Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 485–489
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Effects of calcium and of the Vitamin D system on skeletal and calcium homeostasis: lessons from genetic models☆

David Goltzman*, Dengshun Miao, Dibyendu K. Panda, Geoffrey N. Hendy

Calcium Research Laboratory, McGill University Health Centre, 687 Pine Avenue West, Room H4.67, Montreal, Que., Canada H3A 1A1

Abstract

Targeted deletion of genes encoding the 1,25-dihydroxyVitamin D [1,25$(OH)_2$D]-synthesizing enzyme, 25 hydroxyVitamin D-1α-hydroxylase [1α(OH)ase or CYP27B1], and of the nuclear receptor for 1,25$(OH)_2$D, the Vitamin D receptor (VDR), have provided useful mouse models of the inherited human diseases, Vitamin D-dependent rickets types I and II. We employed these models and double null mutants to examine the effects of calcium and of the 1,25$(OH)_2$D/VDR system on skeletal and calcium homeostasis. Optimal dietary calcium absorption required both 1,25$(OH)_2$D and the VDR. Skeletal mineralization was dependent on adequate ambient calcium but did not directly require the 1,25$(OH)_2$D/VDR system. Parathyroid hormone (PTH) secretion was also modulated primarily by ambient serum calcium but the enlarged parathyroid glands which the mutants exhibited and the widened cartilaginous growth plates could only be normalized by the combination of calcium and 1,25$(OH)_2$D, apparently independently of the VDR. Optimal osteoclastic bone resorption and osteoblastic bone formation both required an intact 1,25$(OH)_2$D/VDR apparatus. The results indicate that calcium cannot entirely substitute for Vitamin D in skeletal and mineral homeostasis but that the two agents have discrete and overlapping functions.

Keywords: Vitamin D; Skeleton; Calcium homeostasis

1. Introduction

The Vitamin D metabolite 1α,25-dihydroxyVitamin D [1,25$(OH)_2$D] remains the most potent active form of Vitamin D known to date. It is synthesized by the mitochondrial enzyme 25-hydroxyVitamin D-1α-hydroxylase [1α(OH)ase or CYP27B1] which is expressed in several fetal and adult tissues [1–3]. The circulating concentrations of 1,25$(OH)_2$D are determined, however, by the activity of the renal enzyme. Parathyroid hormone (PTH) and hypocalcemia are potent activators of the renal 1α(OH)ase enzyme, whereas 1,25$(OH)_2$D itself, as well as hypercalcemia, have been shown to be potent inhibitors. The 25-hydroxyVitamin D-24-hydroxylase enzyme [24(OH)ase or CYP24] can 24 hydroxylate either the substrate for 1,25$(OH)_2$D production, that is 25-hydroxyVitamin D, or 1,25$(OH)_2$D itself, thereby limiting the circulating concentration of the active metabolite [1–3]. This enzyme is upregulated by 1,25$(OH)_2$D and downregulated by PTH. The genomic actions of 1,25$(OH)_2$D occur by binding of the ligand to the Vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which then heterodimerizes with the retinoid X receptor (RXR), and this dimer binds to response elements (VDREs) on target genes. Co–regulators are then mobilized to alter gene transcription [4]. A number of non-genomic rapid effects of 1,25$(OH)_2$D have been described. These may involve interaction with a putative membrane receptor, stimulation of protein kinase C, and increase in intracellular calcium, and activation of mitogen activated protein-kinase (MAP-kinase) [5]. The Vitamin D system has been implicated in the modulation of growth and differentiation of a variety of cells and tissues, however, its major association has been with skeletal function and mineral metabolism [3].

Recently several groups have developed mouse models of targeted deletion of the 1α(OH)ase [1α(OH)ase$^{-/-}$] [6,7] and of the VDR (VDR$^{-/-}$) [8–10] and in doing so have provided mouse models of the inherited human disorders, Vitamin D dependent rickets (VDDR) type I [11] and type II [12], respectively. Studies in these models have focused primarily on examining the consequences to skeletal and mineral metabolism. We previously noted the reproductive and immune defects of the animals with 1α(OH)ase deficiency [6] and others have also reported reproductive abnormalities in mice with genetic ablation of the VDR [8,9]. The interpretation of the mechanisms underlying the phenotypes in these mouse mutants has been greatly influenced by reports of the normalization of the skeletal abnormalities if serum

