BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 395–412

0007-1188/09
www.brjpharmacol.org

THEMED SECTION: ADVANCES IN NUTRITIONAL PHARMACOLOGY

REVIEW

Potential for vitamin D receptor agonists in the treatment of cardiovascular disease

JR Wu-Wong

Abbott, Abbott Park, IL, USA

Vitamin D_3 is made in the skin and modified in the liver and kidney to form the active metabolite, 1,25-dihydroxyvitamin D_3 (calcitriol). Calcitriol binds to a nuclear receptor, the vitamin D receptor (VDR), and activates VDR to recruit cofactors to form a transcriptional complex that binds to vitamin D response elements in the promoter region of target genes. During the past three decades the field has focused mainly on the role of VDR in the regulation of parathyroid hormone, intestinal calcium/phosphate absorption and bone metabolism; several VDR agonists (VDRAs) have been developed for the treatment of osteoporosis, psoriasis and hyperparathyroidism secondary to chronic kidney disease (CKD). Emerging evidence suggests that VDR plays important roles in modulating cardiovascular, immunological, metabolic and other functions. For example, data from epidemiological, preclinical and clinical studies have shown that vitamin D and/or 25(OH)D deficiency is associated with increased risk for cardiovascular disease (CVD). However, VDRA therapy seems more effective than native vitamin D supplementation in modulating CVD risk factors. In CKD, where decreasing VDR activation persists over the course of the disease and a majority of the patients die of CVD, VDRA therapy was found to provide a survival benefit in both pre-dialysis and dialysis CKD patients. Although VDR plays an important role in regulating cardiovascular function and VDRAs may be potentially useful for treating CVD, at present no VDRA is approved for CVD, and also no serum markers, beside parathyroid hormone in CKD, exist to indicate the efficacy of VDRA in CVD.
British Journal of Pharmacology (2009) **158,** 395–412; doi:10.1111/j.1476-5381.2009.00171.x; published online 9 April 2009

This article is part of a themed section on Advances in Nutritional Pharmacology. To view all articles in this section visit http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: vitamin D; vitamin D receptor; vitamin D receptor agonists; paricalcitol; calcitriol; doxercalciferol; vitamin D deficiency; cardiovascular disease; chronic kidney disease

Abbreviation: CKD, chronic kidney disease; CVD, cardiovascular disease; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism; VDR, vitamin D receptor; VDRA, VDR agonist or activator

Introduction

It is well known that humans can acquire vitamin D_2 or D_3 via food or vitamin D supplement and also make vitamin D_3 in the skin by exposing to sunshine. However, vitamin D_3 is not immediately active, but needs to be converted to 25-hydroxyvitamin D_3 ($25(OH)D_3$) by 25-hydroxylase in the liver, followed by further hydroxylation by 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) to form the active hormone, 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$ or calcitriol) (Figure 1). The second hydroxylation step by CYP27B1 occurs mainly in the kidney, which results in the production of circulating (endocrine) $1\alpha,25(OH)_2D_3$. Calcitriol synthesis can also occur in extra-renal cells and tissues, which does not significantly contribute to endocrine $1\alpha,25(OH)_2D_3$ levels and is considered primarily to have an autocrine and/or paracrine function (Hewison *et al.*, 2007). The binding of calcitriol ($1\alpha,25(OH)_2D_3$) or its analogs to the vitamin D receptor (VDR), a nuclear receptor, activates VDR to recruit cofactors to form a transcriptional complex that binds to vitamin D response elements in the promoter region of target genes to regulate gene transcription (Andress, 2006; Wu-Wong *et al.*, 2006c) (Figure 1). Calcitriol is extremely potent. In healthy individuals, the average level of 25(OH)D in blood circulation is ~30 $ng \cdot mL^{-1}$ (~75 $nmol \cdot L^{-1}$), while the

Correspondence: J Ruth Wu-Wong, RA37, AP34, 100 Abbott Park Road, Abbott Park, IL 60064, USA. E-mail: ruth.wu-wong@abbott.com
Received 29 September 2008; revised 24 November 2008; accepted 5 January 2009

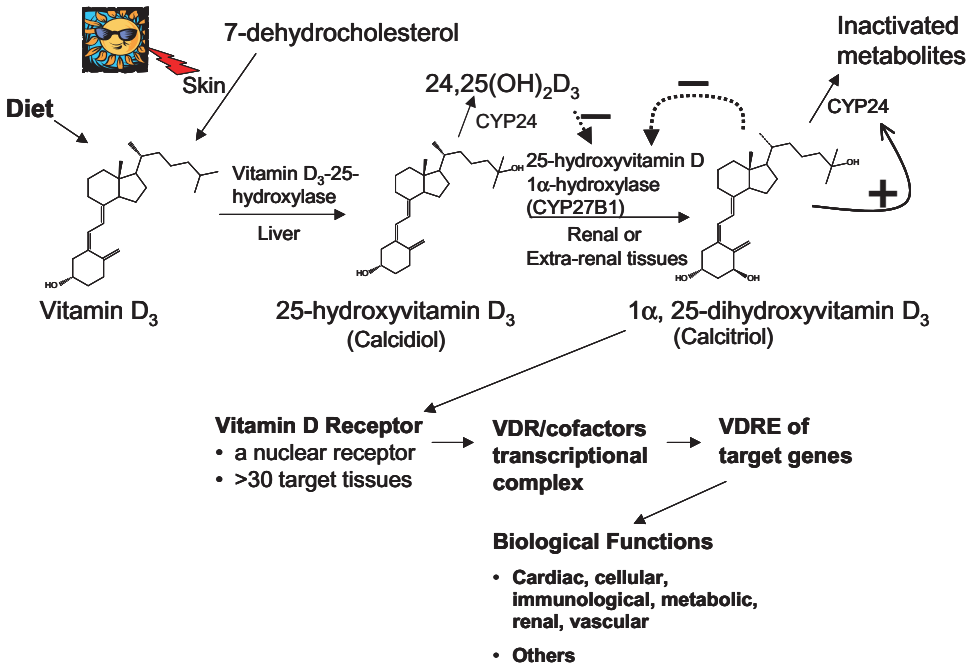

Figure 1 Vitamin D_3 is converted to 25-hydroxyvitamin D_3 ($25(OH)D_3$) in the liver, and then converted to the active metabolite, 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$ or calcitriol), by 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) in either renal or extra-renal tissues. Calcitriol binds to and activates vitamin D receptor (VDR) to regulate the expression of target genes. Calcitriol is metabolized by 25-hydroxyvitamin D-24-hydroxylase (24-OHase, CYP24) into excreted metabolites and also down-regulates CYP27B1 via a feedback mechanism. VDR is present in more than 30 tissues and may be involved in modulating diverse biological effects.

level of calcitriol is maintained at 1–45 $pg \cdot mL^{-1}$ (equivalent to 0.002–0.1 $nmol \cdot L^{-1}$). The VDR signalling pathway is dependent on the availability of $1,25(OH)_2D$, the level of which is tightly regulated. A host of proteins and enzymes such as vitamin D-binding protein, the putative liver 25-hydroxylase, CYP27B1 and 25-hydroxyvitamin D-24-hydroxylase (24-OHase or CYP24), along with others such as megalin (an endocytic receptor responsible for the resorption of vitamin D-binding protein in the kidney) and FGF23 (known to inhibit CYP27B1), weave themselves into a complicated network to maintain a balance among the vitamin D, $25(OH)D$ and $1,25(OH)_2D$ levels. In addition, contributing to the complexity of the VDR signalling system is the presence of numerous membrane proteins, cytosolic factors and transcription co-activators/co-repressors that are often associated with the regulation of nuclear receptors. For further information on this subject, please refer to an excellent review by Ebert *et al.* (2006).

During the past three decades, a majority of the studies in the VDR field have focused on elucidating its role in mineral homeostasis such as regulation of parathyroid hormone (PTH), intestinal calcium and phosphate absorption and bone metabolism (Andress, 2006). Consequently, it is now well recognized that vitamin D deficiency results in defective intestinal absorption of calcium and phosphate and skeletal disorders. Furthermore, calcitriol (the endogenous VDR activator) and its analogs such as paricalcitol and doxercalciferol have been developed to treat hyperparathyroidism secondary to chronic kidney disease (CKD) (Brown and Slatopolsky, 2007), osteoporosis (Cheskis *et al.*, 2006) and psoriasis (Fogh and Kragballe, 2004); the structures of several of these drugs are shown in Figure 2. However, it is important to note that VDR has been found in more than 30 tissues including smooth muscle cells, pancreatic β-cells, monocytes, keratinocytes, etc., suggesting that VDR may be involved in modulating many different functions beyond regulation of mineral homeostasis (Nagpal *et al.*, 2005).

This paper will attempt to review preclinical and clinical studies to investigate the involvement of VDR in cardiovascular function and also the efficacy of vitamin D and VDRAs (VDR agonists or activators) in cardiovascular disease (CVD). Because hypertension, diabetes, atherosclerosis, vascular calcification and CKD are well-recognized risk factors for CVD, discussions will focus on papers linking VDRAs to these risk factors.

VDR and cardiovascular function: early studies

The involvement of vitamin D in normal cardiovascular function in the rats was known more than 20 years ago. Rats maintained on a vitamin D-deficient diet exhibited increased systolic blood pressure (BP) and serum creatine phosphokinase, which coincided with a reduction in serum calcium (Weishaar and Simpson, 1987a,b). Ventricular and vascular muscle contractile function was also markedly enhanced. The changes in cardiac contractile function could not be reversed by restoration of serum calcium to normal levels, suggesting that it might be mediated by vitamin D independent of calcium. VDR was also identified in the human heart (O'Connell and Simpson, 1996). However, results from *in vitro* studies investigating the role of VDR in regulating cardiomyocyte functions have not been straightforward. For example,

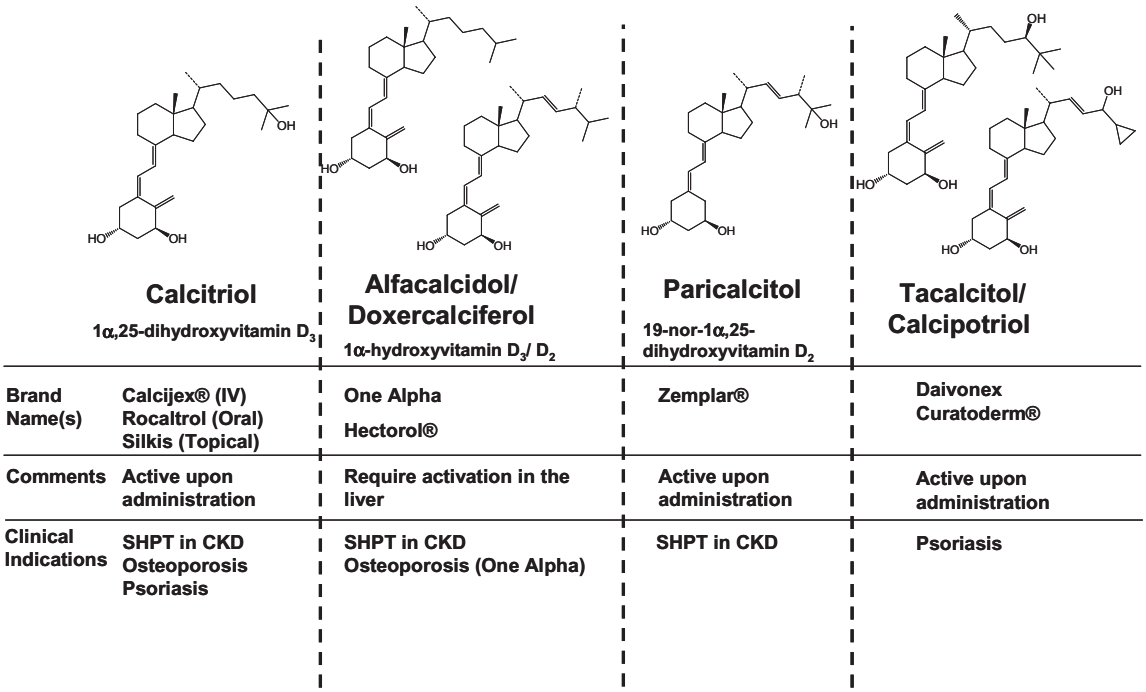

Figure 2 The structures and characteristics of some of the vitamin D analogs currently used to treat osteoporosis, psoriasis and hyperparathyroidism secondary to chronic kidney disease (CKD) (SHPT).

in one study calcitriol was shown to induce hypertrophy in neonatal rat cardiomyocytes (O'Connell *et al.*, 1997), but in other studies calcitriol was capable of antagonizing endothelin-stimulated hypertrophy of neonatal rat cardiomyocytes (Wu *et al.*, 1995; Wu *et al.*, 1996).

