Vitamin D in chronic kidney disease: A systemic role for selective vitamin D receptor activation

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Hyperparathyroidism occurs in most patients during the progression of chronic kidney disease (CKD) and one of its initiating events, reduced serum levels of 1,25-dihydroxyvitamin D, results from a decrease in renal 1α hydroxylase activity, which converts 25-hydroxyvitamin D to its activated form. The combination of persistently high parathyroid hormone (PTH) and low 1,25-dihydroxyvitamin D is associated with bone loss, cardiovascular disease, immune suppression and increased mortality in patients with end-stage kidney failure. Recent studies in dialysis patients suggest that paricalcitol, a selective activator of the vitamin D receptor (VDR), is associated with a more favorable efficacy to side effect profile than calcitriol, with less morbidity and better survival. One hypothesis derived from such studies suggests that systemic activation of VDRs may have direct effects on the cardiovascular system to decrease mortality in CKD. Although current guidelines for regulating serum calcium, phosphate and PTH recommend specific interventions at the various stages of CKD to prevent or postpone irreversible parathyroid disease and decrease cardiovascular morbidity and mortality, emerging data suggest that vitamin D therapy may prolong survival in this patient population by mechanisms that are independent of calcium, phosphate and PTH. It is suggested that a re-evaluation of current treatment recommendations is needed and that future research should focus on mechanisms that distinguish potential tissue specific benefits of selective VDR activators in patients with CKD.

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KEYWORDS: vitamin D; vitamin D receptor activation; chronic kidney disease; cardiovascular disease

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VITAMIN D TREATMENT AND SURVIVAL IN DIALYSIS PATIENTS

Two recent epidemiologic studies revealed a potentially important systemic role for vitamin D receptor (VDR) activation in the survival of dialysis patients.[1,2] The first study revealed that the use of the selective VDR activator, paricalcitol, was associated with an adjusted 16% survival benefit when compared to the use of calcitriol.[1] This was observed not only in patients who remained on their respective treatments throughout the evaluation period, but also in that subgroup who switched from calcitriol to paricalcitol (Figure 1). In the second retrospective study, patients who received injectable vitamin D (calcitriol or paricalcitol) had a 20–25% higher survival rate than those who did not receive injectable vitamin D over the same 2-year period (Figure 2).[2] All-cause mortality, as well as cardiovascular mortality, was less in the group receiving injectable vitamin D, after adjusting for potential confounders. Interestingly, the survival benefit of vitamin D was apparent across all quintiles of calcium, phosphate and parathyroid hormone (PTH) (Figure 2), particularly after using marginal structural analysis to account for their time-dependent changes during the evaluation period.[2] This suggests that the use of pulsatile calcitriol or paricalcitol therapy may possibly mitigate the deleterious effects of elevated phosphate, calcium and PTH on mortality.[3] Although only one randomized comparison between injectable calcitriol and paricalcitol has been performed in this patient population, the results from the study show that paricalcitol was more effective than calcitriol in suppressing PTH, while having fewer episodes of sustained elevated calcium and phosphate as identified in the *post hoc* analysis.[3] Thus, although the potentially better side effect profile of paricalcitol may explain, in part, why its use is associated with lower morbidity and mortality,[1] the suggestion that pulsatile vitamin D in general may also be associated with improved survival (compared to those not taking injectable vitamin D),[2] suggests that widespread VDR activation may contribute to improved outcomes in patients with chronic kidney disease (CKD).

POTENTIAL MECHANISMS FOR A SURVIVAL BENEFIT FROM SYSTEMIC VDR ACTIVATION

Vascular calcification

Because at least half of the deaths among dialysis patients are attributed to cardiovascular disease, the potential benefit of

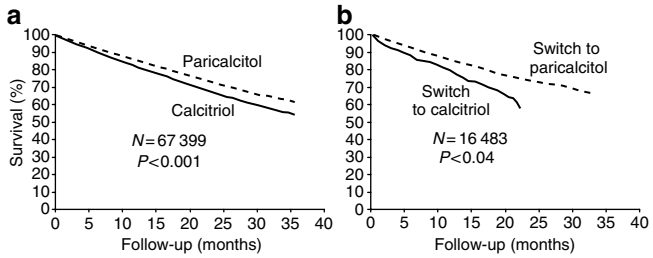

Figure 1 | (a) Survival curves in dialysis patients treated with either injectable paricalcitol or calcitriol. (**b**) Survival curves in dialysis patients who switched from calcitriol to paricalcitol or from paricalcitol to calcitriol. (From Teng *et al.*[1] with permission.)

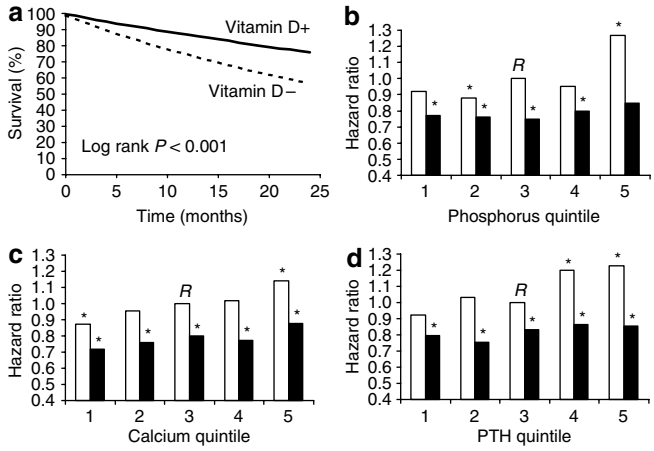

Figure 2 | (a) Survival curves in dialysis patients treated with injectable vitamin D (black) compared to patients not treated with injectable vitamin D (white). Mortality hazard ratios across all quintiles of phosphate (**b**), calcium (**c**) and PTH (**d**). The first quintile represents the lowest levels and the fifth quintile represents the highest levels. (From Teng *et al.*[2] with permission.)

