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Review

Inferences from genetically modified mouse models on the skeletal actions of vitamin D

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ABSTRACT

Vitamin D has pleiotropic extra-skeletal effects which have been noted in mouse models of deletion of either the 25-hydroxy vitamin D 1α-hydroxylase enzyme, cyp27b1 (1OHase$^{-/-}$ mice) or of the vitamin D receptor (*Vdr*$^{-/-}$ mice); these may be preventable or reversible by either restoring normal signaling of the 1,25(OH)$_2$D/VDR system, or in some cases by restoring normal mineral homeostasis. However, effects on skeletal and mineral homeostasis are clearly the major phenotype observed in humans with loss-of-function mutations in either CYP27B1 or VDR. In mouse phenocopies of these human disorders, correction of hypocalcemia and hypophosphatemia reduce elevated circulating parathyroid hormone concentrations and normalize impaired bone mineralization, but restoration of normal 1,25(OH)$_2$D/VDR signaling may be required for optimal bone formation. Induction of high endogenous 1,25(OH)$_2$D concentrations in genetically modified mouse models may cause increased bone resorption and decreased mineralization. Transgenic *Vdr* overexpression and conditional *Vdr* deletion in cells of the osteoblastic lineage have also provided insights into the stages of osteoblast differentiation which may mediate these actions. These anabolic and catabolic effects of the 1,25(OH)$_2$D system on bone may therefore be a function of both the ambient concentration of circulating 1,25(OH)$_2$D and the stage of differentiation of the osteoblast.

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Contents

1. Introduction

With the advent of technology to either overexpress or specifically delete genes in whole animals, either globally (i.e., in all cells and tissues) or conditionally (i.e., in specific cells), the application of this technology to engineering of mouse models has provided a wealth of new understanding of whole animal

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physiology. The area of vitamin D biology has benefited from new insights gained from manipulation of the vitamin D receptor (VDR) and of the enzymes which metabolize vitamin D and derivatives. These studies, have generally been performed in mice with deletion either of the gene encoding *cyp27b1*, the 25-hydroxyvitamin D 1α-hydroxylase [1OHase], which converts the metabolite 25 hydroxyvitamin D [25OHD] to the active form 1,25 dihydroxyvitamin D [1,25$(OH)_2$D], or with deletion of the gene encoding the vitamin D receptor (*Vdr*). They have demonstrated an array of extra-skeletal effects of vitamin D including, among others, effects on the cardiovascular system [1,2], on brain function [3], on immune function [4–6] and on cell proliferation [7,8,9] and neoplasia [10] In some cases vitamin D deficiency, rather than being the sole cause of the phenotype, may increase susceptibility to the given phenotype as part of a "multiple-hit" pathogenetic pathway [11]. In general these non-skeletal effects may be prevented or eliminated by restoring the levels of ligand/receptor to those required to normalize mineral homeostasis or in some cases vitamin D may function by simply restoring normal mineral homeostasis [12]. These studies therefore provide biological credibility to the observational studies in humans which have reported associations of low levels of vitamin D exposure with a variety of health outcomes [13]. Nevertheless, clinical trials in humans have generally been less convincing in supporting a role of vitamin D in the pathophysiology of these extra-skeletal phenotypes, but a major limitation of these studies has been their use of largely vitamin D sufficient cohorts for examination of treatment effects with vitamin D.

Irrespective of the role of vitamin D in these extra-skeletal effects, human experiments of nature have demonstrated the critical role of the 1,2$(OH)_2$D/VDR system in modulating mineral and skeletal homeostasis. Thus loss-of-function mutations in the *CYP27 B1* gene which result in decreased or absent capacity of affected individuals to synthesise 1,25$(OH)_2$D cause the clinical syndrome of pseudodeficiency rickets (PDDR) or vitamin D resistant rickets, type I [14], whereas loss-of-function mutations in the *VDR* gene result in decreased or absent capacity of the ligand 1,2$(OH)_2$D to act, and cause the syndrome of Hereditary Vitamin D resistant rickets (HVDRR) or vitamin D resistant rickets, type II [15]. In both cases the predominant manifestation is dysregulation of mineral and skeletal homeostasis. The following discussion will therefore be focused on effects of vitamin D in bone.