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +1-514-843-1632; fax: 1-514-843-1712.
E-mail address: david.goltzman@mcgill.ca (D. Goltzman).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.058

calcium is normalized by a rescue diet containing high concentrations of calcium, phosphorus, and lactose [13,14]. These murine studies apparently confirm the reports of the healing of human rickets using intravenous calcium to raise serum calcium, an approach which was used in patients with VDDR II [15]. Nevertheless, $VDR^{-/-}$ mice have elevated $1,25(OH)_2D$ levels [16] which could confound the interpretation of the role of calcium in modulating skeletal homeostasis. We therefore examined $1\alpha(OH)ase^{-/-}VDR^{-/-}$ mice obtained by cross-breeding $1\alpha(OH)ase^{+/-}$ and $VDR^{+/-}$ mice to determine the consequences of 1α(OH)ase deficiency on the $VDR^{-/-}$ phenotype [27]. We also compared the phenotypes of the $1\alpha(OH)ase^{-/-}$, the $VDR^{-/-}$ and the $1\alpha(OH)ase^{-/-}VDR^{-/-}$ when exposed to different calcium diets and after receiving exogenous $1,25(OH)_2D_3$ treatment [27]. All mice were maintained on a high calcium intake (without lactose) until weaning in order to facilitate reproduction. After weaning, mice received either a high calcium intake on which they remained hypocalcemic, a high calcium intake with injections of $1,25(OH)_2D_3$ intraperitoneally three times per week, or a "rescue" diet containing high calcium and lactose. Animals were examined at 4 months of age. We wished to determine if $1,25(OH)_2D$ and the VDR are both necessary and sufficient for VDR action and if extracellular calcium could substitute for the $1,25(OH)_2D$/VDR system in modulating skeletal and mineral metabolism.

2. Calcium absorption

The development of hypocalcemia after weaning, in mice with targeted deletion of the 1α(OH)ase and of the VDR, is well documented to occur on a normal calcium diet. It also was seen, although to a lesser extent, on the high calcium diet we employed, in both $1\alpha(OH)ase^{-/-}$ and $VDR^{-/-}$ mice. This points to the important role of the Vitamin D system in enhancing intestinal calcium absorption. Indeed recent studies have demonstrated the Vitamin D-dependency of novel duodenal calcium channel proteins such as calcium transport protein 1 [17]. Nevertheless several studies [13,14,18], including our own [27] have documented the capacity of a rescue diet containing lactose to eliminate this hypocalcemia. This points to the important role of dietary lactose in increasing transport of calcium at least in the rodent intestine, independent of the $1,25(OH)_2D$/VDR system and may account for the apparent absence of hypocalcemia in these mouse models which ingest lactose-containing maternal milk prior to weaning. The precise mechanism of the lactose effect remains to be determined. The presence of hypocalcemia in both the $1\alpha(OH)ase^{-/-}$ mice with intact VDR but deficient $1,25(OH)_2D$ production and in the $VDR^{-/-}$ mice with elevated endogenous levels of $1,25(OH)_2D$ but deficient VDR suggests that both $1,25(OH)_2D$ and the VDR are necessary for optimal intestinal absorption of calcium. In our studies we employed a $VDR^{-/-}$ model (generously provided by Dr. Marie Demay of Mass. General Hospital, Boston, MA) in which a 5 kb fragment of genomic DNA encoding the second zinc finger of the receptor DNA-binding domain was deleted. The presence of hypocalcemia in these $VDR^{-/-}$ animals on the normal calcium or a lactose-free high calcium diet therefore confirms the loss of function of this VDR deletion. This loss of function of the VDR was further confirmed in studies in which $VDR^{-/-}$ mice were fed a diet which causes hypocalcemia and hypocalcemia could not be normalized despite treatment with exogenous $1,25(OH)_2D_3$. In contrast, $1\alpha(OH)ase^{-/-}$ mice on the same diet, treated with the same dose of $1,25(OH)_2D_3$ did normalize serum calcium both in our studies and others [19]. Intestinal calcium absorption, therefore, appears to require both $1,25(OH)_2D$ and the VDR.

3. Effect on the 1α(OH)ase and on the 24(OH)ase enzymes

We assessed, in our models, the genetic regulation of the 1α(OH)ase and of the 24-hydroxyVitamin D-24-hydroxylase [24(OH)ase], two key enzymes involved in $1,25(OH)_2D$ metabolism Gene expression of renal 1α(OH)ase was elevated and 24(OH)ase was suppressed in $VDR^{-/-}$ mice, resulting in high circulating $1,25(OH)_2D$ concentrations. Thus in the absence of VDR, 1α(OH)ase could not be suppressed or 24(OH)ase stimulated by endogenous (or exogenous) $1,25(OH)_2D$. Treatment with exogenous $1,25(OH)_2D_3$ of the $1\alpha(OH)ase^{-/-}$ mouse which retained a normal VDR, enhanced 24(OH)ase expression. This effect of $1,25(OH)_2D_3$ was not seen in the mutants lacking a VDR. These studies, therefore, suggest that both $1,25(OH)_2D$ and the VDR are required for regulation of gene expression of both the 1α(OH)ase and 24(OH)ase in vivo [1–3]. However, the actions of the $1,25(OH)_2D$/VDR system also normalized serum calcium, and elimination of hypocalcemia with a rescue diet normalized 1α(OH)ase levels and 24(OH)ase in mutant mice despite the persistant defects in the $1,25(OH)_2D$/VDR system. This, therefore, demonstrates a calcium effect on gene expression of these enzymes independent of the $1,25(OH)_2D$/VDR system. Whether this effect of calcium is entirely indirect, by suppressing ambient PTH concentrations [1–3], or is partly direct, remains to be determined.

4. Parathyroid gland function

Calcium is known to inhibit parathyroid hormone secretion via the calcium sensing receptor (CaSR) and also to decrease parathyroid cell growth [20]. $1,25(OH)_2D$ has also been shown to inhibit PTH synthesis [21] and secretion [22] and has been reported to inhibit parathyroid cell growth in vitro [23]. On a normal or lactose-free high calcium diet, when hypocalcemia is present, increased circulating PTH concentrations and enlarged parathyroid glands

have been described in the 1α(OH)ase$^{-/-}$ mice [6,27], and the VDR$^{-/-}$ [8,27] mice, and we found similar increases in the 1α(OH)ase$^{-/-}$VDR$^{-/-}$ double mutants [27]. Serum PTH levels were lower in the hypocalcemic VDR$^{-/-}$ mice which had elevated endogenous 1,25(OH)$_2$D levels than in the 1α(OH)ase$^{-/-}$ mice or double mutants and treatment of 1α(OH)ase$^{-/-}$ mice with exogenous 1,25(OH)$_2$D$_3$ reduced PTH into the low normal range even in the presence of normocalcemia. Nevertheless on the rescue diet, serum PTH concentrations fell in all mutants suggesting that raising the ambient calcium could alone normalize PTH secretion although 1,25(OH)$_2$D even in the absence of VDR could have an additive effect.

Parathyroid gland size was smaller in the VDR deleted (VDR$^{-/-}$) mice with elevated endogenous 1,25(OH)$_2$D than in the 1α(OH)ase$^{-/-}$ and the 1α(OH)ase$^{-/-}$VDR$^{-/-}$ mice [27]. The glands remained moderately enlarged in these latter mutants which lacked endogenous 1,25(OH)$_2$D even when hypocalcemia was rectified by the rescue diet. Treatment of 1α(OH)ase$^{-/-}$ mice with exogenous 1,25(OH)$_2$D$_3$ normalized serum calcium and also normalized parathyroid gland size. Exogenous 1,25(OH)$_2$D$_3$ could not, however, normalize parathyroid gland size in mutants deficient in VDR who remained hypocalcemic. Consequently both calcium and 1,25(OH)$_2$D appear to act co-operatively to diminish PTH production and parathyroid gland size. The results in the VDR$^{-/-}$ mice we employed suggest that 1,25(OH)$_2$D may act independently of the VDR in these particular roles with respect to parathyroid function.