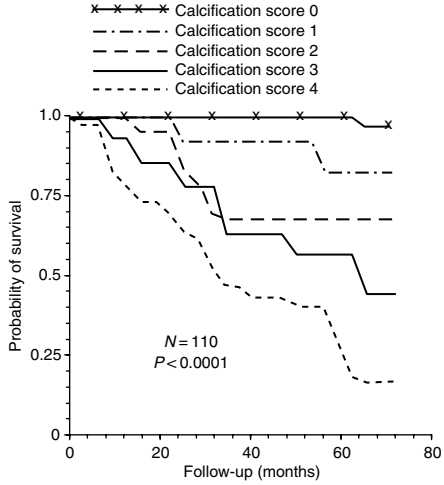

Figure 3 | Probability of survival in dialysis patients as a function of calcification score. (From Blacher *et al.*[11] with permission.)

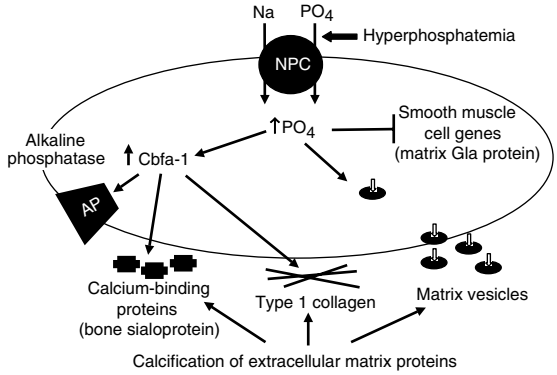

Figure 4 | Hypothesis of how hyperphosphatemia may cause arterial calcification by altering the VSMC phenotype into an osteoblast-like cell capable of calcifying its extracellular matrix. Phenotype changes also include decreased expression of VSMC genes, such as MGP, which inhibits calcification. (Modified from Giachelli CM: *J Am Soc Nephrol* 15:2959, 2004.)

vitamin D treatment is likely to include mechanisms related to the development of vascular calcification, atherosclerosis and cardiac dysfunction. Several studies in patients with CKD have now correlated arterial calcification with the presence of coronary artery disease,[4] peripheral vascular disease,[5] left ventricular hypertrophy [6] and mortality.[5–9] Although it is still not clear how calcified arteries may cause acute cardiovascular events, recent studies have documented increased mortality rates in dialysis patients who have arterial calcifications[7] (Figure 3). Increased pulse pressure, left ventricular hypertrophy and arrythmias, resulting from arterial stiffness, have been suggested as potential effects of arterial calcification that may lead to cardiovascular disease and death.[9–11] The recent finding that significant coronary artery calcification is present in patients of stages 3 and 4 CKD,[12] a population which is also at high risk for cardiovascular events,[13] underscores the importance of early diagnosis and treatment.

A current hypothesis for the development of arterial calcification in dialysis patients has centered on the effects of high phosphate levels[14] or uremic serum[15,16] on vascular smooth muscle cells (VSMC) that reside within the medial portion of the arterial wall (Figure 4). In this model, elevated serum phosphate increases the intracellular phosphate concentration, which stimulates the transcription factor, core binding factor alpha 1 (cbfα1). Cbfα1 has its primary role in cartilage cells and osteoblasts to promote bone formation through its stimulation of type I collagen and non-collagenous proteins (e.g. bone sialoprotein, osteocalcin, osteopontin) into the surrounding matrix to support the calcification process.[17] Owing to its abnormal expression of cbfα1, the VSMC phenotype becomes more like that of an osteoblast-like cell and subsequent calcification of the extracellular matrix becomes amplified by excess phosphate and calcium loading,[18] hypercalcemia[19] and low serum fetuin levels.[20] Although cbfα1 may have a central role in promoting calcification, other potential inducers of calcification include

Table 1 | Potential inducers and inhibitors of arterial calcification in CKD

Inducers
Phosphate
Uremic serum
Core binding factor alpha1 (cbfα1)
Bone morphogenetic protein-2 (BMP-2)
PTH
Msx2
β-Catenin
Interleukin-1β and -6
Transforming growth factor-alpha (TGF-α)
Type I collagen
Cyclic AMP
Oxidative stress
Hypercalcemia (high-dose vitamin D)
Inhibitors
Matrix gla protein (MGP)
Osteopontin (phosphorylated)
Type IV collagen
Phosphonoformic acid (PFA)
Parathyroid hormone-related protein (PTHrP)
C-natriuretic protein
High-density lipoprotein (HDL)
Bone morphogenetic protein-7

Table 2 | Potential ameliorative effects of VDR activation on arterial calcification

Calcification	VDR activation
Inducers	
Type I collagen	↓
Cbfα1	↓
BMP-2	↓
β-catenin	↓ Activation
Interleukin-Iβ, IL-6, TGF-α	↓
Inhibitors	
Matrix gla protein (MGP)	↑
Osteopontin	↑
C-natriuretic peptide (CNP)	↑ CNP-receptors

bone morphogenetic protein-2,[21] interleukin-1 beta (IL-1β),[22] IL-6,[22] type I collagen,[23] cyclic adenosine monophosphate,[24] tumor necrosis factor-α,[25]oxidative stress,[26] Msx2 transcription factor[27] and hypercalcemia from high-dose vitamin D[28] (Table 1).