VDR and CYP27B1 gene-ablation studies in mice

One important preclinical study demonstrating the involvement of VDR in the cardiovascular system came from the VDR knockout (KO) mice, which were hypertensive and their heart weight/body weight ratios were also significantly higher (Li *et al.*, 2002). In addition, the renal renin mRNA level of adult VDR KO mice was more than threefold higher than that of wild-type (WT). The plasma angiotensin II (ANGII) level was also increased, likely due to increased renin activity. In a subsequent paper, the same group reported that the size of left ventricular cardiomyocytes in VDR KO mice was markedly increased compared with WT (Xiang *et al.*, 2005). Levels of atrial natriuretic peptide (ANP) mRNA and circulating ANP and the cardiac renin mRNA level were significantly increased in the VDR KO mice. These data suggest that VDR is involved in regulating cardiovascular functions, at least in part, through modulation of the renin–angiotensin system (RAS).

Similar observations were made in mice lacking CYP27B1, a key enzyme involved in the synthesis of calcitriol (Zhou *et al.*, 2008). The vehicle-treated CYP27B1 KO mice developed hypertension, cardiac hypertrophy and impaired cardiac function along with an up-regulation of the RAS in both renal and cardiac tissues, which were normalized by calcitriol treatment. In CYP27B1 KO mice on the rescue diet containing a high concentration of calcium and lactose, the serum calcium and phosphorus levels were normalized, but abnormalities in BP, cardiac structure-function and the RAS remained.

In addition to showing that the renin expression and plasma ANGII production were increased significantly in VDR KO mice, Li *et al.* also reported that calcitriol treatment suppressed renin mRNA expression in As4.1 cells, a JG cell-like cell line that was derived from kidney tumours of SV40 T antigen transgenic mice and maintains a high level of renin synthesis (Li *et al.*, 2002). A follow-up report by Li's group demonstrated that calcitriol directly suppressed renin gene expression via a mechanism in which the activated VDR binds to CREB, blocks the formation of the CREB/CREB/CBP/p300 complex and prevents CREB from binding to CRE in the renin promoter region (Yuan *et al.*, 2007). From the proposed model (Yuan *et al.*, 2007), all VDRAs capable of activating VDR shall have similar effects on renin suppression. Indeed, we found that paricalcitol, calcitriol and $1\alpha,25(OH)_2D_2$ (25-OH-doxercalciferol, the active form of doxercalciferol) are similar in potency in suppressing renin mRNA expression (IC_{50} = 3.5, 3.6 and 2.9 nmol·L^{-1} respectively) (Figure 3). It is worth noting that paricalcitol is known to be approximately fivefold less potent than calcitriol in suppressing PTH and ~10-fold less hypercalcemic and is often dosed approximately fourfold higher (Slatopolsky *et al.*, 2003). The potential implication of this observation is that, at equipotent PTH suppressing doses, paricalcitol is expected to provide a better effect in modulating renin expression because patients are exposed to a higher dose of paricalcitol.

The findings by Li *et al.* from the VDR KO mice were not completely reproduced by others. Simpson *et al.* (2007) were able to confirm that the heart in VDR KO mice on the rescue diet was hypertrophied. However, no difference was observed in systolic or mean BP in WT (+/+), KO (–/–) or HETERO (+/–) mice at 3 and 6 months, but systolic BP was decreased in the KO mice (vs. WT) at 9 months of age. Plasma renin activity seemed elevated but not significantly different in KO (vs.

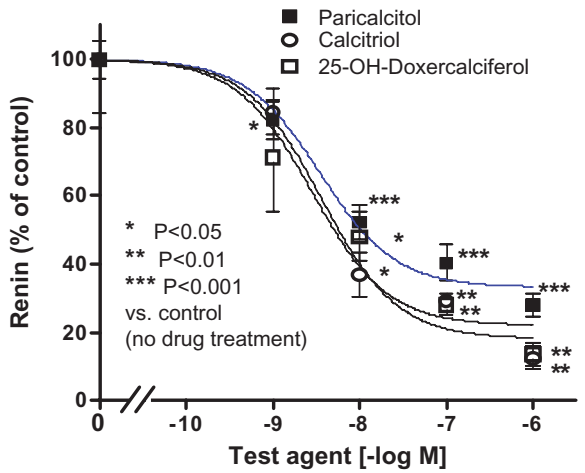

Figure 3 Effect of calcitriol, paricalcitol and 25-OH-doxercalciferol on renin suppression. As 4.1 cells were transfected with human vitamin D receptor cDNA-pcDNA3. After overnight incubation, cells were treated with paricalcitol, calcitriol or 25-OH-doxercalciferol at indicated concentrations for 24 h. Sample analysis was as described previously (Nakane *et al.*, 2007). Statistical comparisons were performed by one-way ANOVA, Dunnett's *t*-test.

WT), and no significant differences in plasma ANGII or aldosterone levels were observed. These data do not support the idea that elevated renin/ANGII and/or hypertension is the mediator for cardiovascular abnormalities in VDR KO mice. Rahman *et al.* (2007) also confirmed the cardiac hypertrophic phenotype in the VDR KO mice. Furthermore, tissue inhibitors of metalloproteinases such as TIMP-1 and TIMP-3 were significantly under-expressed, while metalloproteinases such as MMP-2 and MMP-9 were up-regulated in VDR KO mice. Extracellular matrix remodelling mediated by matrix metalloproteinases is known to contribute to progressive left ventricular remodelling, dilation and heart failure. The data suggest that MMPs and TIMPs expression may be regulated by VDR, and modulation of heart extracellular matrix metabolism may be one of the mechanisms mediating VDR's cardiovascular functions.

Interestingly the VDR KO mice also displayed a phenotype of increased thrombogenic activity. Platelet aggregation was enhanced significantly in normocalcemic VDR KO mice compared with WT and hypocalcemic VDR KO mice (Aihara *et al.*, 2004). The gene expression of antithrombin in the liver and thrombomodulin in the aorta, liver and kidney was down-regulated in both hypo- and normocalcemic VDR KO mice, while tissue factor mRNA expression in the liver and kidney was up-regulated independent of the plasma calcium level. Consequently VDR KO mice exhibited multi-organ thrombus formation after lipopolysaccharide injection. These results suggest that the VDR system may play a physiological role in the maintenance of antithrombotic homeostasis. Consistent with the observations made by Aihara *et al.*, we found that VDRAs up-regulated thrombomodulin and down-regulated plasminogen activator inhibitor-1 in human aortic smooth muscle cells (Figure 4). Similar results were observed in human coronary artery smooth muscle cells (Wu-Wong *et al.*, 2007). These results demonstrate that VDRAs may suppress thrombogenicity.

VDRAs affect cardiovascular functions in hypertensive animal models

In spontaneously hypertensive rats (SHR) with impaired endothelial function, oral cholecalciferol (vitamin D_3) treatment significantly improved the endothelium-dependent vascular relaxation and hyperpolarization induced by acetylcholine (Borges *et al.*, 1999). By comparing the effects of N(omega)-nitro-L-arginine (the NO synthesis inhibitor) and apamin (an inhibitor of Ca^{2+}-dependent K^+ channels), the authors concluded that cholecalciferol treatment may modulate the pathway involving endothelium-derived hyperpolarizing factor, but not nitric oxide. A follow-up study by the same group found that that vasodilator effects of bradykinin on the mesenteric vascular bed were significantly decreased in SHR likely due to impaired Ca^{2+}-dependent K^+ channels, while cholecalciferol treatment restored the hyperpolarizing response to bradykinin (Borges *et al.*, 2002). Interestingly, the observation seemed unique for SHR because the reduced vasodilatory response to bradykinin in Wistar Kyoto rats was not corrected by cholecalciferol treatment (Borges *et al.*, 2002).

Paricalcitol, a calcitriol analog, was tested in the Dahl-salt-sensitive (DSS) rat model (Bodyak *et al.*, 2007). The DSS rat is an established animal model in which high-salt diet induces hypertension, cardiac hypertrophy and heart failure. DSS rats became vitamin D-deficient during the development of cardiac dysfunction. Paricalcitol therapy prevented the appearance of both pathological and echocardiographic evidence of cardiac hypertrophy and cardiac dysfunction. In addition, serum brain natriuretic peptide and cardiac ANF mRNA expression levels were normalized after paricalcitol treatment. One interesting observation made in this study was that the effect of paricalcitol in the DSS rat was independent of BP control.

Recently calcitriol was studied in spontaneously hypertensive heart failure rats that possess one or two copies of the corpulent gene (cp), a mutant form of the leptin receptor cp/+. Increased dietary salt intake induces left ventricular hypertrophy and fibrosis in these rats with severe hypertension. Calcitriol treatment in these rats fed a high-salt diet resulted in a reduction in heart weight, myocardial collagen levels, left ventricular diameter and cardiac output despite higher serum leptin levels (Mancuso *et al.*, 2008).

Does VDR play a role in diabetic animal models?

Some early studies show that VDR is present in pancreatic islets (Ishida and Norman, 1988) and calcitriol seems essential for normal insulin release (Norman *et al.*, 1980; Chertow *et al.*, 1983). Calcitriol increases insulin secretion and improves glucose tolerance in vitamin D-deficient animals (Nyomba *et al.*, 1984). However, in VDR KO mice, the results are not consistent. While one group reported impaired glucose tolerance in VDR KO mice (Zeitz *et al.*, 2003), others found no difference (Mathieu *et al.*, 2001). Also, different observations were made in SHR and Wistar rats injected with streptozotocin to induce type II diabetes; cholecalciferol

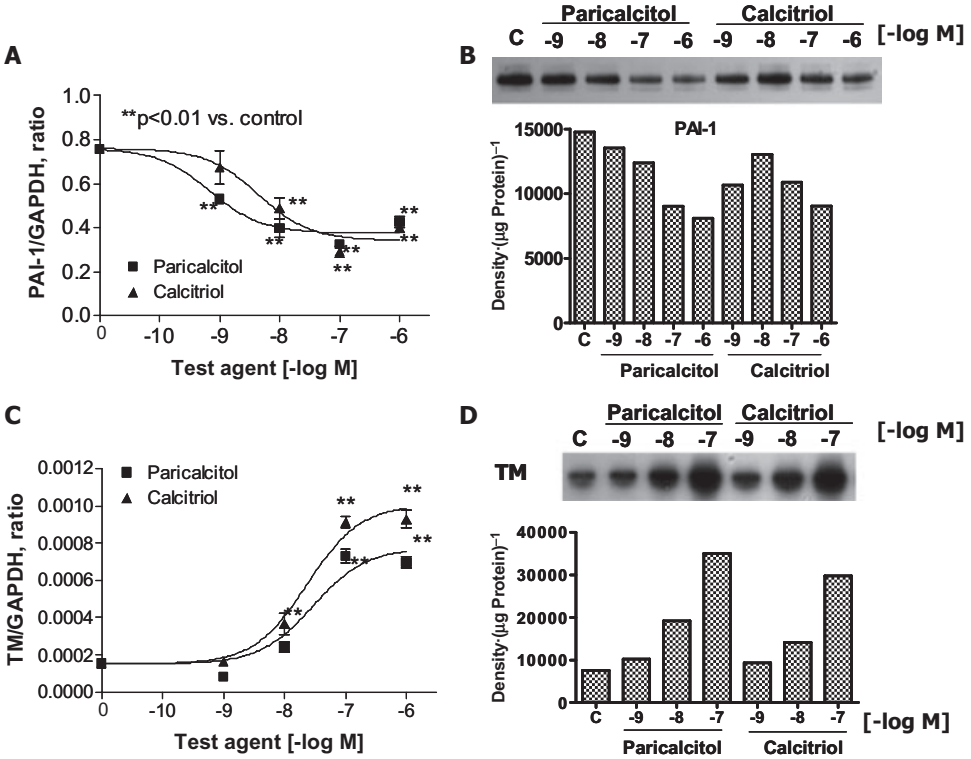

Figure 4 Effects of paricalcitol and calcitriol on the expression of thrombomodulin (TM) and plasminogen activator inhibitor-1 (PAI-1) in human aortic smooth muscle cells. (A) and (C): Primary culture of human aortic smooth muscle cells were treated with paricalcitol or calcitriol at indicated concentrations for 24 h. RNA were isolated and the mRNA level of the genes analysed by real-time RT-PCR. (B) and (D): For Western blotting, cells were treated with paricalcitol or calcitriol at indicated concentrations for 48 h, and samples were analysed with a mouse anti-plasminogen activator inhibitor-1 (PAI-1) monoclonal antibody (1000-fold dilution, Santa Cruz Biotechnology, Santa Cruz, CA) or a mouse anti-thrombomodulin (TM) monoclonal antibody (2000-fold dilution, Santa Cruz Biotechnology) as described previously (Wu-Wong *et al.*, 2007).

supplementation did not change the glucose concentration in the SHR animals, but reduced the blood glucose levels by 40% in Wistar rats (de Souza Santos and Vianna, 2005).

The non-obese diabetic (NOD) mouse, which spontaneously develops type I diabetes, is a widely used animal model for type I diabetes (Atkinson and Leiter, 1999). It has been shown that vitamin D deficiency accelerated the disease progression in NOD mice (Giulietti *et al.*, 2004). When calcitriol was administrated before the onset of insulitis, it effectively prevented the progression of diabetes in NOD mice, but treatment was ineffective when insulitis was well established (Mathieu *et al.*, 1994). Gregori *et al.* (2002) reported that a calcitriol analog was effective in inhibiting interleukin-12 (IL-12) production, blocking pancreatic infiltration of Th1 cells, enhancing CD4(+)CD25(+) regulatory cells and arresting the progression of type 1 diabetes in NOD mice. However, a recent paper showed that, in VDR(–/–) NOD mice, although immune abnormalities were aggravated, disruption of VDR did not alter disease presentation in NOD mice (Gysemans *et al.*, 2008).

Evidence supports a role of VDR in the immune system

Immune abnormalities play key roles in the development of type I diabetes, while inflammation is often associated with insulin resistance and β-cell failure in type II diabetes. Although more studies are needed to demonstrate the link between VDR and the immune/inflammatory mediators in diabetes, evidence exists that VDR is involved in a wide range of immune actions. VDR is present in T lymphocytes, macrophages and thymus tissue, and VDRAs have been shown to promote the differentiation of monocytes into macrophages, prevent dendritic cell maturation (Canning *et al.*, 2001), inhibit delayed-type hypersensitivity reactions, etc. (Mathieu and Adorini, 2002; Palomer *et al.*, 2008).

In the VDR KO mice the immune abnormalities such as impaired macrophage chemotaxis could be fully restored by feeding the animals with lactose-rich or polyunsaturated fat-rich diets to correct hypocalcemia, suggesting that immune defects observed in VDR KO mice are an indirect consequence of VDR disruption (Mathieu *et al.*, 2001). In a separate study, vitamin D-deficient IL-10 KO mice were found to develop accelerated inflammatory bowel disease (IBD). Removing calcium from the diet of these mice increased the severity of IBD. The mice fed either calcium or calcitriol developed an intermediate form of IBD, while the mice fed both calcium and calcitriol had the mildest form of IBD. In the colons, a tumour necrosis factor-α (TNF-α)-inducing transcription factor, lipopolysaccharide-induced TNF-α factor, was inhibited by calcitriol, but not by calcium. The inhibition of several TNF-α-related genes was associated with the decreased colitis in calcitriol-treated IL-10 KO mice (Zhu *et al.*, 2005). Consistent with these observations, VDR/IL-10 double-KO mice exhibited a fulminating form of IBD (Froicu *et al.*, 2003) and

expressed significantly more TNF-α and lipopolysaccharide-induced TNF-α factor than either single-KO strain.

Vitamin D receptor also plays a role in innate immune responses (Liu *et al.*, 2006). Activation of Toll-like receptors in human macrophages up-regulated the expression of VDR and CYP27B1, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *Mycobacterium tuberculosis*. The same study also reported that sera from African-American individuals, who often have increased susceptibility to tuberculosis, had lower serum 25(OH)D levels and consequently were less efficient in inducing cathelicidin messenger RNA expression.

How is VDR involved in atherosclerosis?

The discussion of VDR in immune response and inflammation naturally leads to the subject of atherosclerosis, the principal cause of coronary heart disease, stroke and peripheral vascular disease (Falk, 2006). Atherosclerosis is a process that involves a complex interplay among different factors such as inflammation, thrombosis and various cell types including smooth muscle and endothelial cells. As mentioned above, VDR seems involved in regulating thrombogenic activity, and VDR activation may reduce thrombosis and sustain plaque stability in atherosclerosis. Also, inflammation is linked to plaque vulnerability; VDR activation, through its immunomodulating effects, may inhibit macrophage activation and prevent plaque instability.

Beside inflammation and thrombosis, phenotypic change in smooth muscle cells is an important contributing factor in atherosclerosis. In a study employing DNA microarray technology to assess the gene expression profile in primary culture of human coronary artery smooth muscle cells treated with VDRAs, we found that VDRAs regulated the expression of many genes involved in cell differentiation and proliferation and also down-regulated the expression of natriuretic peptide precursor B, plasminogen activator inhibitor-1 and thrombospondin-1 (Wu-Wong *et al.*, 2006a; Wu-Wong *et al.*, 2007). Furthermore, when we examined the effects of VDRAs on ^{3}H-thymidine incorporation in human coronary artery smooth muscle cells, paricalcitol was as potent as calcitriol in inhibiting thymidine incorporation in a dose-dependent manner. These results suggest that VDR may be involved in the regulation of various events in the vasculature including smooth muscle cell proliferation/differentiation, thrombosis, fibrinolysis, vessel relaxation and endothelial regeneration (Figure 5).

However, inconsistent data do exist. Mitsuhashi *et al.* (1991) reported that, in cultured rat vascular smooth muscle cells (VSMC), calcitriol stimulated the growth of quiescent smooth muscle cells, but diminished the mitogenic response to thrombin. Two other studies showed that, in rat VSMC, calcitriol induced cell proliferation in a dose-dependent manner in quiescent cells and also in cells stimulated to grow (Rebsamen *et al.*, 2002; Cardus *et al.*, 2006). The G1 phase was shortened after calcitriol treatment, and there was an increase in the expression of vascular endothelial growth factor (VEGF). The inhibition of VEGF activity blunted calcitriol-induced VSMC proliferation (Cardus *et al.*, 2006). In the same

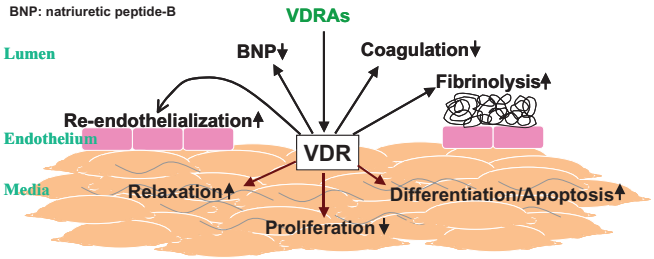

Figure 5 Effects of vitamin D receptor (VDR) activation in the blood vessel. VDR activation results in regulation of genes involved in the cell cycle that leads to inhibition of proliferation and induction of differentiation. BNP (natriuretic peptide B) is down-regulated. Regulation of genes such as thrombomodulin, plasminogen activator inhibitor-1 and thrombospondin-1 by VDRAs (VDR agonists or activators) results in reduced thrombogenicity and increased fibrinolysis. The regulation of type B endothelin receptor, oxytocin receptor and prostaglandin–endoperoxide synthase-1 suggest that VDRAs may also play roles in vessel relaxation and endothelial regeneration. Adapted from Wu-Wong *et al.* (2006a).

paper by Cardus *et al.* (2003), paricalcitol and EB1089, two calcitriol analogs, did not significantly induce cell proliferation in rat VSMC. The discrepancy between human and rat VSMC results may be due to the species difference and/or different culture conditions, which require further investigation.

Some early studies showed that a high concentration of vitamin D in the diet would induce atherosclerosis. Taura *et al.* (1979) reported that coronary atherosclerosis with intimal atheromata and calcified internal elastica occurred in normolipemic swine fed a basal ration supplemented with 31 250 IU, 62 500 IU and 125 000 IU of vitamin D_3 per kilogram of diet for 3 months and then only the basal ration for another 3 months. The incidence of atherosclerotic lesions was proportional to the vitamin D_3 doses. In another study (Kunitomo *et al.*, 1981) rats fed a diet containing 1.5% cholesterol and 1.8 million units of vitamin D_2 per kilogram of diet exhibited deposition of cholesterol and calcium in the aorta and coronary arteries.

The low-density lipoprotein receptor (LDLR) KO mice are known to develop atherosclerotic plaques and calcification when fed a high-fat diet (Towler *et al.*, 1998). Davies *et al.* (2003) showed that, when renal ablation was done in the LDLR KO mice to induce CKD, calcification associated with atherosclerotic plaques was more severe. Recently the same group reported that, in this LDLR KO mice with CKD, calcitriol or paricalcitol at clinically relevant doses reduced neointimal vascular calcium content (Mathew *et al.*, 2008). However, when the concentration of paricalcitol was raised to a high level, it caused more calcification. The authors suggested that VDRAs had a biphasic dosage-dependent effect on the development of atherosclerotic plaques and calcification (Mathew *et al.*, 2008).

Advanced glycation end products (AGEs) are formed from non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. AGEs are often elevated in diabetic and uremic patients and tend to accelerate the occurrence and development of various complications including vascular atherosclerosis. A recent paper (Talmor *et al.*, 2008b) showed that AGEs decreased endothelial nitric

oxide synthase mRNA expression and enzyme activity in human umbilical vein cord endothelial cells, which could be blocked by calcitriol. The effect of calcitriol is likely mediated by its ability to blunt the AGEs-induced elevation of NF-κB-p65 DNA binding activity. The same group also reported that, while lipopolysaccharide induced the expression of receptor of AGE and IL-6 in human umbilical vein cord endothelial cells, calcitriol inhibited the pro-inflammatory parameters mediated through the NF-κB and p38 pathways (Talmor *et al.*, 2008a).

Controversy about vitamin D and its analogs in vascular calcification: cell culture and animal studies

Although vascular calcification is now known to correlate with CVD mortality, especially in patients with CKD and diabetes, the involvement of vitamin D and its analogs in the calcification process is quite controversial. There are two types of vascular calcification, the intimal versus medial calcification (London *et al.*, 2005). Intimal wall calcification is associated with atherosclerosis and occurs at the site of plaque formation. Medial calcification is characterized by a more concentric calcium deposition in the VSMC layer. The consequences of these two types of calcification can be different. In advanced stages of the disease, intimal lesions compromise blood flow, leading to tissue ischaemia and necrosis. Even if the vessels are not obstructed, the atherosclerotic plaques can rupture, resulting in acute ischaemic events. Atherosclerotic plaque and calcification development may be concurrent, but whether calcification actually helps stabilizing the plaque or not remains a subject for debate. On the other hand, the medial layer calcification, also known as Mönckeberg's sclerosis, occurs usually at the internal elastic lamina of the media layer, and its consequence is increased vascular stiffness and reduced vascular compliance, as reflected in increased systolic BP and pulse wave velocity, which then lead to altered coronary perfusion and left ventricular hypertrophy (Blacher *et al.*, 2001).

Until very recently, vascular calcification was considered a passive physicochemical process. However, current knowledge demonstrates that vascular calcification is an active, regulated process that involves many different cellular mechanisms and various factors present in the blood circulation (Giachelli, 2004). Although the mechanisms involved in vascular calcification are still largely unknown, it is thought that disturbances in mineral homeostasis in CKD such as hypercalcemia, hyperphosphatemia and PTH abnormalities may contribute to vascular calcification, especially medial calcification. Because VDR is involved in the regulation of mineral homeostasis, it is perhaps reasonable to assume that vitamin D and its analogs may affect vascular calcification.

Two earlier papers reported that a high dose of vitamin D or vitamin D_3 plus nicotine in the diet induced aortic calcification in the rats (Niederhoffer *et al.*, 1997; Price *et al.*, 2003). Jono *et al.* (1998) demonstrated that calcitriol at 1–100 nmol·L^{-1} induced a dose-dependent increase in calcium accumulation in bovine VSMCs *in vitro*, which was accompanied by an increase in alkaline phosphatase activity, and a down-regulation of PTH-related peptide. However, using the same model of bovine VSMCs, Wolisi and Moe (2005) were unable to replicate the observations. In our own studies we found that, in primary culture of human coronary artery smooth muscle cells, increasing the phosphorus concentration induced a dose-dependent increase in the cellular calcium content in these cells. However, VDRAs such as calcitriol, paricalcitol, or $1\alpha,25(OH)_2D_2$ up to 100 nmol·L^{-1} had no effect on the cellular calcium content (Wu-Wong *et al.*, 2006b).

Chronic kidney disease, likely due to disturbances in mineral homeostasis, aggravates vascular calcification. As mentioned above, calcification associated with atherosclerotic plaques was more severe in the LDLR KO mice with CKD. In other CKD animal models such as the 5/6 nephrectomized (NX) rat, calcitriol induced aortic calcification (Henley *et al.*, 2005). Intriguingly, it seems that different VDRAs may exert differential effects on vascular calcification independent of the serum Ca (calcium), Pi (phosphorus) and CaxPi levels. For example, Hirata *et al.* (2002) showed that calcitriol induced vascular calcification in the NX uremic rats, while $1,25(OH)_2$-22-oxa-calcitriol (OCT), an analog of calcitriol, did not show any effect. They also showed that OCT at a high dose (6.25 μg·kg^{-1}) raised serum Ca, Pi and CaxPi levels to the same level as calcitriol at 0.125 μg·kg^{-1}, but only calcitriol induced aortic calcification.

We have also found that paricalcitol and doxercalciferol exhibited different effects on vascular calcification in the NX uremic rat model (Wu-Wong *et al.*, 2006b). In one study, the uremic rats with hyperphosphatemia were treated with 0.17 μg·kg^{-1} of doxercalciferol or paricalcitol at three times per week, i.p., for 6 weeks. Both drugs at that dose effectively suppressed serum PTH throughout the treatment period, and doxercalciferol was more hypercalcemic than paricalcitol. When the aortic Ca contents were examined, only doxercalciferol treatment resulted in a significant elevation in the aortic Ca content (Figure 6A). Interestingly, paricalcitol and doxercalciferol at a higher dose (0.67 μg·kg^{-1}) raised serum Ca, Pi and CaxPi to similar levels, but the Ca and Pi contents in aorta in the paricalcitol-treated group remained not much different from Sham and NX-vehicle. Follow-up studies showed that aortic compliance as determined by pulse wave velocity was significantly compromised in the doxercalciferol-treated group, but not in the paricalcitol group (Figure 6B) (Noonan *et al.*, 2008). These results suggest that different VDRAs exert differential effects on aortic calcification independent of serum Pi and Ca levels.

Our observations were reproduced by others. Cardus *et al.* (2007) showed that uremic rats treated with very high doses of calcitriol and paricalcitol had elevated serum Ca and Pi. However, severe aortic calcification was only observed in the calcitriol group, but not in the paricalcitol group. There was also a significant increase in pulse pressure in animals treated with calcitriol, likely caused by the extensive calcification. Using the same NX uremic rat model, Mizobuchi *et al.* (2007a) reported that calcitriol and doxercalciferol significantly increased the aortic Ca content, but paricalcitol had no effect. They also found that vascular calcification was independent of the serum CaxPi level. A recent paper by Lopez *et al.* (2008) showed that extraskeletal calcification was present in uremic

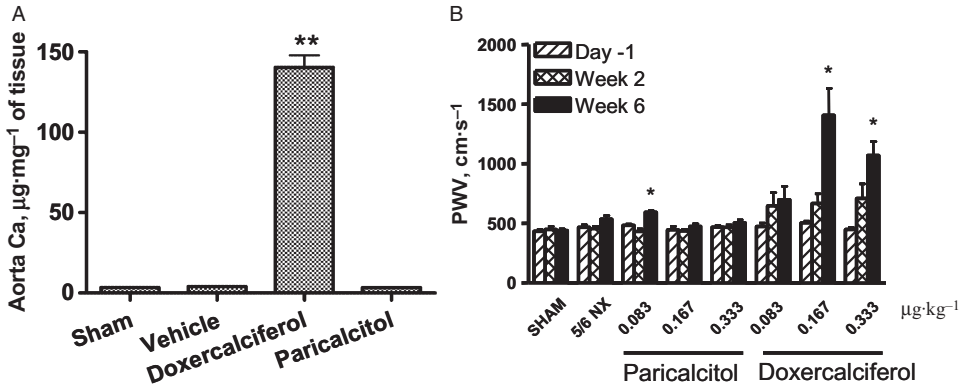

Figure 6 Differential effects of VDRAs (vitamin D receptor agonists or activators) on aortic calcification and pulse wave velocity (PWV). (A) Aortic calcium content in 5/6 nephrectomized uremic rats with hyperphosphatemia treated with vehicle, paricalcitol or doxercalciferol (0.17 μg·kg^{-1}, i.p. three times per week for 6 weeks). Adapted from Wu-Wong *et al.* (2006b). (B) Aortic PWV in 5/6 nephrectomized (NX) uremic rats with hyperphosphatemia treated with vehicle, paricalcitol or doxercalciferol (0.083, 0.167 and 0.333 μg·kg^{-1}, i.p. three times per week for 6 weeks). Data are from Noonan *et al.* (2008). * $p < 0.05$ vs own group, Day-1, ** $p < 0.01$ vs sham.

animals treated with calcitriol (80 ng kg^{-1}) and paricalcitol (240 ng kg^{-1}), but less calcification was observed in the paricalcitol group.

Preclinical studies so far seem to suggest that: (i) both calcitriol or paricalcitol at clinically relevant doses reduced neointimal vascular calcium content in the LDLR KO mice with CKD; (i) VDRAs have no direct effect on inducing Ca accumulation in human smooth muscle cells *in vitro*; and (iii) different analogs may differentially affect vascular calcification independent of serum PTH, Ca and Pi levels in the NX rats with hyperphosphatemia. What is the possible mechanism of action for the different observations? Some of the aforementioned studies have attempted to answer this question. For example, Mizobuchi *et al.* (2007a) showed that calcitriol or doxercalciferol treatment increased the mRNA and protein expression of the bone-related markers Runx2 and osteocalcin in the aorta, whereas paricalcitol did not. Because vascular calcification likely results from an imbalance between numerous inhibitory factors and inducing factors that are present in the cells and in the blood circulation, it is evident that more studies are needed in order to resolve the controversy linking vitamin D and VDRAs to vascular calcification.

CKD animal models: what else can VDRAs do beside suppressing PTH?

It is well recognized that CVD is common among CKD patients (Go *et al.*, 2004). Data from the Unites States Renal Data System show that the risk of CVD death in the young (25–34 years) dialysis patient group is 500 times higher than that in the age-matched general population. Even in the older age segment (45–55 years), it is still 60 times higher than the normal annual mortality (Baigent *et al.*, 2000). Because of these facts, CKD has been considered as an independent risk factor for CVD. As mentioned above, several VDRAs have been developed to treat hyperparathyroidism secondary to CKD. However, is VDR involved in CKD disease progression?

Zhang *et al.* (2008a) recently reported that, when the VDR KO mice were made diabetic by injection of streptozotocin, development of severe albuminuria and glomerulosclerosis was observed, likely due to increased glomerular basement membrane thickening and podocyte effacement. More fibronectin and less nephrin were expressed in the VDR KO mice versus diabetic WT mice. In VDR KO mice, increased renin, angiotensinogen, transforming growth factor-β and connective tissue growth factor accompanied the more severe renal injury. A follow-up study by the same group (Zhang *et al.*, 2008b) demonstrated that combination therapy with an AT1 receptor blocker and a VDRA markedly ameliorated renal injury in the streptozotocin-induced diabetes model. It is worth noting that the RAS is likely the main pathway involved in the development of diabetic nephropathy in this animal model, while the RAS pathway may be just one of the many factors contributing to disease progression in CKD in humans.

Calcitriol was shown to reduce urinary protein and IL-6 excretion, reduce glomerular diameters, decrease neutrophil and monocyte accumulation and attenuate glomerular cells proliferation in anti-Thy-1.1. nephritis rats, an experimental model of mesangial proliferative glomerulonephritis (Panichi *et al.*, 2001). In a mouse model of obstructive nephropathy characterized by predominant tubulointerstitial lesions, paricalcitol reduced infiltration of T cells and macrophages in the obstructed kidney, accompanied by a decreased expression of RANTES and TNF-α (Tan *et al.*, 2008). In the same model paricalcitol treatment resulted in a reduced interstitial volume, decreased collagen deposition and repressed mRNA expression of fibronectin and type I and type III collagens. Paricalcitol also suppressed renal transforming growth factor-β-1 and its type I receptor expression, restored VDR abundance and inhibited cell proliferation and apoptosis after obstructive injury, suggesting that paricalcitol attenuated renal interstitial fibrosis in obstructive nephropathy (Tan *et al.*, 2006).

In the NX uremic rat model, 1,25(OH)$_2$-22-oxa-calcitriol treatment significantly suppressed urinary albumin excretion, prevented increases in serum creatinine and serum urea nitrogen and inhibited glomerular cell number, glomerulosclerosis ratio and glomerular volume (Hirata *et al.*, 2002). Mizobuchi *et al.* (2007b) reported that there was improvement in creatinine clearance and the excretion of urinary protein in NX rats

treated with enalapril, paricalcitol or enalapril + paricalcitol. Interestingly, enalapril normalized BP, but paricalcitol had no effect. An earlier study by Schwarz *et al.* (1998) reported that calcitriol reduced glomerular volume, glomerulosclerosis index and albuminuria in the NX rats. They went on to demonstrate that the effect of calcitriol was independent of PTH by treating parathyroidectomized NX rats without or with calcitriol and observed similar antiproliferative actions of calcitriol. A recent paper by Freundlich *et al.* (2008) reported that treating the NX rats with paricalcitol for 8 weeks with the drug given immediately after renal ablation surgery prevented the increase in the mRNA and protein levels of factors such as angiotensinogen, renin, renin receptor, the angiotensin type 1 receptor and VEGF in the remnant kidney. In addition, glomerular and tubulointerstitial damage, hypertension, proteinuria and the deterioration of renal function resulting from renal ablation were significantly improved in animals receiving paricalcitol.

The 'non-genomic' effects of VDRAs

It is generally acknowledged that most of the effects of VDRAs are mediated by their binding to VDR, which then activates the receptor to recruit various cofactors to form a transcriptional complex, leading to modulation of target genes and a cascade of different signal transduction pathways. However, it may be of interest to note that there are reports showing the 'non-genomic' or 'rapid responsive' effects of calcitriol. An excellent review on this subject was published by Norman (2006). Although the function of the 'rapid response' of calcitriol remains largely unknown, there are at least two papers showing that calcitriol acutely modulates contractile function in myocytes isolated from adult rat hearts (Green *et al.*, 2006), and also regulate vascular tone by reducing calcium influx into the endothelial cells and decreasing the production of endothelium-derived contracting factors (Wong *et al.*, 2008).

What have we learned from epidemiological studies about vitamin D in humans?

Epidemiological studies have long observed that there is some correlation among altitude, season and cardiovascular disorder. For example, Mortimer *et al.* (1977) reported that there was a reduction in mortality associated with coronary heart disease in men residing at high altitude. Enquselassie *et al.* (1993) showed that both fatal and non-fatal coronary events were more likely to occur in winter and spring than at other times of the year, while Zittermann *et al.* (2005) pointed out that there was a relationship between death rates from ischaemic heart disease and geographical latitude in men and women from different European countries. Different hypotheses such as variations in temperature or respiratory disease prevalence have been proposed to explain the effects of season and latitude on cardiovascular disorder. However, season and latitude affects the intensity of solar UVB light, which is required for the cutaneous synthesis of vitamin D_3. Rostand showed that ultraviolet light exposure affected geographical and racial BP (Rostand, 1997), while Krause *et al.* (1998) demonstrated directly that serial whole-body irradiation with an artificial UVB source, but not with a UVA source, could reduce BP in patients with untreated mild hypertension. It is therefore possible that the effect of season and latitude on CVD might be related to solar UVB exposure and the vitamin D level (Scragg, 1981; Webb *et al.*, 1988). Zittermann *et al.* (2005) has promptly proposed that an insufficient vitamin D status might contribute to the worldwide high prevalence of CVD.

Regarding diabetes, an important risk factor for CVD, Pittas *et al.* (2007) conducted a meta-analysis of existing observational studies and clinical trials on this subject and reported that there was a relatively consistent association between low 25(OH)D levels and prevalence of type 2 diabetes mellitus or metabolic syndrome. As an example, one of the studies cited in the meta-analysis was from Chiu *et al.* (2004) that, in 126 healthy, glucose-tolerant subjects living in California, the 25(OH)D concentration was positively correlated with insulin sensitivity index and negatively correlated with first- and second-phase insulin responses. An independent negative relation of 25(OH)D concentration with plasma glucose concentration was also observed during the oral glucose tolerance test (Chiu *et al.*, 2004).

Evidence exists to show the direct link between vitamin D deficiency and increased CVD risk. A study analysing data from the Third National Health and Nutrition Examination Survey (Martins *et al.*, 2007) found that the adjusted prevalence of hypertension, diabetes mellitus, obesity and high serum triglyceride levels was significantly higher in the group of individuals with the lowest level of serum 25(OH)D (<21 ng·mL^{-1}) versus those with the highest level of serum 25(OH)D (≥ 37 ng·mL^{-1}). In a few prospective cohort studies the relative risk of incident hypertension was significantly higher when the plasma 25(OH)D levels were <15 ng·mL^{-1} compared with those whose levels were ≥ 30 ng·mL^{-1} (Forman *et al.*, 2007). The RAS is now well recognized as a key player in regulating BP and fluid balance. Two earlier studies showed that plasma renin activity was significantly higher in individuals with a lower level of calcitriol (Resnick *et al.*, 1986; Burgess *et al.*, 1990) and vice versa. Because free intracellular calcium concentration can inhibit renin secretion by the kidney, it was postulated that a higher calcitriol concentration might result in an increase in intracellular calcium concentration, leading to a decrease in renin secretion. However, recent findings from VDR KO mice and As4.1 cell culture studies as discussed above suggest that calcitriol directly suppresses renin expression.

Several recent papers reported consistent findings. In a prospective cohort study of 3258 patients scheduled for coronary angiography, patients with their 25(OH)D and calcitriol levels separated into quartiles were followed for 7.7 years to look at all-cause and cardiovascular deaths (Dobnig *et al.*, 2008). Multivariate-adjusted hazard ratios for all-cause mortality and cardiovascular mortality were higher for patients in the lower two 25(OH)D quartiles (median, 7.6 and 13.3 ng·mL^{-1}) compared with patients in the highest 25(OH)D quartile (median, 28.4 ng·mL^{-1}). Similar results were obtained for patients in the lowest calcitriol quartile. Low 25(OH)D levels were also correlated with markers of inflammation, oxidative burden and cell adhesion (Dobnig *et al.*, 2008). The Health Professionals

Follow-up Study reported that, during 10 years of follow-up of 18 225 men free of diagnosed CVD at blood collection, men with 25(OH)D at ≤15 ng·mL^{-1} were at increased risk for myocardial infarction versus those with 25(OH)D at ≥30 ng·mL^{-1}. Men with intermediate 25(OH)D levels at 22.6–29.9 ng·mL^{-1} were also at elevated risk versus those with 25(OH)D at ≥30 ng·mL^{-1} (Giovannucci *et al.*, 2008). Wang *et al.* (2008b) showed that, in a study of 1739 Framingham Offspring Study participants without prior CVD with a mean follow-up of 5.4 years, the risk of a cardiovascular event such as a heart attack, heart failure or stroke was twofold higher in the individuals with 25(OH)D at ≤15 ng·mL^{-1} versus those with higher levels of 25(OH)D. A similar observation was made by Melamed *et al.* (2008) that the lowest quartile of 25(OH)D level (<17.8 ng·mL^{-1}) is independently associated with increased all-cause mortality in the general population.

The results from epidemiological studies suggest that there is a strong correlation between low 25(OH)D levels and increased CVD risk.

Does vitamin D supplementation reduce CVD risk in humans?

The results from interventional studies testing native vitamin D perhaps are not as consistent as those from epidemiological studies. Scragg *et al.* (1995) gave individuals from general practitioner age-sex registers in Cambridge (UK) a single oral dose of 2.5 mg cholecalciferol and followed them up for 5 weeks. Neither BP nor serum cholesterol concentrations were altered. However, in the study the serum 25(OH)D levels only increased by 7.2–18 nmol·L^{-1} (equivalent to ~7.2 ng·mL^{-1}). As a comparison, when 148 elderly women were supplemented with 1200 mg calcium plus 800 IU (20 μg) vitamin D_3 or 1200 mg calcium alone daily for 8 weeks, the group with vitamin D_3 + calcium resulted in a 72% increase in serum 25(OH)D (from 10 to 17 ng·mL^{-1}), a 17% decrease in serum PTH, a 9.3% decrease in systolic BP and a 5.4% decrease in heart rate (Pfeifer *et al.*, 2001). In another study, Forman *et al.* (2005) examined the association between intake of vitamin D and the risk of incident hypertension among participants of three large and independent prospective cohorts with each cohort followed for ≥8 years and found that higher intake of vitamin D was not associated with a lower risk of incident hypertension. Yet a recent paper by Wang *et al.* (2008a) found that, in a prospective cohort of 28 886 US women aged ≥45 years, the risk of hypertension decreased in the higher quintiles of dietary calcium and dietary vitamin D, but did not change with calcium or vitamin D supplements.

Regarding diabetes, in a systematic review and meta-analysis study, Zipitis and Akobeng (2008) reported that the risk of type 1 diabetes was significantly reduced in infants supplemented with vitamin D; there was some evidence of a dose–response effect. In the meta-analysis by Pittas *et al.* (2007), evidence from trials with vitamin D and/or calcium suggested that combined vitamin D and calcium supplementation might help in the prevention of type 2 diabetes in populations at high risk. A recent paper by Sugden *et al.* (2008) demonstrated that, in a double-blind, parallel group, placebo-controlled randomized trial, a single large dose of vitamin D_2 (100 000 IU) raised serum 25(OH)D levels and improved endothelial function as measured by flow-mediated vasodilatation of the brachial artery in patients with type 2 diabetes mellitus. However, it has been reported that, in three cases of British Asians with vitamin D deficiency and non-insulin-dependent diabetes mellitus, vitamin D supplementation actually resulted in an increase in insulin resistance and a deterioration of glycaemic control (Taylor and Wise, 1998). Pittas *et al.* (2007) also added a cautious note in their paper stating that most of the intervention studies were short in duration with few subjects and also used different formulations of vitamin D and calcium. Clearly more studies are required in order to understand the effect of vitamin D supplementation on diabetes.

C-reactive protein (CRP) is an inflammatory marker that is associated with coronary heart disease, inflammation and the metabolic syndrome (Abraham *et al.*, 2007). There were at least two studies showing that vitamin D supplementation reduced serum CRP levels. Timms *et al.* (2002) compared three monthly injections of cholecalciferol at high (50 000 IU, equivalent to a 14 μg daily dose) or low (500 IU, equivalent to a 0.14 μg daily dose) dosage on serum CRP levels over 1 year in 171 healthy British Bangladeshi adults. Initial 25(OH)D levels were 21.8 nmol·L^{-1} and 20.7 nmol·L^{-1} in the high- and low-dose groups respectively. Mean CRP levels decreased by 39.7% and 4.8% in the high- and low-dose groups, but the mean increase in serum 25(OH)D levels was very similar in both groups (16.7 vs. 12.3 nmol·L^{-1}). In a study by Van den Berghe *et al.* (2003), patients with prolonged critical illness were given daily vitamin D supplement of either 200 IU (low dose) or 500 IU (high dose) during the first 10 days after intensive care unit admission. High dose of vitamin D slightly increased the serum 25(OH)D level, but not the calcitriol level. Serum CRP, 40-fold higher versus matched controls at baseline, decreased significantly with time in the intensive care unit in both high- and low-vitamin D groups, but the decrease in CRP was more profound in the high-dose vitamin D group.

Autier and Gandini (2007) conducted a meta-analysis of 18 randomized controlled trials to investigate whether vitamin D supplementation was associated with reduced mortality. As pointed out in the editorial commentary for the paper (Giovannucci, 2007), some interesting findings were made in this analysis. First, vitamin D supplementation at doses ranging from 300 to 2000 IU·day^{-1} seems quite safe. Second, although a majority of the trials included in the analysis was not studying mortality as their primary end points, the meta-analysis found a 7% reduction in mortality from any cause in individuals randomized to vitamin D. Third, there was a 1.4–5.2-fold difference in serum 25(OH)D levels between the intervention and control groups, suggesting that vitamin D supplementation raised 25(OH)D levels.

Interventional studies in humans: effects of native vitamin D versus VDRAs

Vitamin D is essential for mineral metabolism, and low levels are associated with impaired skeletal metabolism (Lips *et al.*,

2006). Emerging evidence suggests that there is a link between bone disorders and CVD, likely mediated through mineral abnormalities and soft tissue calcification (Raggi and Kleerekoper, 2008). Although controlled interventional trials with vitamin D supplement (and calcium) yielded no consistent results in terms of the prevention of extravertebral fractures (Jackson *et al.*, 2007), treatment with VDRAs such as alfacalcidol seems to exert better effects (Scharla, 2006). This observation is further supported by a comparative meta-analysis (Richy *et al.*, 2008), in which 14 trials were included with 21 268 patients randomized to native vitamin D, VDRAs or placebo. When focusing on studies featuring the highest methodological quality, a statistically significant lower level of risk for falls was observed in the VDRA group versus the native vitamin D group (a 3.5-fold difference). A similar observation was made by MacLean *et al.* (2008) looking at bone fractures

Regarding bone disorders and vascular calcification, as bone is the main reservoir of calcium and phosphate in the body, it is conceivable that abnormal bone remodelling with uncoupled bone-forming and resorptive activities will result in an excess of calcium and phosphate ions leaking into circulation, which then predisposes to soft tissue calcification and accelerates adverse cardiovascular events. Bone disorders are very common in CKD. The high turnover bone disease in CKD triggered by high PTH is likely one critical factor contributing to excessive vascular calcification and increased risk of adverse cardiovascular events in CKD. On the other hand, adynamic bone disease, an event associated with low PTH and markedly impaired bone-forming activity, also results in increased soft tissue calcification (London *et al.*, 2004; Hruska *et al.*, 2007; London *et al.*, 2007). Just like in the cell culture and animal studies, there is considerable controversy regarding VDRAs and soft tissue calcification in CKD patients. Calcitriol and its analogs, commonly used to treat secondary hyperparathyroidism (SHPT) in CKD, are thought to induce vascular calcification because they may over-suppress PTH, leading to adynamic bone disease, and also because they may cause hypercalcemia and hyperphosphatemia, two important risk factors for vascular calcification. However, Watson *et al.* (1997) found that there was an inverse correlation between the serum calcitriol level and coronary artery calcification in two patient populations (173 subjects) at high and moderate risk for coronary heart disease. Another study (Doherty *et al.*, 1997) examining 283 asymptomatic subjects with risk factors for CKD reported that serum calcitriol independently and inversely predicted coronary calcification quantity. A review by Wolisi and Moe (2005) indicated that therapy using calcitriol and its analogs has not been demonstrated to be associated with vascular calcification in CKD. London *et al.* (2007) found that, in stage 5 CKD (or end-stage CKD), the serum 25(OH)D and calcitriol levels were negatively correlated with aortic pulse wave velocity and positively correlated with brachial artery distensibility and flow-mediated dilation. Therefore, a disruption in the delicate balance among Ca, P, PTH, bone and the calcitriol/VDR system can lead to increased vascular calcification. In the normal population, vitamin D may adequately control for bone-forming activities thereby reducing soft tissue calcifications (Holick, 2004). In a disease state such as CKD where decreased calcitriol/VDR activation reduces bone-forming properties, calcium deposition in soft issues may be aggravated (Goltzman, 2007).

As mentioned above, CKD patients experience many different abnormalities including abnormal levels of calcium, phosphorus, PTH or vitamin D metabolism. Levin *et al.* (2007) reported from an outpatient cohort cross-sectional study conducted in 153 centres that, when kidney function declines in patients with CKD, a decrease in the serum calcitriol level was the first to occur, before other changes in serum calcium, phosphate, PTH or 25(OH)D can be observed. Significant differences in the mean and median values of calcitriol and PTH were seen across deciles of estimated glomerular filtration rate (eGFR), but the rise in PTH was only observed following a decrease in calcitriol. These observations seem consistent with the idea that treating calcitriol deficiency may be more important than correcting 25(OH)D deficiency.

Perhaps it is necessary to briefly recap the controversy regarding vitamin D or 25(OH)D versus VDRAs as therapeutic agents, especially for CKD patients. Epidemiological studies as mentioned above provide strong evidence that low 25(OH)D levels are associated with increased risk for various diseases. However, vitamin D supplementation does not always provide consistent therapeutic benefits. The potency of 25(OH)D for VDR is ~1000-fold less than calcitriol, but the 25(OH)D level in blood circulation is also ~1000-fold higher than calcitriol. Emerging evidence shows that many extrarenal cells/tissues such as vascular cells and cardiomyocytes express CYP27B1 and are capable of converting 25(OH)D to calcitriol (Segersten *et al.*, 2002). Although it is thought that calcitriol suppresses PTH via its endocrine effect, Ritter *et al.* (2006) demonstrated that 25(OH)D could be converted to calcitriol by bovine parathyroid cells, leading to PTH suppression. The issue is, of course, whether 25(OH)D or native vitamin D is as effective as calcitriol and other VDRAs in treating diseases in CKD patients. At early stages of CKD vitamin D (ergocalciferol or cholecalciferol) is able to reduce PTH (Al-Badr and Martin, 2008), but native vitamin D therapy seems inadequate for late stages of CKD. One earlier study (Dusso *et al.*, 1988) examined the efficacy of 25(OH)D on reducing PTH in haemodialysis patients and found that 200 $\mu g \cdot day^{-1}$ of 25(OH)D given orally for 2 weeks increased serum 25(OH)D and $1,25(OH)_2D$ levels, but did not cause any significant change in PTH. The current thinking in the nephrology community is that, in general, ergocalciferol or cholecalciferol can increase 25(OH)D and/or $1,25(OH)_2D$ levels and suppress PTH in CKD 3 patients, but has no significant effects in late stages (stage 4/5) of CKD (Al-Aly *et al.*, 2007; Zisman *et al.*, 2007). The question remains unanswered is that whether ergocalciferol or cholecalciferol can reduce CVD risk and improve survival because the rate of CVD events in stage 3 CKD is still >11-fold higher than the general population (Go *et al.*, 2004). Thus, although reduced 25(OH)D levels are associated with increased risk for CVD, diabetes, metabolic syndrome and other disorders, the efficacy of vitamin D or 25(OH)D as therapeutic agents may be dependent on disease state, which requires further exploration.

Currently studies investigating the effect of VDRAs on diabetes or CVD in CKD patients are rather limited. A paper by Mak (1998) found that, in diabetic patients on maintenance haemodialysis, serum glucose concentrations during oral

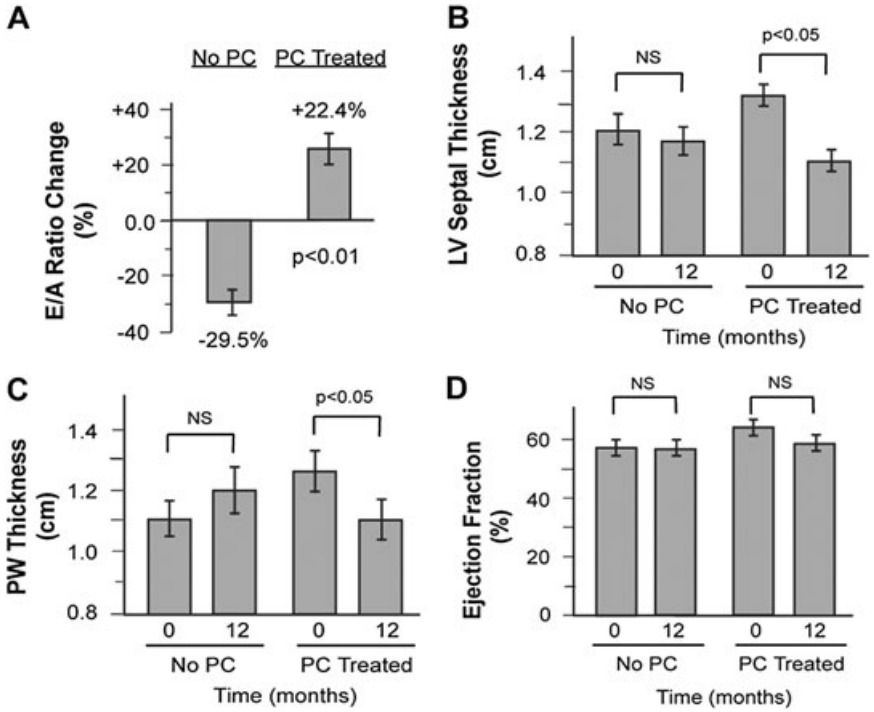

Figure 7 Echocardiogram parameters in haemodialysis patients. Changes in E/A ratio (A), left ventricular (LV) septal thickness (B), posterior wall (PW) thickness (C) and ejection fraction (D) in patients with (n = 15) and without (n = 6) paricalcitol (PC) treatment (average dose: 13 ± 7 μg·week^{-1}; duration of treatment: 4.3 ± 1.2 months). Data are from Bodyak *et al.* (2007).

glucose tolerance test were normalized following 4 weeks of intravenous calcitriol therapy. A small-scale clinical study testing the effect of calcitriol on myocardial hypertrophy in haemodialysis patients (Park *et al.*, 1999) found that 15 weeks of treatment with calcitriol showed pronounced reductions in interventricular wall thickness, left ventricular posterior wall thickness and left ventricle mass index. In addition, plasma renin activity, and plasma ANGII and ANP concentrations were significantly reduced. A follow-up report by the same group (Kim *et al.*, 2006) showed that calcitriol treatment in haemodialysis CKD patients resulted in regression of myocardial hypertrophy and a reduction in the QTc interval and dispersion without biochemical and haemodynamic changes. It has also been shown that VDRA therapy significantly reduced the rates of hospitalization (many associated with cardiovascular events), with paricalcitol providing more benefit than calcitriol (14% fewer hospitalization per year and 9.17 fewer hospital days per year) (Dobrez *et al.*, 2004). Bodyak *et al.* (2007) reported that paricalcitol increased E/A ratios, improved diastolic function and was associated with a 15% and 11% reduction in septal and posterior wall thickness in 15 dialysis patients (Figure 7).

A few studies investigated whether VDRA therapy would slow CKD disease progression. Agarwal *et al.* (2005) showed that, in three double-blind, randomized, placebo-controlled studies to evaluate the safety and efficacy of oral paricalcitol in 220 stages 3 and 4 CKD patients with SHPT, 51% of the paricalcitol patients (vs. 25% placebo patients) had reduction in proteinuria. Proteinuria is a marker of cardiovascular and renal disease in patients with CKD, and reduction in proteinuria has been associated with improved cardiovascular and renal outcomes. A recent paper by the same group (Alborzi *et al.*, 2008) reported that, in CKD stage 2/3 patients, paricalcitol reduced albuminuria and CRP independent of PTH or BP. In an open-label prospective uncontrolled study (Szeto *et al.*, 2008), patients with immunoglobulin A (IgA) nephropathy were treated with calcitriol for 12 weeks on top of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy. Calcitriol therapy resulted in a significant decrease in proteinuria with time and also a progressive decrease in urine protein/creatinine ratio. Oral calcitriol, when evaluated in human renal transplant recipients, reduced the rate of loss of graft function and prolonged graft survival (O'Herrin *et al.*, 2002). In a pilot trial 24 CKD patients were randomly allocated to 0, 1 or 2 μg of oral paricalcitol for 1 month. The treatment/baseline ratio of 24 h albumin excretion rate was significantly reduced in both doses of paricalcitol versus placebo (Alborzi *et al.*, 2008). This study also examined CRP and found that paricalcitol treatment reduced CRP with a dose-dependent effect.

VDRA therapy associated with survival benefit in CKD patients

During the past few years numerous studies have found that VDRA therapy is associated with survival benefits for CKD patients. Table 1 is a summary of human studies examining mortality and VDRA therapy in CKD patients. The common theme from these studies is that VDRAs, although mainly prescribed to treat SHPT, were associated with a significant

Table 1 Observational studies examining outcomes associated with VDR agonist therapy in CKD patients

Study	*Patients*	*Therapy*	*Results*
Teng *et al.* (2003)	67 399 prevalent haemodialysis patients in the USA	Injectable paricalcitol vs. calcitriol	16% lower all-cause mortality in the paricalcitol group; improved survival among patients switching from calcitriol to paricalcitol
Shoji *et al.* (2004)	242 prevalent haemodialysis patients in Japan	Daily dose of alfacalcidol vs. non-users	Reduced mortality from cardiovascular disease in the users; no difference in mortality from non-cardiovascular disease between the two groups
Teng *et al.* (2005)	51 037 prevalent haemodialysis patients in the USA	Any injectable VDRA vs. no treatment	20% lower all-cause mortality in the VDRA group
Melamed *et al.* (2006)	1007 incident haemodialysis and peritoneal dialysis patients in the USA	Injectable calcitriol vs. no treatment	26% lower all-cause mortality in the calcitriol group vs. no treatment
Kalantar-Zadeh *et al.* (2006); Lee *et al.* (2007)	58 058 prevalent haemodialysis patients in the USA	Injectable paricalcitol vs. no treatment	Improved survival associated with any dose of paricalcitol use in time-dependent models
Tentori *et al.* (2006)	7731 prevalent haemodialysis patients in the USA	Injectable paricalcitol, doxercalciferol or calcitriol vs. no treatment	In all models mortality was higher for patients with no VDRA treatment; mortality was similar for paricalcitol vs. doxercalciferol; in adjusted models, mortality was not statistically different among three VDRAs
Wolf *et al.* (2007)	825 incident US haemodialysis patients	Any injectable VDRA vs. no treatment	Low vitamin D levels associated with increased mortality; untreated vitamin D-deficient patients at significantly increased risk for early mortality
Wolf *et al.* (2008)	9303 incident US haemodialysis patients (5110 non-Hispanic White, 979 Hispanic White, 3214 Black)	Any injectable VDRA vs. no treatment	Treated Black patients had 16% lower mortality vs. White patients; untreated Black patients had 35% higher mortality vs. White
Shinaberger *et al.* (2008)	34 307 maintenance haemodialysis patients in the USA	Injectable paricalcitol	Higher weekly paricalcitol dosage (normalized by per unit of serum PTH) associated with greater survival
Naves-Diaz *et al.* (2008)	Haemodialysis patients (7703 treated vs. 8801 untreated) from six Latin America countries	Oral VDRA vs. no treatment	Survival advantage observed in the group that had received oral VDRA in 36 of the 37 strata studied including that with the highest levels of serum calcium, phosphorus and PTH
Shoben *et al.* (2008)	1418 non-dialysis stages 3–4 CKD patients with secondary hyperparathyroidism	Oral calcitriol vs. non-users	Oral calcitriol therapy associated with a 26% lower mortality risk and a 20% lower risk for death or dialysis vs. non-users
Kovesdy *et al.* (2008)	520 male US veterans with stages 3–5 CKD not on dialysis	Oral calcitriol vs. no treatment	Incidence rate ratios for mortality and combined death and dialysis initiation significantly lower in treated patients
Levin *et al.* (2008)	4231 stage 4 CKD patients	Oral VDRA vs. no treatment	VDRA use associated with improved survival vs. no treatment

CKD, chronic kidney disease; PTH, parathyroid hormone; VDR, vitamin D receptor; VDRA, VDR agonist or activator.

survival benefit for CKD patients, either on dialysis or predialysis. Also, the survival benefit for CKD patients seems to be independent of mineral metabolism and PTH and may be different for different VDRAs with paricalcitol associated with better survival than calcitriol (Teng *et al.*, 2003).

One interesting point worth mentioning is that the report by Wolf *et al.* (2008) may provide a partial answer to a conundrum in the CKD field. It is known that the mortality rates for Black are higher than those for White in the general population (Murray *et al.*, 2006), but Black on dialysis seem to have a survival advantage compared with White (Robinson *et al.*, 2006). Wolf *et al.* reported that, in a prospective cohort of non-Hispanic White, Hispanic White and Black incident haemodialysis patients, Black patients had 16% lower mortality compared with White patients, but the difference was lost when adjusted for the dosage of VDRAs. In contrast, Black patients not treated with any VDRA had 35% higher mortality compared with untreated White patients. They proposed an explanation that the survival advantage for Black in dialysis may be due to VDRA therapy because PTH was usually higher among Black patients, and consequently they were most likely to receive a higher dose of VDRA.

This, of course, brings up many questions and debates. For example, what is the mechanism of action for the survival benefit of VDRAs in CKD? Which VDRAs shall be used? For any VDRA, what dosage is needed for its survival benefit in CKD? One of the limitations of the studies listed in Table 1 is that all of them are observational studies, although a recent paper by Vervloet and Twisk (2008) took a closer look of several of these studies from a statistician's point of view and concluded that results from these observational studies appear robust and consistent. Only positive results from a randomized trial will give a definite answer whether VDRAs truly provide a survival benefit to CKD patients. Before results from such randomized trials become available, shall physicians, especially nephrologists, ignore the existing data and not to give VDRAs to CKD patients with normal PTH levels? Or shall physicians prescribe these drugs to improve the

outcomes of the CKD patients? Unfortunately no clear answers to these questions can be obtained at present.

Discussion/conclusion

The preclinical and clinical data suggest that VDR is involved in regulating cardiovascular functions and vitamin D and its analogs are potentially useful for treating CVD. Many of the clinical studies considered a level of 25(OH)D at $\leq$15 ng·mL^{-1} deficient, while a level between 15 and 30 ng·mL^{-1} is borderline and a level more than 30 ng·mL^{-1} is perhaps necessary in order to reduce CVD risk. A word of caution is that, although most studies seem to suggest that 30–40 ng·mL^{-1} is an acceptable target for adequate vitamin D function, the optimal level of 25(OH)D is not known. Assuming a correlation between low 25(OH)D levels and increased CVD risk is true, then in theory vitamin D supplementation shall raise 25(OH)D levels and reduce CVD risks. However, discrepancy exists regarding the beneficial effects of vitamin D supplementation on hypertension, diabetes, inflammation markers and mortality. Even on the very basic question of whether vitamin D supplementation raises serum 25(OH)D levels, there is inconsistency from study to study. One concern is whether the serum 25(OH)D level is a good indicator for VDR function. After all, 25(OH)D is at least 1000-fold less potent than its active metabolite, calcitriol, in activating VDR. Perhaps one may ask: why not measuring the calcitriol level directly? A simplified answer is that the calcitriol level in blood circulation is extremely low and difficult to determine accurately.

Calcitriol and its analogs have been tested extensively in numerous preclinical animal models and used clinically for >20 years to treat various human diseases such as hyperparathyroidism secondary to CKD, osteoporosis and psoriasis. Results from both preclinical and clinical studies seem to suggest that different VDRAs have differential effects and, depending on the doses used in the studies, very different outcomes may be obtained. It seems that an adequate supply of calcitriol, the endogenous hormone, is required for proper VDR activation and maintenance of cardiovascular health. When calcitriol is deficient such as during the development of CKD, VDR activation and normal cardiovascular function are compromised. While it is necessary to correct calcitriol deficiency, it can be 'overdone' because the therapeutic window for calcitriol is quite narrow, and the correction of VDR activation deficiency may be better accomplished by analogs of calcitriol that have a wider safety margin. In addition, as exemplified in the studies examining the effects of VDRAs on renin, Runx2 and osteocalcin gene expression, these analogs at clinically relevant doses may regulate different genes differentially. Thus, at equipotent PTH suppressing doses, some analogs may have better effects in modulating certain gene expression than others, leading to better outcomes. This explanation of course is overly simplified and will certainly require more studies to confirm.

For the sake of stimulating further discussions, an even more provoking idea is brought forward here. Can it be that a deficiency in VDR activation, not just vitamin D deficiency, is the culprit for the cardiovascular problem experienced by the general population? In other words, there may be a pathological condition called 'VDR hormone deficiency disease' that needs to be treated. If there is indeed a VDR hormone deficiency disease, how shall it be treated? If it can be treated with VDRAs, how will one know whether the deficiency has been corrected after therapy? In CKD patients with SHPT, PTH is a useful marker for VDRA therapy. However, if the PTH level is normal, how to gauge whether VDR has been properly activated after VDRA therapy? It is perhaps evident from the papers cited in this review that a majority of the human studies measured 25(OH)D, but not vitamin D or calcitriol. One reason is of course the technical difficulty involved in accurately determining the level of vitamin D or calcitriol. Another reason may be due to the fact that there seems a lack of correlation between the calcitriol and 25(OH)D levels, and also between vitamin D intake and the calcitriol level. Even if the calcitriol level can be determined accurately, what is the optimal concentration of calcitriol for cardiovascular health in a disease state without elevated PTH? How about the aging process? Emerging evidence suggests that calcitriol is not only an endocrine hormone, but also a paracrine/autocrine hormone because extra-renal tissues also express CYP27B1 and are capable of converting 25(OH)D into a high concentration of calcitriol at the local sites. Studies have shown that the cutaneous synthesis of vitamin D_3 declines when one ages. Is it possible that the activity of CYP27B1 in both renal and extra-renal tissue also goes down when one ages? Can it be possible that aging as one of the risk factors for CVD is partially due to VDR hormone deficiency?

Just like the numerous questions arising from the observation that VDRA therapy is associated with survival benefit in CKD patients, no answers to these questions can be found at present. However, as the field evolves and we come to understand more about the role of VDR in various biological and pathophysiological functions in the human body, perhaps very soon we will be able to answer some of these questions and even treat this 'VDR hormone deficiency disease' if such a disease dose exist.

Conflict of interest

J Ruth Wu-Wong is an employee of Abbott that sells Calcijex and Zemplar.

References

Abraham J, Campbell CY, Cheema A, Gluckman TJ, Blumenthal RS, Danyi P (2007). C-reactive protein in cardiovascular risk assessment: a review of the evidence. *J Cardiometab Syndr* **2**: 119–123.

Agarwal R, Acharya M, Tian J, Hippensteel RL, Melnick JZ, Qiu P *et al*. (2005). Antiproteinuric effect of oral paricalcitol in chronic kidney disease. *Kidney Int* **68**: 2823–2828.

Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T *et al*. (2004). Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. *J Biol Chem* **279**: 35798–35802.

Al-Aly Z, Qazi RA, Gonzalez EA, Zeringue A, Martin KJ (2007). Changes in serum 25-hydroxyvitamin D and plasma intact PTH levels following treatment with ergocalciferol in patients with CKD. *Am J Kidney Dis* **50**: 59–68.

Al-Badr W, Martin KJ (2008). Vitamin D and kidney disease. *Clin J Am Soc Nephrol* **3**: 1555–1560.

Alborzi P, Patel NA, Peterson C, Bills JE, Bekele DM, Bunaye Z *et al.* (2008). Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. *Hypertension* **52**: 249–255.

Andress DL (2006). Vitamin D in chronic kidney disease: a systemic role for selective vitamin D receptor activation. *Kidney Int* **69**: 33–43.

Atkinson MA, Leiter EH (1999). The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med* **5**: 601–604.

Autier P, Gandini S (2007). Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* **167**: 1730–1737.

Baigent C, Burbury K, Wheeler D (2000). Premature cardiovascular disease in chronic renal failure. *Lancet* **356**: 147–152.

Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM (2001). Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* **38**: 938–942.

Bodyak N, Ayus JC, Achinger S, Shivalingappa V, Ke Q, Chen YS *et al.* (2007). Activated vitamin D attenuates left ventricular abnormalities induced by dietary sodium in Dahl salt-sensitive animals. *Proc Natl Acad Sci USA* **104**: 16810–16815.

Borges AC, Feres T, Vianna LM, Paiva TB (1999). Effect of cholecalciferol treatment on the relaxant responses of spontaneously hypertensive rat arteries to acetylcholine. *Hypertension* **34**: 897–901.

Borges AC, Feres T, Vianna LM, Paiva TB (2002). Cholecalciferol treatment restores the relaxant responses of spontaneously hypertensive rat arteries to bradykinin. *Pathophysiology* **8**: 263–268.

Brown AJ, Slatopolsky E (2007). Drug insight: vitamin D analogs in the treatment of secondary hyperparathyroidism in patients with chronic kidney disease. *Nat Clin Pract Endocrinol Metab* **3**: 134–144.

Burgess ED, Hawkins RG, Watanabe M (1990). Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. *Am J Hypertens* **3**: 903–905.

Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA (2001). 1-alpha,25-Dihydroxyvitamin D3 (1,25(OH)(2)D(3)) hampers the maturation of fully active immature dendritic cells from monocytes. *Eur J Endocrinol* **145**: 351–357.

Cardus A, Gallego C, Muray S, Marco MP, Parisi E, Aldea M *et al.* (2003). Differential effect of vitamin D analogues on the proliferation of vascular smooth muscle cells. *Nefrologia* **23**: 117–121.

Cardus A, Parisi E, Gallego C, Aldea M, Fernandez E, Valdivielso JM (2006). 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. *Kidney Int* **69**: 1377–1384.

Cardus A, Panizo S, Parisi E, Fernandez E, Valdivielso JM (2007). Differential effects of vitamin D analogs on vascular calcification. *J Bone Miner Res* **22**: 860–866.

Chertow BS, Sivitz WI, Baranetsky NG, Clark SA, Waite A, Deluca HF (1983). Cellular mechanisms of insulin release: the effects of vitamin D deficiency and repletion on rat insulin secretion. *Endocrinology* **113**: 1511–1518.

Cheskis BJ, Freedman LP, Nagpal S (2006). Vitamin D receptor ligands for osteoporosis. *Curr Opin Investig Drugs* **7**: 906–911.

Chiu KC, Chu A, Go VL, Saad MF (2004). Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* **79**: 820–825.

Davies MR, Lund RJ, Hruska KA (2003). BMP-7 is an efficacious treatment of vascular calcification in a murine model of atherosclerosis and chronic renal failure. *J Am Soc Nephrol* **14**: 1559–1567.

Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B *et al.* (2008). Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* **168**: 1340–1349.

Dobrez DG, Mathes A, Amdahl M, Marx SE, Melnick JZ, Sprague SM (2004). Paricalcitol-treated patients experience improved hospitalization outcomes compared with calcitriol-treated patients in real-world clinical settings. *Nephrol Dial Transplant* **19**: 1174–1181. Epub 5 March 2004.

Doherty TM, Tang W, Dascalos S, Watson KE, Demer LL, Shavelle RM *et al.* (1997). Ethnic origin and serum levels of 1alpha,25-dihydroxyvitamin D3 are independent predictors of coronary calcium mass measured by electron-beam computed tomography. *Circulation* **96**: 1477–1481.

Dusso A, Lopez-Hilker S, Rapp N, Slatopolsky E (1988). Extra-renal production of calcitriol in chronic renal failure. *Kidney Int* **34**: 368–375.

Ebert R, Schutze N, Adamski J, Jakob F (2006). Vitamin D signaling is modulated on multiple levels in health and disease. *Mol Cell Endocrinol* **248**: 149–159.

Enquselassie F, Dobson AJ, Alexander HM, Steele PL (1993). Seasons, temperature and coronary disease. *Int J Epidemiol* **22**: 632–636.

Falk E (2006). Pathogenesis of atherosclerosis. *J Am Coll Cardiol* **47**: C7–12.

Fogh K, Kragballe K (2004). New vitamin D analogs in psoriasis. *Curr Drug Targets Inflamm Allergy* **3**: 199–204.

Forman JP, Bischoff-Ferrari HA, Willett WC, Stampfer MJ, Curhan GC (2005). Vitamin D intake and risk of incident hypertension: results from three large prospective cohort studies. *Hypertension* **46**: 676–682.

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC *et al.* (2007). Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* **49**: 1063–1069.

Freundlich M, Quiroz Y, Zhang Z, Zhang Y, Bravo Y, Weisinger JR *et al.* (2008). Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. *Kidney Int*

Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT (2003). A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol Endocrinol* **17**: 2386–2392.

Giachelli CM (2004). Vascular calcification mechanisms. *J Am Soc Nephrol* **15**: 2959–2964.

Giovannucci E (2007). Can vitamin D reduce total mortality? *Arch Intern Med* **167**: 1709–1710.

Giovannucci E, Liu Y, Hollis BW, Rimm EB (2008). 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* **168**: 1174–1180.

Giulietti A, Gysemans C, Stoffels K, van Etten E, Decallonne B, Overbergh L *et al.* (2004). Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. *Diabetologia* **47**: 451–462.

Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY (2004). Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* **351**: 1296–1305.

Goltzman D (2007). Use of genetically modified mice to examine the skeletal anabolic activity of vitamin D. *J Steroid Biochem Mol Biol* **103**: 587–591.

Green JJ, Robinson DA, Wilson GE, Simpson RU, Westfall MV (2006). Calcitriol modulation of cardiac contractile performance via protein kinase C. *J Mol Cell Cardiol* **41**: 350–359.

Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L (2002). A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* **51**: 1367–1374.

Gysemans C, van Etten E, Overbergh L, Giulietti A, Eelen G, Waer M *et al.* (2008). Unaltered diabetes presentation in NOD mice lacking the vitamin D receptor. *Diabetes* **57**: 269–275.

Henley C, Colloton M, Cattley RC, Shatzen E, Towler DA, Lacey D *et al.* (2005). 1,25-Dihydroxyvitamin D3 but not cinacalcet HCl (Sensipar/Mimpara) treatment mediates aortic calcification in a rat model of secondary hyperparathyroidism. *Nephrol Dial Transplant* **20**: 1370–1377.

Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P *et al.* (2007). Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in

human health and disease. *J Steroid Biochem Mol Biol* **103**: 316–321.

Hirata M, Makibayashi K, Katsumata K, Kusano K, Watanabe T, Fukushima N *et al*. (2002). 22-Oxacalcitriol prevents progressive glomerulosclerosis without adversely affecting calcium and phosphorus metabolism in subtotally nephrectomized rats. *Nephrol Dial Transplant* **17**: 2132–2137.

Holick MF (2004). Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* **79**: 362–371.

Hruska KA, Saab G, Mathew S, Lund R (2007). Renal osteodystrophy, phosphate homeostasis, and vascular calcification. *Semin Dial* **20**: 309–315.

Ishida H, Norman AW (1988). Demonstration of a high affinity receptor for 1,25-dihydroxyvitamin D3 in rat pancreas. *Mol Cell Endocrinol* **60**: 109–117.

Jackson C, Gaugris S, Sen SS, Hosking D (2007). The effect of cholecalciferol (vitamin D3) on the risk of fall and fracture: a meta-analysis. *Qjm* **100**: 185–192.

Jono S, Nishizawa Y, Shioi A, Morii H (1998). 1,25-Dihydroxyvitamin D3 increases *in vitro* vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation* **98**: 1302–1306.

Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS *et al*. (2006). Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int* **70**: 771–780.

Kim HW, Park CW, Shin YS, Kim YS, Shin SJ, Kim YS *et al*. (2006). Calcitriol regresses cardiac hypertrophy and QT dispersion in secondary hyperparathyroidism on hemodialysis. *Nephron Clin Pract* **102**: c21–c29.

Kovesdy CP, Ahmadzadeh S, Anderson JE, Kalantar-Zadeh K (2008). Association of activated vitamin D treatment and mortality in chronic kidney disease. *Arch Intern Med* **168**: 397–403.

Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM (1998). Ultraviolet B and blood pressure. *Lancet* **352**: 709–710.

Kunitomo M, Kinoshita K, Bando Y (1981). Experimental atherosclerosis in rats fed a vitamin D, cholesterol-rich diet. *J Pharmacobiodyn* **4**: 718–723.

Lee GH, Benner D, Regidor DL, Kalantar-Zadeh K (2007). Impact of kidney bone disease and its management on survival of patients on dialysis. *J Ren Nutr* **17**: 38–44.

Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA *et al*. (2007). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int* **71**: 31–38.

Levin A, Djurdjev O, Beaulieu M, Er L (2008). Variability and risk factors for kidney disease progression and death following attainment of stage 4 CKD in a referred cohort. *Am J Kidney Dis* **52**: 661–671.

Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP (2002). 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* **110**: 229–238.

Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G *et al*. (2006). The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *J Intern Med* **260**: 245–254.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR *et al*. (2006). Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* **311**: 1770–1773.

London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC (2004). Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol* **15**: 1943–1951.

London GM, Marchais SJ, Guerin AP, Metivier F (2005). Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia. *Curr Opin Nephrol Hypertens* **14**: 525–531.

London GM, Guerin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ *et al*. (2007). Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol* **18**: 613–620.

Lopez I, Mendoza FJ, Aguilera-Tejero E, Perez J, Guerrero F, Martin D *et al*. (2008). The effect of calcitriol, paricalcitol, and a calcimimetic on extraosseous calcifications in uremic rats. *Kidney Int* **73**: 300–307.

MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M *et al*. (2008). Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. *Ann Intern Med* **148**: 197–213.

Mak RH (1998). 1,25-Dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. *Kidney Int* **53**: 1353–1357.

Mancuso P, Rahman A, Hershey SD, Dandu L, Nibbelink KA, Simpson RU (2008). 1,25-Dihydroxyvitamin-D3 treatment reduces cardiac hypertrophy and left ventricular diameter in spontaneously hypertensive heart failure-prone (cp/+) rats independent of changes in serum leptin. *J Cardiovasc Pharmacol* **51**: 559–564.

Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R *et al*. (2007). Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* **167**: 1159–1165.

Mathew S, Lund RJ, Chaudhary LR, Geurs T, Hruska KA (2008). Vitamin D receptor activators can protect against vascular calcification. *J Am Soc Nephrol* **19**: 1509–1519.

Mathieu C, Adorini L (2002). The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med* **8**: 174–179.

Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R (1994). Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. *Diabetologia* **37**: 552–558.

Mathieu C, Van Etten E, Gysemans C, Decallonne B, Kato S, Laureys J *et al*. (2001). *In vitro* and *in vivo* analysis of the immune system of vitamin D receptor knockout mice. *J Bone Miner Res* **16**: 2057–2065.

Melamed ML, Eustace JA, Plantinga L, Jaar BG, Fink NE, Coresh J *et al*. (2006). Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: a longitudinal study. *Kidney Int* **70**: 351–357.

Melamed ML, Michos ED, Post W, Astor B (2008). 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* **168**: 1629–1637.

Mitsuhashi T, Morris RC Jr, Ives HE (1991). 1,25-dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. *J Clin Invest* **87**: 1889–1895.

Mizobuchi M, Finch JL, Martin DR, Slatopolsky E (2007a). Differential effects of vitamin D receptor activators on vascular calcification in uremic rats. *Kidney Int* **72**: 709–715.

Mizobuchi M, Morrissey J, Finch JL, Martin DR, Liapis H, Akizawa T *et al*. (2007b). Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *J Am Soc Nephrol* **18**: 1796–1806.

Mortimer EA Jr, Monson, RR, MacMahon B (1977). Reduction in mortality from coronary heart disease in men residing at high altitude. *N Engl J Med* **296**: 581–585.

Murray CJ, Kulkarni SC, Michaud C, Tomijima N, Bulzacchelli MT, Iandiorio TJ *et al*. (2006). Eight Americas: investigating mortality disparities across races, counties, and race-counties in the United States. *PLoS Med* **3**: e260.

Nagpal S, Na S, Rathnachalam R (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* **26**: 662–687.

Nakane M, Ma J, Ruan X, Kroeger PE, Wu-Wong R (2007). Mechanistic analysis of VDR-mediated renin suppression. *Nephron Physiol* **107**: 35–44.

Naves-Diaz M, Alvarez-Hernandez D, Passlick-Deetjen J, Guinsburg A, Marelli C, Rodriguez-Puyol D *et al*. (2008). Oral active vitamin D is

associated with improved survival in hemodialysis patients. *Kidney Int* **74**: 1070–1078.

Niederhoffer N, Bobryshev YV, Lartaud-Idjouadiene I, Giummelly P, Atkinson J (1997). Aortic calcification produced by vitamin D3 plus nicotine. *J Vasc Res* **34**: 386–398.

Noonan W, Koch K, Nakane M, Ma J, Dixon D, Bolin A *et al*. (2008). Differential effects of vitamin D receptor activators on aortic calcification and pulse wave velocity in uraemic rats. *Nephrol Dial Transplant* **23**: 3824–3830

Norman AW (2006). Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology* **147**: 5542–5548.

Norman AW, Frankel JB, Heldt AM, Grodsky GM (1980). Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* **209**: 823–825.

Nyomba BL, Bouillon R, De Moor P (1984). Influence of vitamin D status on insulin secretion and glucose tolerance in the rabbit. *Endocrinology* **115**: 191–197.

O'Connell TD, