The unsettled science of nonrenal calcitriol production and its clinical relevance

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The primary function of vitamin D in higher vertebrates is to regulate mineral homeostasis through direct actions on intestinal calcium (Ca) and phosphate (P) absorption, bone mineral resorption, and renal mineral reabsorption. Vitamin D itself is inactive and must undergo sequential modification via two specific chemical reactions, first in the liver to 25(OH)D3 by the enzyme CYP2R1, and then in the kidney to 1,25-dihydroxyvitamin D3 [1,25(OH)2D3 or calcitriol] by the tightly regulated enzyme CYP27B1 (1). Calcitriol, whose level is also modulated via renal CYP24A1–mediated degradation, is then secreted into the blood as an active endocrine hormone and delivered to distant target tissues.

Production of calcitriol in nonrenal target tissues

Research over the past several decades has suggested that the conversion of 25(OH)D3 to calcitriol also occurs in a myriad of nonrenal tissues and cells (NRTCs) that include the skin, parathyroid glands, bone cells, both cardiovascular and immune cells, and many others (2). The basis for this idea stems from initial immunocytotoxic observations that indicate that CYP27B1 is also expressed in NRTCs, albeit at very low levels relative to levels in the primary renal source. Not surprisingly, these observations stimulated new research aimed at determining details of this previously undescribed source of calcitriol (3). This work has led to an important hypothesis proposing that renal endocrine calcitriol is predominantly linked to […]

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The importance of adequate blood levels of vitamin D

Ironically, a current conflict exists within the field as to what might constitute adequate levels of circulating 25(OH)D₃, the substrate for calcitriol production, in healthy humans, and of supplemental vitamin D required to maintain those levels. Indeed, while the Institute of Medicine has concluded that 25(OH)D₃ levels at or above 20 ng/mL are adequate to protect the skeleton, the Endocrine Society has concluded that 30 ng/mL or more is appropriate (9). Although this issue remains unresolved and is not the topic of this Viewpoint, the absence of an appreciation for how these levels might impact the differential synthesis of calcitriol in the kidneys and in NRTCs has led to considerable uncertainty as to how clinical trials and the parameters of those trials should be established. This is particularly true relative to the effects of vitamin D in cancer prevention, cardiovascular protection, immune disease, and diabetes, as well as in other maladies for which a protective, noncalcemic role for vitamin D has been indicated. Despite the potential relevance of this information, numerous randomized clinical trials (RCTs) have been conducted over the past several decades; unfortunately, results from the majority of these have not been particularly illuminating. Commentary on the details of these trials, most of which showed no effect, has been extensive and will not be considered here (10); three recent trials, however, merit reference. The recently concluded VITAL trial (VITamin D and OmegA-3 TriaL), aimed at assessing the ability of supplemental vitamin D to prevent cancer and cardiovascular disease, resulted in the general absence of an impact of the vitamin on either of these diseases, although a modest potential effect on cancer was noted upon careful secondary evaluation (10, 11). A second trial conducted to determine the impact of high-dose supplemental vitamin D on prediabetic progression did not reach statistical significance (12). Finally, a clinical trial aimed at assessing the impact of supplemental vitamin D on patients with tuberculosis also found no

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The mechanistic in vivo work alluded to NRTC production of calcitriol (8). Unfortunately, virtually all the issues noted above that have resulted in uninformative clinical trial outcomes with other diseases (3) have been previously defined for the kidney are generally either few or absent in NRTCs; viable alternatives for the latter will have to be explored (3). This issue is particularly critical, since blood levels of unbound 25(OH)D3 are postulated to be essential for cellular uptake, as advanced by advocates of the free-hormone hypothesis (15, 16). Resolution of these issues will be essential for understanding the role of calcitriol production in NRTCs and perhaps for the design of future clinical studies aimed at assessing the levels of vitamin D supplementation necessary to maintain health and combat specific disease states.

Models for characterizing features of NRTC calcitriol production
A unique animal model is required to assess the features of NRTC production of calcitriol described above. Several characteristics of such a model include (a) a highly attenuated circulating endocrine calcitriol level facilitated through selective downregulation of Cyp27b1 expression in the kidneys, (b) full retention of Cyp27b1 expression and regulation in NRTCs, and (c) an ability to precisely regulate circulating levels of either vitamin D3 or 25(OH)D3 experimentally under conditions of tightly controlled mineral metabolism. Although a kidney-selective Cyp27b1-null mouse is not yet available and global Cyp27b1-null mice do not retain NRTC production of calcitriol (17), a Cyp27b1 pseudo-null mouse with the above characteristics has recently been generated (7). This mouse strain was derived directly from our identification of a kidney-specific genomic module that controls Cyp27b1 expression in mice. Accordingly, deletion of this module led to a dramatic loss of basal and regulated expression of renal Cyp27b1 in these mice, yet was without effect on Cyp27b1 expression in NRTCs. This Cyp27b1 pseudo-null mouse exhibits hypocalcemia, hyperparathyroidism, low FGF23 expression, and very low levels of
circulating calcitriol as well as an aberrant Cyp27b1-null–like skeleton. Importantly, while key elements of this phenotype are normalized when systemic mineral metabolism and low levels of renal Cyp24a1 are fully restored through dietary means, detectable blood levels of calcitriol are completely eliminated (7). This model will be extremely useful for exploring the missing features of NRTC production of calcitriol, as enumerated above, and assessing the role of calcitriol in both health and disease. It is our view that the details obtainable through this avenue of investigation in the mouse will likely advance our current understanding of the synthesis of calcitriol in NRTCs and provide potential clinically relevant insights as well.

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