactating women and preterm neonates are some of the highest risk groups for developing vitamin D deficiency. The most concerning pregnancy-related outcomes of vitamin D deficiency for pregnant mothers include increased risk of gestational diabetes, preeclampsia, and small-for-gestational-age infants (1). These maternal complications alone have been demonstrated to cause significant morbidities in the newborn period and into childhood (2). However, one of the most studied and well-documented consequences of maternal vitamin D deficiency is an increased incidence of childhood asthma and recurrent wheezing, suggesting that vitamin D has an important role in the developing lung. Consistent with this idea, strong epidemiologic data demonstrate an increased incidence of childhood wheezing and allergies in children born to vitamin D–deficient mothers (3). Higher maternal vitamin D intake during pregnancy was inversely associated with wheeze prevalence in children 3 and 5 years old, suggesting that maternal vitamin D status strongly modulates the risk of early wheeze phenotypes (4, 5). A recent analysis of two large randomized controlled trials of vitamin D supplementation of pregnant women in order to prevent childhood wheeze and asthma reported a beneficial effect of vitamin D supplementation in only the pregnant women with higher vitamin D levels at study entry as compared with pregnant women with lower vitamin D levels at study entry. These findings suggest that the treatment of perinatal vitamin D deficiency and its consequences is not simply related to vitamin D replacement (6). This is further highlighted by a recent meta-analysis of randomized controlled trials examining vitamin D and pregnancy outcomes, which failed to demonstrate measurable effects of vitamin D supplementation in pregnancy on childhood asthma (7). These disappointing clinical findings are in stark contrast with past preclinical studies that consistently showed profound adverse effects of maternal vitamin D deficiency as well as benefits from vitamin D supplementation in the prevention of neonatal and childhood respiratory morbidities.

The many epidemiologic studies linking perinatal vitamin D deficiency to various pregnancy, newborn, and childhood-related morbidities have led researchers to focus on the direct effects of vitamin D on the developing and newborn lung using various preclinical models of vitamin D deficiency and supplementation. Vitamin D has multiple effects in the developing fetus, including bone growth, the immune system, and brain development. In addition, more recent preclinical studies demonstrate that vitamin D is an important regulator of lung growth *in utero* (8). Past studies have largely focused on the association of maternal vitamin D with abnormal airway development and bronchial hyperreactivity (9). Preclinical studies have shown that $1,25(OH)_2$ D, the hormonally active form of vitamin D, plays a role in normal lung airway development, and this is due to enhanced epithelial cell maturation (10, 11), inhibition of airway smooth muscle proliferation (12), modulation of lipofibroblast proliferation and differentiation (13), and the regulation of genes involved in bronchial smooth muscle cells, including genes previously implicated in asthma predisposition and pathogenesis (14).

More recently, vitamin D has been shown to play a role in distal lung and vascular development (15). In addition to its role in normal lung development, vitamin D has been shown to have protective effects on lung development in models of perinatal lung injury leading to bronchopulmonary dysplasia, the chronic lung disease of prematurity (16). Although there have been many advancements in the mechanistic understanding of vitamin D's role in lung development from preclinical studies this past decade, there remains a great deal to be discovered about how perinatal vitamin D deficiency changes the lung microenvironment and alters local resident cellular interactions.

In this issue of the *Journal*, Sakurai and colleagues (pp. 521–531) demonstrate that maternal vitamin D deficiency has a significant and persistent effect on resident lung cells of the newborn rodent (17). They found that vitamin D deficiency decreased basal proliferation rates of resident lung mesenchymal stem cell (LMSC) numbers compared with vitamin D–supplemented animals. In addition to the decreased basal proliferation of LMSCs seen in vitamin D deficiency, Sakurai and colleagues also report that LMSCs isolated during perinatal vitamin D deficiency exhibited increased Wnt signaling and myogenic potential. This research further highlights the growing story of maternal vitamin D deficiency and impaired distal lung airspace development by demonstrating impaired LMSC–AT-2 cell interactions in vitamin D–deficient offspring. Interestingly, the authors report deleterious effects of both vitamin D deficiency and high-level supplementation on LMSC function and differentiation patterns. This highlights the importance of precise dosing of vitamin D, and although vitamin D is generally believed to have a wide safety profile, its specific cellular effects likely require more precise, organ-specific concentrations of vitamin D.

As stem cells have shown significant promise in terms of lung repair, this work is especially relevant because it extends our knowledge of the influence of perinatal vitamin D deficiency on the differentiation potential of stem cells. Mesenchymal stem cells are particularly appealing for therapeutic use because they are easily isolated, have immunomodulatory properties, do not give rise to teratomas, and may contribute to the regeneration of many different types of lung tissues. Sakurai and colleagues' findings of increased myogenic phenotype of LMSCs isolated from perinatal vitamin D–deficient animals is important because it shows that the microenvironment of the LMSC

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has a significant influence on resident LMSC differentiation. Overall, these exciting findings give the scientific community continued reason to explore the mechanisms of the effect of perinatal vitamin D deficiency on the developing lung despite the underwhelming results from clinical vitamin D treatment studies. Establishing mechanistic links between antenatal vitamin D deficiency and disease pathogenesis—and determining how these factors intercede in the respiratory outcomes after birth—are essential for the prevention of asthma and other late respiratory morbidities.