Inhibitors of calcification have recently been identified from gene knockout models and cell culture studies. Osteopontin[29] and matrix gla protein (MGP)[29] are both potent inhibitors of arterial calcification as demonstrated in mice that have been made genetically deficient in these proteins and which subsequently develop significant calcification. Moreover, mice containing double knockout disruptions of both osteopontin and MGP show more arterial calcification than with single protein deletions of either,[29] indicating that both are important in preventing the calcification process by different mechanisms. Importantly, osteopontin needs to be in its phosphorylated form to inhibit calcification.[30] The mechanism for the inhibitory effect of MGP may be related, in part, to its binding and neutralization of bone morphogenetic protein-2;[31] gamma-carboxylation of MGP is necessary for it to inhibit calcification[32] and to bind to bone morphogenetic protein-2.[31] Other inhibitors of *in vitro* induced calcification include type IV collagen,[23] BMP-7,[33] phosphonoformic acid through its inhibition of the sodium-phosphate co-transporter in VSMC,[14] parathyroid hormone-related protein,[34] C-natriuretic protein[34] and high-density lipoprotein.[22]

Activation of VDRs by vitamin D therapy may directly mitigate cardiovascular disease by inhibiting the production of proteins that are either necessary for arterial calcification or by stimulating proteins that inhibit mineralization (Table 2). For example, VDRs are present in VSMCs, and 1,25-dihydroxyvitamin D treatment of osteoblastic cells is known to inhibit the synthesis of type I collagen,[35] which is the major 'scaffold' for calcification of the extracellular matrix. Perhaps more importantly, other studies of cultured osteoblastic cells show that a vitamin D response element is present within the promoter region of cbfα1 and that stimulation with 1,25-dihydroxyvitamin D reduces cbfα1 synthesis.[36] Moreover, inhibition of bone morphogenetic protein-2 production by 1,25-dihydroxyvitamin D in cultured osteoblasts has been recently documented.[37] Calcitriol also inhibits circulating levels of IL-1β and IL-6,[38] which are not only implicated in calcification but also have important roles in mediating the inflammatory response to atheroma formation (see below). Treatment of cells *in vitro* with 1,25-dihydroxyvitamin D also stimulates the production of MGP,[39] osteopontin[35] and natriuretic peptide receptor-C.[40]

Atherosclerosis

Although VDR activation may mitigate the effects of uremia-induced arterial calcification, emerging data support a potential role for VDR activation in preventing or ameliorating the pathogenesis of atherosclerosis. Current models of atherosclerosis include the inter-related functions of T lymphocytes and macrophages as initial stimulators of intimal thickening and plaque formation in susceptible arteries.[41] Th1 lymphocytes infiltrate into the subendothelial space, in response to oxidized low-density lipoprotein, and secrete interferon-γ, which is a potent macrophage activator (Figure 5). Activated macrophages in turn secrete the cytokines IL-1β, IL-6 and tumor necrosis factor-α, which further enhance monocyte recruitment, increase the oxidation of low-density lipoprotein and promote the production of membrane metalloproteinases (MMPs)that function to destabilize the plaque to cause rupture and thrombosis within the lumen. Th1 cells produce interferon-γ, which suppresses Th2 lymphocytes, cells that are antiatherogenic through their production of IL-10, which inhibits macrophage activation.[41]

The potential ameliorative effects of VDR activation on the pathogenesis of atherosclerosis may occur by enriching the Th2 cell population of lymphocytes (Table 3). For

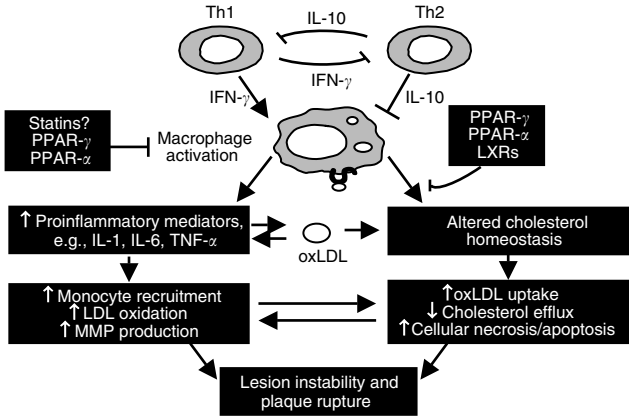

Figure 5 | Role of T-lymphocytes and macrophages in the development of atherosclerosis. (From Li and Glass[41] with permission.)

Table 3 | Potential ameliorative effects of VDR activation on arterial disease

Arterial disease	VDR activation
Atheroma formation	
Inducers	
Th1 cells	↓ (by ↓IFNγ)
IL-1β, IL-6	↓
Inhibitors	
Th2 cells	↑ (by ↑IL-10)
IL-4	↑
Thrombogenesis	↓

example, calcitriol treatment of cells results in marked inhibition of interferon-γ[42] and upregulation of IL-10.[43] In addition, IL-1β and IL-6 are inhibited by 1,25-dihydroxyvitamin D,[38] which would also mitigate or inhibit macrophage activation and prevent plaque instability. Although VDR activation is a major stimulus for IL-10 production, it also stimulates IL-4 synthesis[44], which is important in promoting the anti-atherogenic function of Th2 cells. An additional mechanism by which VDR activation may sustain plaque stability is by preventing thrombosis as demonstrated in VDR-knockout mice that develop arterial thromboses in association with downregulation of antithrombin and thrombomodulin and upregulation of tissue factor.[45]

Whereas these studies suggest possible mechanisms for a beneficial effect of VDR activation at the cellular level, studies in animals and humans also show potential ameliorative effects on the cardiovascular system by other mechanisms. These include VDR effects on bone and the parathyroid glands as well as on cardiac function.