2. Effect of reduction in the levels of active vitamin D or the vitamin D receptor on bone

In previous studies, we examined the phenotype of homozygous mice with global deletion of cyp27b1 [1OHase-/- mice] which are phenocopies of PDDR [16] and compared them to mice with homozygous global deletion of the *Vdr* [$Vdr^{-/-}$ mice] which are phencopies of HVDRR [17]. We simultaneously compared them to homozygous compound mutants [1OHase$^{-/-}$$Vdr^{-/-}$] and to wild-type littermates. In view of the fact that the mutant mice we studied displayed reduced fertility, mice of each genotype were maintained on a high calcium diet rather than normal chow after weaning, which may again distinguish our study from those of others [18]. Some of the animals that we studied on the high calcium intake were also treated with exogenous 1,25$(OH)_2D_3$. Alternatively, mice of each genotype received a rescue diet from the time of weaning. The rescue diet contained high calcium (2%), high phosphorus (1.25%) and 20% lactose to facilitate vitamin D independent calcium absorption. At 16 weeks of age, on the high calcium diet, hypocalcemia, hypophosphatemia and elevated serum parathyroid hormone (PTH) concentrations were observed which were eliminated by treating the 1OHase$^{-/-}$ mice with 1,25 $(OH)_2$D, but not by treating the two VDR deficient models [$Vdr^{-/-}$ and 1OHase$^{-/-}$$Vdr^{-/-}$ mice] with the active vitamin D ligand (Table 1). In all three genetic models the rescue diet alone normalized serum calcium, phosphorus and PTH, irrespective of whether the mice lacked the active ligand or the receptor or both. Similar results with respect to mineral and PTH status have been found in other studies of 1OHase$^{-/-}$ [19] and $Vdr^{-/-}$ [20] mice treated with a rescue diet.

2.1. Indirect anabolic effects of the 1,25$(OH)_2$D/VDR system on bone

In bone, we observed a large excess of unmineralized bone matrix or osteoid in all genetically modified mice on the high calcium intake. The increased osteoid volume was reduced to wild-type levels in 1(OH) ase$^{-/-}$ mice that received exogenous 1,25 $(OH)_2D_3$ (but not in the models that lacked a VDR). Osteoid volumes were also reduced to wild-type levels in all genetically modified models by the use of the rescue diet alone irrespective of whether the mice were deficient in the active vitamin D ligand or the VDR or both (Table 1). Unmineralized bone can occur due to absence of sufficient calcium and phosphorus to serve as a substrate for amorphous calcium-phosphate and hydroxyapatite formation in bone matrix however elevated concentrations of PTH have also been reported to inhibit mineralization by increasing the expression of mineralization inhibitors such as matrix gla protein [21]. Consequently normalization of serum calcium and phosphorus levels may have been sufficient to provide adequate substrate for bone matrix mineralization and may have indirectly contributed to improved mineralization by reducing elevated PTH levels. Therefore, the effects of the 1,25$(OH)_2$D/VDR system to promote bone mineralization and eliminate osteomalacia appear to be indirect by facilitating normal calcium and phosphorus absorption.

The 1,25$(OH)_2$D/VDR system facilitates increased active, saturable calcium absorption under low or normal calcium conditions, by inducing the apical membrane epithelial calcium entry channel TRPV6 which mediates the rate-limiting step in transcellular calcium transport, as well as by inducing the calcium-binding protein calbindin-D_{9k} ($CaBP_{9k}$), which is thought to "buffer" and/or mediate the transit of intracellular absorbed calcium [22,23]. The extrusion of calcium at the basolateral membrane appears less vitamin D-dependent, is likely constitutive in part, and is carried

Table 1
Effect of diet on serum minerals, serum pth, bone mineralization and bone matrix in different genetic models.

Genetic model	High Ca diet					High Ca diet + 1,25$(OH)_2D_3$					Rescue diet				
	Ser Ca	Ser P	Ser PTH	Bone miner	Bone matrix	Ser Ca	Ser P	Ser PTH	Bone miner	Bone matrix	Ser Ca	Ser P	Ser PTH	Bone miner	Bone matrix
WT	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
1(OH) ase$^{-/-}$	↓	↓	↑	↓	↑	→	→	→	→	→	→	→	→	→	↓
$Vdr^{-/-}$	↓	↓	↑	↓	↑	↓	↓	↑	↓	↑	→	→	→	→	↓
1(OH) ase$^{-/-}$$Vdr^{-/-}$	↓	↓	↑	↓	↑	↓	↓	↑	↓	↑	→	→	→	→	↓

WT: wild-type; Ser Ca: serum calcium; Ser P: serum phosphorus; Ser PTH: serum PTH; Bone miner: bone mineralization; →: normal levels; ↓: reduced levels; ↑: elevated levels. Results are from genetic mouse models studied at 16 weeks.