The treatment of vitamin D deficiency with vitamin D supplementation can correct conditions such as rickets; however, the developmental consequences of *in utero* vitamin D deficiency for organs and cells may not be amenable to such typical postnatal replacement strategies. Data from developmental lung gene expression studies that examined vitamin D–related pathways indicate that vitamin D has effects on lung development in the first trimester (18); this may partially explain why postnatal supplementation is less effective for lung health. Thus, as observed in studies of folate supplementation for the prevention of neural tube defects, maternal vitamin D supplementation for optimal fetal lung development likely needs to be started before conception to be effective. Although many of the clinical trials of vitamin D supplementation in vitamin D–deficient pregnant individuals have not revealed striking effects on changes in disease phenotypes, preclinical studies have consistently demonstrated a deleterious effect of vitamin D deficiency on organ development. Pregnancy is known to place a significant stress on vitamin D metabolism; however, we lack information about the specific dosing and critical timing of vitamin D strategies needed to prevent lung abnormalities. Because of ethical concerns about modulating the vitamin D status of pregnant mothers, this knowledge can only be determined by additional, high-quality preclinical studies that can then inform clinical trial development, allow for selective strategies for clinical care, and lead to the development of novel therapeutic interventions with vitamin D. ■

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References

1. Nassar N, Halligan GH, Roberts CL, Morris JM, Ashton AW. Systematic review of first-trimester vitamin D normative levels and outcomes of pregnancy. *Am J Obstet Gynecol* 2011; 205:208.e1–208.e7.
2. Poindexter BB, Feng R, Schmidt B, Aschner JL, Ballard RA, Hamvas A, *et al.*; Prematurity and Respiratory Outcomes Program. Comparisons and limitations of current definitions of bronchopulmonary dysplasia for the Prematurity and Respiratory Outcomes Program. *Ann Am Thorac Soc* 2015;12:1822–1830.
3. Litonjua AA. Vitamin D deficiency as a risk factor for childhood allergic disease and asthma. *Curr Opin Allergy Clin Immunol* 2012;12:179–185.
4. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S, *et al.* Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007;85:853–859.
5. Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, *et al.* Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007;85:788–795.
6. Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, Stokholm J, *et al.* Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: a combined analysis of two randomized controlled trials. *PLoS One* 2017;12:e0186657.
7. Bialy L, Fenton T, Shulhan-Kilroy J, Johnson DW, McNeil DA, Hartling L. Vitamin D supplementation to improve pregnancy and perinatal outcomes: an overview of 42 systematic reviews. *BMJ Open* 2020;10: e032626.
8. Nguyen M, Trubert CL, Rizk-Rabin M, Rehan VK, Besançon F, Cayre YE, *et al.* 1,25-Dihydroxyvitamin D3 and fetal lung maturation: immunogold detection of VDR expression in pneumocytes type II cells and effect on fructose 1,6 bisphosphatase. *J Steroid Biochem Mol Biol* 2004;89-90: 93–97.
9. Saadoon A, Ambalavanan N, Zinn K, Ashraf AP, MacEwen M, Nicola T, *et al.* Effect of prenatal versus postnatal vitamin d deficiency on pulmonary structure and function in mice. *Am J Respir Cell Mol Biol* 2017; 56:383–392.
10. Nadeau K, Montermini L, Mandeville I, Xu M, Weiss ST, Sweezey NB, *et al.* Modulation of Lgl1 by steroid, retinoic acid, and vitamin D models complex transcriptional regulation during alveolarization. *Pediatr Res* 2010;67:375–381.
11. Nguyen TM, Guillozo H, Marin L, Tordet C, Koite S, Garabedian M. Evidence for a vitamin D paracrine system regulating maturation of developing rat lung epithelium. *Am J Physiol* 1996;271:L392–L399.
12. Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, *et al.* Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol* 2009;158:1429–1441.
13. Sakurai R, Shin E, Fonseca S, Sakurai T, Litonjua AA, Weiss ST, *et al.* 1alpha,25(OH)2D3 and its 3-epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L496–L505.
14. Bossé Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. *Physiol Genomics* 2007;29: 161–168.
15. Mandell EW, Ryan S, Seedorf GJ, Gonzalez T, Smith BJ, Fleet JC, *et al.* Maternal vitamin D deficiency causes sustained impairment of lung structure and function and increases susceptibility to hyperoxia-induced lung injury in infant rats. *Am J Respir Cell Mol Biol* 2020;63:79–91.
16. Mandell E, Seedorf G, Gien J, Abman SH. Vitamin D treatment improves survival and infant lung structure after intra-amniotic endotoxin exposure in rats: potential role for the prevention of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L420–L428.
17. Sakurai R, Singh H, Wang Y, Harb A, Gornes C, Liu J, *et al.* Effect of perinatal vitamin D deficiency on lung mesenchymal stem cell differentiation and injury repair potential. *Am J Respir Cell Mol Biol* 2021;65:521–531.
18. Kho AT, Sharma S, Qiu W, Gaedigk R, Klanderman B, Niu S, *et al.* Vitamin D related genes in lung development and asthma pathogenesis. *BMC Med Genomics* 2013;6:47.