VDR ACTIVATION, BONE LOSS AND CARDIOVASCULAR DISEASE

Low levels of serum 1,25-dihydroxyvitamin D are responsible for increased PTH production, decreased intestinal calcium and phosphorus absorption, reduced bone formation and increased bone resorption. Elevated PTH increases myocardial cell calcium concentration in uremic animal models of heart disease[45,46] and parathyroidectomy in dialysis patients improves left ventricular hypertrophy[47] and long-term survival rates.[48] The combination of high PTH and low 1,25-dihydroxyvitamin D levels in predialysis CKD patients results in high bone turnover[49] with bone loss,[50] and treatment with vitamin D compounds lowers PTH and bone turnover toward normal levels.[49] Because VDR activation was recently shown in mouse knockout models to be required to promote bone formation[51] and to maintain normal bone-forming capability by inhibiting osteoblast apoptosis,[52] the bone accretion effects of vitamin D treatment in CKD are likely to be due to the combination of enhanced bone formation and reduced bone resorption. Thus, from a bone perspective, optimizing circulating PTH and 1,25-dihydroxyvitamin D levels into the normal range in early and moderate CKD may be required to maintain normal bone remodeling and prevent adynamic bone disease. The finding that vitamin D treatment prevents bone loss in patients with stages 3 and 4 CKD[53] is consistent with this notion (Figure 6).

The potential role of bone loss as a contributor to arterial calcification in CKD has not been fully explored. However, recent longitudinal data in postmenopausal women without

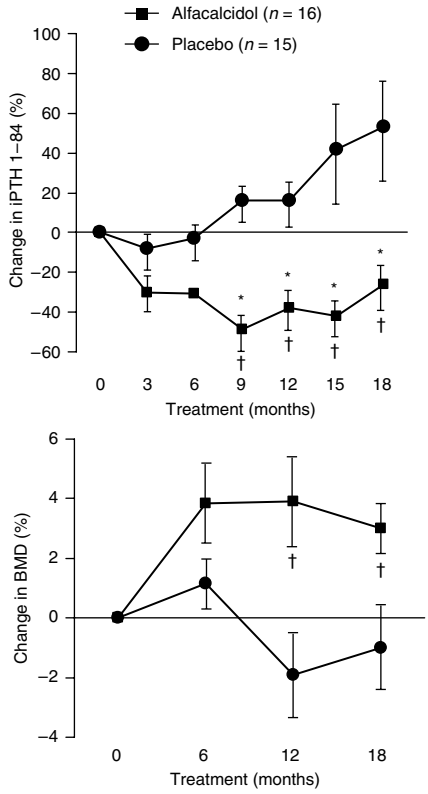

Figure 6 | PTH and bone density response to treatment with alphacalcidol in patients with stages 3 and 4 CKD. (From Rix *et al.*[53] with permission.)

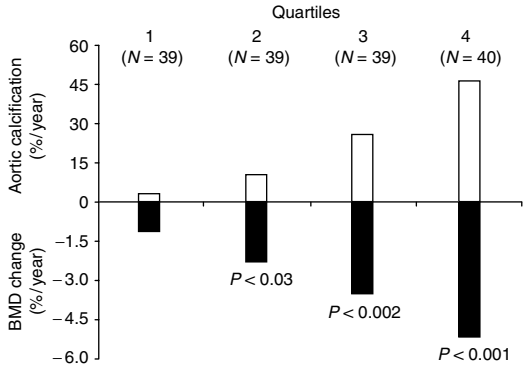

Figure 7 | Association of aortic calcification with increased rates of bone loss in a longitudinal study of post-menopausal women. (From Schulz *et al.*[54] with permission.)

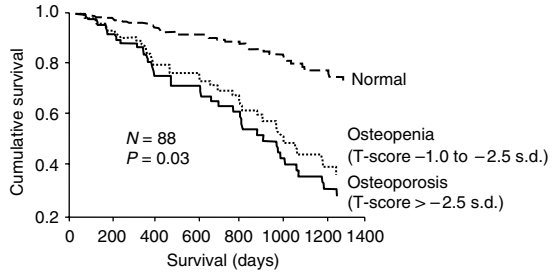

Figure 8 | Cumulative survival in dialysis patients with normal or low bone mineral density. (From Taal *et al.*[55] with permission.)

known CKD reveal a significant correlation between elevated rates of bone loss and increased coronary artery calcification (Figure 7).[55] This finding may have relevance in the dialysis population because low hip bone mineral density (Figure 8) and excessive coronary artery calcification[7] have both been associated with decreased survival. From these data, it follows that it may be important to develop treatment strategies that prevent bone loss in an effort to decrease arterial calcification and cardiovascular disease in CKD patients.