5. Development of the cartilaginous growth plate

On a normal or high calcium lactose-free intake, all three hypocalcemic mutant mouse models, i.e., 1α(OH)ase$^{-/-}$, VDR$^{-/-}$, and 1α(OH)ase$^{-/-}$VDR$^{-/-}$ mice, develop characteristic rachitic changes in long bones, i.e., enlarged and distorted cartilaginous growth plates with widened hypertrophic zones [6–9,27]. These abnormalities appear less severe in VDR$^{-/-}$ mice with elevated endogenous 1,25(OH)$_2$D levels than in the other two mutants again suggesting that 1,25(OH)$_2$D may modulate cartilage function independent of the VDR. Indeed non-genomic effects of 1,25(OH)$_2$D have been reported in cartilage cells [5]. Nevertheless, 1,25(OH)$_2$D per se cannot normalize the growth plate if hypocalcemia is not normalized, i.e., in the VDR$^{-/-}$ and 1α(OH)ase$^{-/-}$VDR$^{-/-}$ where intestinal calcium absorption is defective. Furthermore, elimination of hypocalcemia with the rescue diet does not completely normalize the growth plate in the mouse models that have deficient endogenous 1,25(OH)$_2$D, i.e., in the 1α(OH)ase$^{-/-}$ and 1α(OH)ase$^{-/-}$VDR$^{-/-}$ mice. Consequently both calcium and 1,25(OH)$_2$D together appear necessary for normal development of the cartilaginous growth plate, however, the effect of 1,25(OH)$_2$D appears independent of the VDR. Previous studies in double null mutants of the VDR and of retinoid X receptor gamma (RXR gamma) have suggested the existence of a unique VDR in the cartilaginous growth plate with which 1,25(OH)$_2$D$_3$ may interact [24]. Our findings are consistent with this observation.

6. Skeletal mineralization

Mineralization of both cartilage and bone is severely impaired in all mutants, i.e., 1α(OH)ase$^{-/-}$, VDR$^{-/-}$ and 1α(OH)ase$^{-/-}$VDR$^{-/-}$ models, that are hypocalcemic on either a lactose-free, normal or high calcium intake [27]. The extent of demineralization is similar whether animals have high endogenous 1,25(OH)$_2$D levels or not. Furthermore cartilage and bone mineralization is normalized in all models when hypocalcemia is eliminated by the rescue diet [14,18,27]. Administration of exogenous 1,25(OH)$_2$D$_3$ normalizes mineralization only when serum calcium is normalized, i.e., in the 1α(OH)ase$^{-/-}$ model [19,27]. Consequently the major determinant of skeletal mineralization appears to be the ambient concentration of extracellular calcium and the 1,25(OH)$_2$D/VDR system appears to play no direct role in this process.

7. Bone and cartilage remodeling

Osteoblast numbers, bone formation, and bone volume are markedly increased in all hypocalcemic animals, i.e., 1α(OH)ase$^{-/-}$ mice, VDR$^{-/-}$ mice, and 1α(OH)ase$^{-/-}$VDR$^{-/-}$ double mutants, on either a lactose-free, normal calcium intake [6–8,27] or a lactose-free, high calcium intake. This appears to be due to the “anabolic” effect of PTH which is markedly elevated in association with the severe secondary hyperparathyroidism in these animals. Increased serum alkaline phosphatase reflects the increased osteoblastic stimulation by PTH and is normalized when PTH is normalized by eliminating the secondary hyperparathyroidism. The increased bone volume is largely due to increased osteoid as a result of the ambient hypocalcemia. Interestingly, a sustained elevation of increased PTH is generally associated with increased osteoclastic bone resorption as well as increased bone formation. Nevertheless, osteoclast number and resorbing surface are not elevated in these models compared to wild-type animals suggesting an “inappropriate” response to the increased PTH. Furthermore, in our studies [27] a decrease in the average osteoclast size was seen in the 1,25(OH)$_2$D and/or VDR deficient mutant animals with secondary hyperparathyroidism. This suggests, therefore, that there is uncoupling of bone turnover in the presence of a defective 1,25(OH)$_2$D/VDR system and the relatively low resorption may contribute, with increased osteoblast activity, to the increased bone volume. Indeed, previous studies with osteoclast-generating models in vitro have shown that osteoblastic cells from VDR$^{-/-}$ mice in co-culture with normal spleen cells,

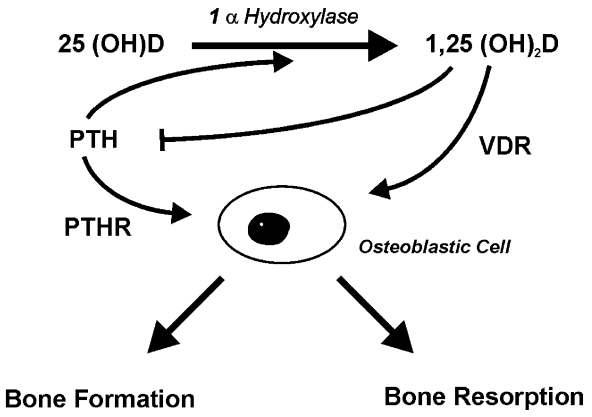

Fig. 1. PTH and 1,25(OH)$_2$D both stimulate bone formation and resorption. PTH enhances production of 1,25(OH)$_2$D and 1,25(OH)$_2$D inhibits parathyroid gland size. PTH, via the PTH receptor (PTHR), and 1,25(OH)$_2$D via the VDR, act on osteoblastic cells to increase osteoblastic bone formation and also to increase bone resorption.

cannot sustain 1,25(OH)$_2$D$_3$-stimulated osteoclast production although PTH could [25]. Therefore, although PTH and local modulators of bone resorption may sustain a "normal" level of osteoclastic resorption in these models an intact 1,25(OH)$_2$D/VDR system is required for an appropriate osteoclastic response to increased PTH.

In view of the fact that osteoclast/chondroclast production at the chondro–osseous junction may also be defective, diminished removal of hypertrophic chondrocytes may occur in this region leading to altered cartilage growth plate remodeling. Therefore the enlargement of the cartilaginous growth plate, and notably the hypertrophic zone, may also be in part due to reduced activity of the 1,25(OH)$_2$D/VDR system on the chondroclast/osteoclast system [14].

In our studies we also found that in all mutant models, [1α(OH)ase$^{-/-}$, VDR$^{-/-}$ and 1α(OH)ase$^{-/-}$VDR$^{-/-}$] when hypocalcemia and secondary hyperparathyroidism were prevented by the rescue diet, osteoblast numbers, mineral apposition rate and bone volume were suppressed below levels seen in wild-type mice [27]. This suggests that the 1,25(OH)$_2$D/VDR system may exert an "anabolic" effect which is necessary to sustain basal bone forming activity and which is unmasked when the defective 1,25(OH)$_2$D/VDR system exists in the presence of normal PTH. This inhibition of bone formation was not previously observed in either VDR$^{-/-}$ or 1α(OH)ase$^{-/-}$ mice on the rescue diet and may reflect the older age of our mice at the time of analysis. Nevertheless, previous studies have pointed to an anabolic effect of 1,25(OH)$_2$D [26]. Our studies suggest therefore that 1,25(OH)$_2$D may exert effects on bone turnover analogous to those of PTH (Fig. 1).

8. Conclusions

In summary, genetic models of the Vitamin D/VDR system have provided powerful probes for dissecting in vivo actions of components of this endocrine system on skeletal and mineral metabolism. When coupled with controlled environmental exposure to specific diets and Vitamin D, distinctions between the actions of the Vitamin D system and the actions of the calcium ion may be revealed. The studies have shown discrete effects of calcium and of the 1,25(OH)$_2$D/VDR system on calcium absorption, on mineralization of bone, on parathyroid growth and hormone production, on cartilage development and remodeling, and on bone turnover (Table 1).

Table 1
Influence of calcium (Ca) 1,25(OH)$_2$D and the VDR on skeletal and mineral homeostatic functions

Functions	Effectors		
	1,25(OH)$_2$D/VDR	Ca	Ca + 1,25(OH)$_2$D
Calcium absorption	↑		
Osteoblast activity	↑		
Osteoclast activity	↑		
Mineralization of bone		↑	
PTH Secretion		↓	
Parathyroid gland size			↓
Cartilaginous growth plate development			↑

↑ means increases and ↓ means decreases.

Acknowledgements

This work was supported by operating grants to D. Goltzman (MOP-5775), D. Miao (IMH-63263) and G.N. Hendy (MOP-57730) from the Canadian Institutes for Health Research and to D. Goltzman (012243) from the National Cancer Institute of Canada and to G.N. Hendy from the Kidney Foundation of Canada.

References

[1] R. Bouillon, W.H. Okamura, A.W. Norman, Structure–function relationships in the Vitamin D endocrine system, Endocr. Rev. 6 (2) (1995) 200–257.
[2] G. Jones, S.A. Strugnell, H.F. DeLuca, Current understanding of the molecular actions of Vitamin D, Physiol. Rev. 78 (4) (1998) 1193–1231.
[3] Al. Sutton, P.N. MacDonald, Vitamin D: more than a "bone-a-fide" hormone, Mol. Endocrinol. 17 (5) (2003) 777–791.
[4] M.R. Haussler, G.K. Whitfield, C.A. Haussler, J.C. Hsieh, P.D. Thompson, S.H. Selznick, C.E. Dominguez, P.W. Jurutka, The nuclear Vitamin D receptor: biological and molecular regulatory properties revealed, J. Bone Miner. Res. 13 (3) (1998) 325–349.
[5] A.W. Norman, W.H. Okamura, J.E. Bishop, H.L. Henry, Update on biological actions of 1 alpha, 25(OH)(2)-Vitamin D(3) (rapid effects) and 24R,25(OH)(2)-Vitamin D(3), Mol. Cell Endocrinol. 197 (1–2) (2002) 1–13.
[6] D.K. Panda, D. Miao, M.L. Tremblay, J. Sirois, R. Farookhi, G.N. Hendy, D. Goltzman, Targeted ablation of the 25-hydroxyVitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction, Proc. Natl. Acad. Sci. U.S.A. 98 (13) (2001) 7498–7503.

[7] O. Dardenne, J. Prud'homme, A. Arabian, F.H. Glorieux, R. St-Arnaud, Targeted inactivation of the 25-hydroxyVitamin D(3)-1(alpha)-hydroxylase gene (CYP27B1) creates an animal model of pseudoVitamin D-deficiency rickets, Endocrinology 142 (7) (2001) 3135–3141.
[8] Y.C. Li, A.E. Pirro, M. Amling, G. Delling, R. Baron, R. Bronson, M.B. Demay, Targeted ablation of the Vitamin D receptor: an animal model of Vitamin D-dependent rickets type II with alopecia, Proc. Natl. Acad. Sci. U.S.A. 94 (18) (1997) 9831–9835.
[9] T. Yoshizawa, Y. Handa, Y. Uematsu, S. Takeda, K. Sekine, Y. Yoshihara, T. Kawakami, K. Arioka, H. Sato, Y. Uchiyama, et al., Mice lacking the Vitamin D receptor exhibit impaired bone formation, Nat. Genet. 16 (4) (1997) 391–396.
[10] R.G. Erben, D.W. Soegiarto, K. Weber, U. Zeitz, M. Lieberherr, R. Gniadecki, G. Moller, J. Adamski, R. Balling, Deletion of deoxyribonucleic acid binding domain of the Vitamin D receptor abrogates genomic and nongenomic functions of Vitamin D, Mol. Endocrinol. 16 (7) (2002) 1524–1537.
[11] D. Fraser, S.W. Kooh, H.P. Kind, M.F. Holick, Y. Tanaka, H.F. DeLuca, Pathogenesis of hereditary vitamin-D-dependent rickets. An inborn error of Vitamin D metabolism involving defective conversion of 25-hydroxyVitamin D to 1 alpha, 25-dihydroxyVitamin D, N. Engl. J. Med. 289 (16) (1973) 817–822.
[12] S. Beer, M. Tieder, D. Kohelet, O.A. Liberman, E. Vure, G. Bar-Joseph, D. Gabizon, Z.U. Borochowitz, M. Varon, D. Modai, Vitamin D resistant rickets with alopecia: a form of end organ resistance to 1,25-dihydroxy Vitamin D, Clin. Endocrinol. 14 (4) (1981) 395–402.
[13] Y.C. Li, M. Amling, A.E. Pirro, M. Priemel, J. Meuse, R. Baron, G. Delling, M.B. Demay, Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in Vitamin D receptor-ablated mice, Endocrinology 139 (10) (1998) 4391–4396.
[14] M. Amling, M. Priemel, T. Holzmann, K. Chapin, J.M. Rueger, R. Baron, M.B. Demay, Rescue of the skeletal phenotype of Vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses, Endocrinology 140 (11) (1999) 4982–4987.
[15] S. Balsan, M. Garabedian, M. Larchet, A.M. Gorski, G. Cournot, C. Tau, A. Bourdeau, C. Silve, C. Ricour, Long-term nocturnal calcium infusions can cure rickets and promote normal mineralization in hereditary resistance to 1,25-dihydroxyVitamin D, J. Clin. Invest. 77 (5) (1986) 1661–1667.
[16] Y. Sakai, J. Kishimoto, M.B. Demay, Metabolic and cellular analysis of alopecia in Vitamin D receptor knockout, J. Clin. Invest. 107 (8) (2001) 961–966.
[17] S.J. Van Cromphaut, M. Dewerchin, J.G. Hoenderop, I. Stockmans, E. Van Herck, S. Kato, R.J. Bindels, D. Collen, P. Carmeliet, R. Bouillon, et al., Duodenal calcium absorption in Vitamin D receptor-knockout mice: functional and molecular aspects, Proc. Natl. Acad. Sci. U.S.A. 98 (23) (2001) 13324–13329.
[18] O. Dardenne, J. Prud'homme, S.A. Hacking, F.H. Glorieux, R. St-Arnaud, Correction of the abnormal mineral ion homeostasis with a high-calcium, high-phosphorus, high-lactose diet rescues the PDDR phenotype of mice deficient for the 25-hydroxy Vitamin D-1alpha-hydroxylase (CYP27B1), Bone 32 (4) (2003) 332–340.
[19] O. Dardenne, J. Prud'homme, S.A. Hacking, F.H. Glorieux, R. St-Arnaud, Rescue of the pseudo-Vitamin D deficiency rickets phenotype of CYP27B1-deficient mice by treatment with 1,25-dihydroxyVitamin D3: biochemical, histomorphometric, and biomechanical analyses, J. Bone Miner. Res. 18 (4) (2003) 637–643.
[20] E.M. Brown, R.J. MacLeod, Extracellular calcium sensing and extracellular calcium signaling, Physiol. Rev. 81 (1) (2001) 239–297.
[21] J. Russell, J. Silver, L.M. Sherwood, The effects of calcium and Vitamin D metabolites on cytoplasmic mRNA coding for pre-proparathyroid hormone in isolated parathyroid cells, Trans. Assoc. Am. Physicians 97 (1984) 296–303.
[22] Y.L. Chan, C. McKay, E. Dye, E. Slatopolsky, The effect of 1,25-dihydroxycholecalciferol on parathyroid hormone secretion by monolayer cultures of bovine parathyroid cells, Calcif. Tissue Int. 38 (1986) 27–32.
[23] R. Kremer, I. Bolivar, D. Goltzman, G.N. Hendy, Influence of calcium and 1,25-dihydroxycholecalciferol on proliferation and proto-oncogene expression in primary cultures of bovine parathyroid cells, Endocrinology 125 (2) (1989) 935–941.
[24] N. Yagishita, Y. Yamamoto, T. Yoshizawa, K. Sekine, Y. Uematsu, H. Murayama, Y. Nagai, W. Krezel, P. Chambon, T. Matsumoto, et al., Aberrant growth plate development in VDR/RXR gamma double null mutant mice, Endocrinology 142 (12) (2001) 5332–5341.
[25] S. Takeda, T. Yoshizawa, Y. Nagai, H. Yamato, S. Tukumoto, K. Sekine, S. Kato, T. Matsumoto, T. Fujita, Stimulation of osteoclast formation by 1,25-dihydroxyVitamin D requires its binding to Vitamin D receptor (VDR) in osteoblastic cells: studies using VDR knockout mice, Endocrinology 140 (2) (1999) 1005–1008.
[26] J.P. Van Leeuwen, M. van Driel, G.J. van den Bemd, H.A. Pols, Vitamin D control of osteoblast function and bone extracellular matrix mineralization, Crit. Rev. Eukaryot Gene Expr. 11 (1–3) (2001) 199–226.
[27] D.K. Panda, D. Miao, I. Bolivar, J. Li, R. Huo, G.N. Hendy, D. Goltzman, Inactivation of the 25-hydroxyVitamin D 1α–hydroxylase and Vitamin D receptor demonstrates independent and interdependent effects of calcium and Vitamin D on skeletal and mineral homeostasis, J. Biol. Chem. 179 (2004) 16754–16766.