There are other potential mechanisms where VDR activation may ameliorate or prevent cardiovascular disease (Table 4). Decreased VDR activity increases circulating renin levels and blood pressure[56] and causes left ventricular and mycocyte hypertrophy in genetically manipulated mouse models.[57] Interestingly, earlier clinical studies had established a significant relationship between low circulating levels of 1,25-dihydroxyvitamin D and elevated serum renin.[58] Treatment with 1,25-dihydroxyvitamin D also decreases endothelium-induced atrial natriuretic peptide levels while ameliorating cardiac myocyte hypertrophy.[59] VDR activation downregulates atrial natriuretic peptide transcription by nuclear interactions that do not involve retinoid X receptor -VDR heterodimerization.[60,61] VDR activation is also important in downregulating endothelin receptors in cultured osteoblasts,[62] which may help explain how vitamin D treatment prevents or mitigates endothelin-induced cardiac remodeling and left ventricular dysfunction[63,64] as well as

Table 4 | Potential ameliorative effects of VDR activation on effectors of cardiac dysfunction

Organ/cell dysfunction	Putative effectors	VDR activation
Cardiac and myocyte hypertrophy	Renin–angiotensin	↓
	Atrial naturetic peptide (ANP)	↓
	Endothelin troponin T	↓
Left ventricular hypertrophy	PTH, ANP and renin–angiotensin	↓
	PTH	↓

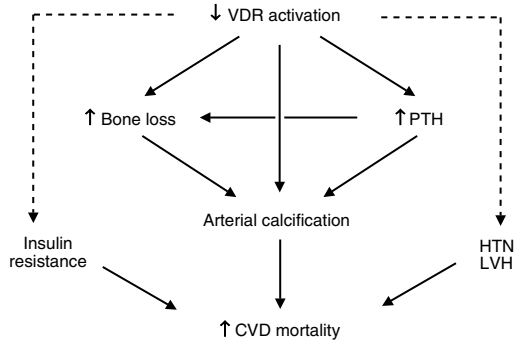

Figure 9 | Hypothetical mechanisms of how decreased VDR activation may lead to arterial calcification and cardiovascular mortality in CKD, by indirect (increased PTH secretion and bone loss) and direct (permitting increased cbfα1 and type I collagen expression and decreased MGP and osteopontin expression) effects. Emerging data also suggest direct effects of VDR activation to improve insulin resistance and prevent hypertension and left ventricular hypertrophy.

arterial vascular calcification.[65] Recent clinical studies have shown that calcitriol-induced reductions in PTH,[66,67] atrial natriuretic peptide and renin–angiotensin II[66] are associated with amelioration of left ventricular hypertrophy in patients receiving dialysis.

A working hypothesis (Figure 9) that includes these factors suggests that VDR activation (by treatment with injectable vitamin D) may play a role in preventing uremic-induced arterial calcification through inhibition of cbfα1 and type I collagen synthesis and stimulation of the calcification inhibitor, MGP. VDR activation would also have indirect roles in preventing calcification through its inhibition of PTH-stimulated bone loss and its direct stimulation of bone formation.[51,52] How bone loss in uremia contributes to arterial calcification is still a mystery but plausible mechanisms include internal calcium loading (calcium shift from bone to vessel) and/or the release of bone growth factors, such as BMP-2, which has been implicated as a pathogenic factor in some models of arterial calcification.[31]

VITAMIN D IN THE PATHOGENESIS OF SECONDARY HYPERPARATHYROIDISM

Several studies have clearly documented that early declines in the glomerular filtration rate (GFR) result in falling serum

1,25-dihydroxyvitamin D and increase PTH before there are changes in serum phosphate and calcium.[68–70] In most patients, 1,25-dihydroxyvitamin D levels decline to the lower limit of normal in late stage 2 CKD (stage 2 CKD: estimated GFR, 60–89 ml/min/1.73 m^2), and by the time patients have progressed through stage 3 CKD (estimated GFR, 30–59 ml/min/1.73 m^2) many have low levels of 1,25-dihydroxyvitamin D and elevated PTH. Patients with stage 4 (estimated GFR, 15–29 ml/min/1.73 m^2) and stage 5 (estimated GFR, <15 ml/min/1.73 m^2) CKD have the worst combination of abnormalities with hyperphosphatemia and hypocalcemia further stimulating PTH secretion, independently of low 1,25-dihydroxyvitamin D levels. It is also during these later stages that bone loss,[50] cardiovascular events[3] and death[13] reach their highest prevalence before dialysis is initiated.

A major determinant of low 1,25-dihydroxyvitamin D production is the reduction in renal mass, which results in less 1α hydroxylase being available for converting 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Recently, circulating levels of fibroblast growth factor-23 (FGF-23) have also been implicated as a potential early change responsible for depressed 1,25-dihydroxyvitamin D.[71] Later in the course of CKD progression, hyperphosphatemia reversibly suppresses 1α hydroxylase activity and is therefore a remediable factor that can raise 1,25-dihydroxyvitamin D levels when appropriately treated.[72] Metabolic acidosis[73] and uremic toxins,[74] which also suppress 1α hydroxylase activity and 1,25-dihydroxyvitamin D synthesis, would be expected to have a growing impact in stages 4 and 5 CKD when their accumulation becomes maximal (Figure 10).

Independent of CKD progression is the issue of 25-hydroxyvitam D deficiency, which is prevalent even in subjects without kidney disease.[75,76] Interestingly, low substrate levels of 25-hydroxyvitamin D are associated with low 1,25-dihyroxyvitamin D levels[77] except for those with normal renal function.[78] Thus, there is interest in knowing whether treatment with vitamin D (ergocalciferol or cholecalciferol), to raise 25-hydroxyvitamin D levels, will lower PTH in CKD patients. Some doubt exists, however, about the potential success of ergocalciferol monotherapy as cellular uptake of 25-hydroxyvitamin D may be dependent on combined

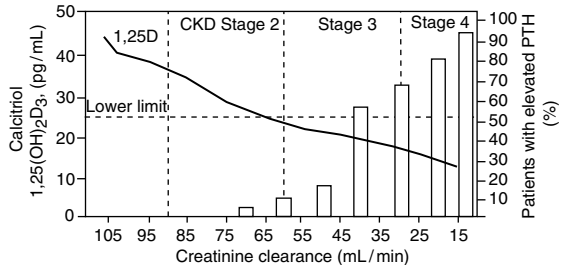

Figure 10 | Progression of 1,25-dihydroxyvitamin D deficiency and hyperparathyroidism among patients with CKD stages 1–4. The 1,25-dihydroxyvitamin D levels represent the mean values from several studies (from Pitts *et al.*,[68] Reichel *et al.*[69] and Kates *et al.*[70]) and do not show the wide variation in individual values along the continuum.

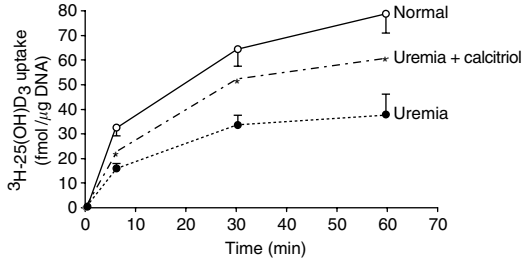

Figure 11 | Uptake of ^{3}H-25-hydroxyvitamin D into monocytes isolated from dialysis patients before and after a 3-week treatment with injectable calcitriol, compared to monocyte ^{3}H-25-hydroxyvitamin D uptake in normal subjects. (From Gallieni *et al.*[79] with permission.)

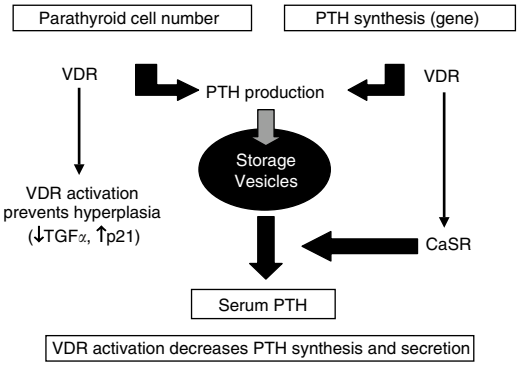

Figure 12 | Determinants of PTH production and secretion. Activation of the VDR inhibits parathyroid gland proliferation, and PTH synthesis while stimulating CaSR expression. Activated CaSR inhibits the secretion of PTH from storage vesicles within parathyroid cells.

therapy with calcitriol to enhance the cellular uptake of 25-hydroxyvitamin D (Figure 11).[79] This notion is also consistent with the findings that 1,25-dihydroxyvitamin D treatment enhances megalin expression[80] and that megalin endocytosis of the 25-hydroxyvitamin D–vitamin D binding protein complex from the glomerular ultrafiltrate is the major mechanism for delivering 25-hydroxyvitamin D to the 1α hydroxylase enzyme in the proximal tubule.[81]

VDR ACTIVATION AND PARATHYROID GLAND GROWTH

Elevated PTH levels in CKD are a product of increased parathyroid cell number and increased PTH synthesis (Figure 12). Activation of the VDR by di-hydroxylated vitamin D compounds controls both of these functions by suppressive mechanisms. Parathyroid gland enlargement occurs initially as a result of diffuse cell proliferation, in association with low circulating 1,25-dihydroxvitamin D levels. As 1,25-dihydroxyvitamin D is known to stimulate VDR synthesis,[82] it is assumed that the low circulating vitamin D levels in CKD contribute directly to the low VDR expression in uremic parathyroid glands.[83] Recent data also show that increased PTH downregulates VDR expression.[84] Studies in uremic animals show that treatment with calcitriol prevents

parathyroid cell proliferation and gland enlargement by inhibiting specific growth factors (TGF-α, epidermal growth factor receptor) and by stimulating proteins (p21, p27) that control the cell cycle to inhibit DNA synthesis.[85,86] VDR activation inhibits PTH synthesis by binding to its vitamin D response element on the PTH gene and inhibiting its transcription.[87] Because 1,25-dihydroxyvitamin D also upregulates calcium sensing receptor (CaSR) synthesis,[88] it is likely that parathyroid gland VDR activation facilitates the effect of calcium on the CaSR to suppress PTH secretion.[89] Whereas parathyroid gland CaSR activity is downregulated in experimental uremia, its expression does not coincide with increased cell proliferation,[90] suggesting that it may not have a direct role in preventing parathyroid hyperplasia, in contrast to the suppressive effects of the VDR.

VDR activation: molecular events

The VDR is found ubiquitously throughout the body and, although present in most organs, its function in many locations has not been established (Table 5). However, within such organs as the parathyroid cell, osteoblast and intestinal enterocyte, VDR action has been carefully examined. The VDR resides within the cell cytosol where it binds preferentially to di-hydroxylated vitamin D compounds. This complex is then rapidly translocated to the nucleus where it first binds to the retinoid X receptor and then to the vitamin D response element located in the promoter region of selected genes (Brown *et al.*[91]). Once bound, the DNA undergoes a conformational change that brings it into contact with distinct nuclear proteins that are cell and gene specific. The gene and its nuclear protein partners determine whether the transcription of the final protein product is either up- or downregulated (Table 6). For example, in osteoblastic bone cells, VDR activation results in the upregulation of osteocalcin and MGP and the downregulation of type 1 collagen and bone sialoprotein.[35] In intestinal cells, the VDR upregulates calbindin, and other calcium transport proteins.[92] In the kidney, VDR expression is responsible for upregulating at least 50 genes (including the CaSR) and downregulating 40 genes (including renin).[93]

In addition to the genomic effects of VDR activation there are well-described non-genomic events that are mediated by a putative cell surface VDR.[94] These effects are induced within minutes upon exposure to activated vitamin D compounds and they involve secondary signaling mechanisms that interact with other signaling networks.[95–98] Potential effects of this type of stimulation include changes in ion channel responses,[96] insulinotropic effects,[97] calcium flux,[97] adipocyte metabolism[98] and antiapoptotic pathways.[52]

Table 5 | Tissue distribution of the VDR

System	Tissue
Endocrine	Parathyroid, pancreatic B cells, thyroid C cells
Cardiovascular	Arterial smooth muscle cells, cardiac myocytes
Musculoskeletal	Osteoblasts, chondryocytes, striated muscle
Gastrointestinal	Esophagus, stomach, intestine
Hepatic	Liver parenchymal cells
Renal	Tubules, JG apparatus (renin), podocytes
Reproductive	Testis, ovary, uterus
Immune	T cells, B cells, bone marrow, thymus
Respiratory	Lung alveolar cells
Epidermis	Keratinocytes, hair follicles
Central nervous system	Brain neurons

Table 6 | Partial list of proteins regulated by VDR activation

Downregulated	Upregulated
PTH (p)	Osteopontin (o)
Cbfα1 (o)	Matrix gla protein (o)
BMP-2 (k,o)	RANK-L (o)
Bone sialoprotein (o)	Calbindin (i, k)
Type I collagen (o)	Type IV collagen (k)
IFN-γ, IL-Iβ, -2, -6, -12(l, s)	IL-10, IL-4 (l)
GM-CSF (l)	Megalin (k)
TNF-α, EGF-receptor (p)	Insulin receptor (m)
Renin (k)	VDR (k, p)
Endothelin receptor (o)	CaSR (k, p)
PCNA (k, p, t)	p21 (p, t)
Cyclin E (f, t)	p27 (t)
Tissue factor (l, k, m)	Antithrombin (li)
PPARϒ2 (ad)	Thrombomodulin (a, li, k,m)
ANP (h)	E2F3 (k)
β-catenin (a, t)	24-hydroxylase (k)
Myogenin (mu)	Insulin-induced gene-1 (ad)

a, aorta; ad, adipocytes; f, fibroblasts; h, heart; i, intestine; k, kidney; l, lymphocytes; li, liver; m, monocytes; mu, muscle; o, osteoblast; p, parathyroid cells; s, serum; t, tumors.

Vitamin D treatment: structural and functional differences of vitamin D compounds

The use of the first generation of activated vitamin D compounds occurred shortly after the discovery that 1,25-dihydroxyvitamin D was the most active vitamin D metabolite.[99] Synthetic 1,25-dihydroxyvitamin D (calcitriol) proved to bind more selectively to the VDR than vitamin D or 25-hydroxyvitamin D, thus establishing it as the most potent form of vitamin D for stimulating intestinal calcium and phosphate absorption. The second-generation compounds involved a side-chain modification, which removed the hydroxyl group from the 25 position [1a-hydroxyvitamin D3 (alphacalcidol), 1a-hydroxyvitamin D2 (doxercalciferol)]. Because these compounds lack the 25-hydroxyl group, they are unable to bind selectively to the VDR and are therefore prohormones. Both require 25-hydroxylation in the liver and both have equivalent potency in animal studies.[100] The third generation is composed of a group of 1- and 25-hydroxylated vitamin D compounds with either ring structure modifications [19-nor-1,25-dihydroxyvitamin D2 (paricalcitol)], or side-chain modifications [22-oxacalcitriol (maxicalcitol)], both of which have less calcemic and less phosphatemic effects when compared to calcitriol.[101,102]

Studies in uremic rats indicate that paricalcitol inhibition of PTH synthesis is similar to calcitriol but without significant elevations in serum calcium and phosphate.[103,104] The mechanism for paricalcitol's low calcemic effect is due to reduced stimulation of intestinal calcium transport proteins

(e.g. calbindin, CAT and PMAT) compared to calcitriol,[92] thus conferring a selective activator function. In comparison studies between paricalcitol and doxercalciferol in normal and uremic rats, similar differences are noted where doxercalciferol induces a greater calcemic and phosphatemic effect due to greater enhancement of intestinal calcium and phosphate absorption.[104] Although head-to-head comparisons of these two compounds have not been performed in patients with CKD, there are indications that paricalcitol has a more beneficial side effect profile than doxercalciferol.[105,106] For example, dialysis patients treated with paricalcitol achieved a 50% reduction in PTH over 12 weeks with serum calcium increasing by 3.5% from baseline ($P<0.02$) and serum phosphate increasing by 8% (p-NS).[104] In contrast, dialysis patients who were treated with doxercalciferol and who also achieved a 50% reduction in PTH over the same time course had a mean serum calcium elevation of 7% ($P<0.01$) and a mean serum phosphate elevation of 19% ($P<0.01$).[106]

Management of hyperparathyroidism in CKD stages 3 and 4

Current recommendations for managing the hyperparathyroidism of CKD have suggested that treatment should begin in stage 3 CKD patients.[107] Opinion-based recommendations for patients with elevated PTH include normalization of 25-hydroxyvitamin D levels using ergocalciferol (vitamin D_2). However, despite the finding that many CKD patients have mild to moderately low 25-hydroxyvitamin D levels,[77] there are no studies in this population which have evaluated the efficacy of ergocalciferol in lowering PTH. In contrast, compounds such as calcitriol,[108,109] doxercalciferol[110] and paricalcitol[111] have been shown to be effective in lowering PTH levels in patients with moderate CKD, although important differences in their side-effect profiles are apparent. For example, a 24-week doxercalciferol treatment resulted in a 5% rise in serum calcium by week 20 ($P<0.012$) and a 6% rise in serum phosphate at week 24 ($P<0.05$) compared to the placebo group,[110] whereas treatment with paricalcitol did not result in elevated serum calcium or phosphate at any time point when compared to placebo controls.[111] These findings are consistent with observations in rats showing that doxercalciferol caused elevated serum calcium and phosphate because of enhanced intestinal absorption, in contrast to comparable doses of paricalcitol, which did not enhance calcium or phosphate absorption or the calcium $\times$ phosphate product in uremia.[104]

Few studies are available that have analyzed the bone response to vitamin D compounds in predialysis CKD. Calcitriol[108,109] and alphacalcidol[49] reduce elevated bone turnover to more normal levels and bone mineral density increases with long-term alphacalcidol treatment.[50] Concomitant with the reduction in PTH-induced high bone turnover, vitamin D treatment also reduces serum bone alkaline phosphatase to more normal levels.[110,111] However, despite the improved changes in bone alkaline phosphatase, studies are still needed to examine the effects of paricalcitol and doxercalciferol on bone histology, bone mineral density, fracture and cardiovascular morbidity.

Adjunctive treatments and soft-tissue calcification: calcium loading and calcimimetics

Hypercalcemia, from high doses of calcitriol, has the potential to induce arterial calcification.[28] Whereas one *in vitro* study has shown that supra-pharmacologic concentrations of calcitriol (10^{-7} M) can directly induce VSMC calcification,[112] other studies have not shown this effect,[113] suggesting that some *in vitro* experiments may not realistically mimic the clinical conditions of CKD. However, excess calcium loading is a major contributor to arterial calcification[17,114,115] and bone loss[116] in dialysis patients and, because the effects of calcium loading are not always reflected by the ambient calcium levels,[17] quantification of the cumulative intake of calcium is more likely to provide reassurance that the patient is not in excessive calcium balance. Although the current recommendation to limit calcium intake to <2 g per day (1500 mg as the calcium binder)[107] is a good initial step toward preventing excess calcium loading, this may still not be sufficient as daily calcium intakes of as low as 1100 to 1350 mg (elemental) are associated with arterial calcification in dialysis patients.[5,115]

The issue of calcium loading also becomes important when adjunctive treatment of severe hyperparathyroidism with CaSR agonists (calcimimetics)[117] is utilized. Calcium levels often decline during calcimimetic therapy by unknown mechanisms and the initial therapeutic response to this side effect has been to raise the serum calcium by calcium loading, either from oral supplements or by employing the use of high dialysate calcium concentrations (>2.5 mEq/l).

A re-evaluation of the K/DOQI guidelines for vitamin D therapy

Stage 5 CKD. Current K/DOQI recommendations for dialysis patients suggest that injectable vitamin D therapy should be stopped when phosphate levels are too high (>5.5 mg/dl), when intact PTH levels are too low (<150 pg/ml) or when the calcium $\times$ phosphate product exceeds 55 mg^2/dl^2.[107] However, in the light of the new data regarding improved survival in dialysis patients at all levels of phosphate and PTH (Figure 2),[2] it appears that these recommendations should be re-evaluated. Although high phosphate is certainly important as a contributor to mortality in dialysis patients,[2] as well as in patients with moderate CKD,[118] the advice to discontinue vitamin D therapy as a method to control phosphate levels would only be appropriate for those active vitamin D compounds that have been shown to stimulate calcium and phosphate absorption significantly (e.g. calcitriol, alphacalcidol, doxercalciferol). Based on the current literature, it appears that the best practice would be to utilize paricalcitol to control PTH secretion, while minimizing calcemic and phosphatemic effects, and to limit dietary indiscretions of phosphate intake while ensuring the use of non-calcium containing phosphate

binders. The goals of managing phosphate levels and vitamin D therapy should not be mutually exclusive but should instead be directed toward maintaining good phosphate control while providing some amount of injectable vitamin D.[1,2] The use of paricalcitol may be preferable to other agents.

Stages 3 and 4 CKD. As suggested by the K/DOQI guidelines, it is important that early treatment with activated vitamin D be incorporated, at least by stage 3 CKD, to fully maximize its inhibitory effect on parathyroid gland growth. Several studies show that activated forms of vitamin D effectively reduce PTH levels in stages 3 and 4 CKD whereas there are no studies showing that treatment with ergocalciferol or cholecalciferol (vitamin D_3) is effective in decreasing PTH in this patient population. Thus, although it is important to improve nutritional stores of 25-hydroxy-vitamin D using ergocalciferol, evidence-based practice should focus on the use of activated vitamin D to ensure sustained control of PTH levels with minimal effects on calcium and phosphate homeostasis. Because moderate elevations of intact PTH (e.g. 60–120 pg/ml) are associated with significant bone loss in this patient population,[50] who are not likely to have adynamic bone turnover, either before or after treatment with activated vitamin D,[49] it makes sense that PTH levels should be maintained within the normal range during stages 3 and 4 CKD in an effort not only to prevent bone loss and the progression of parathyroid gland hyperplasia but also to mitigate the deleterious effects of hyperparathyroidism on cardiovascular function.

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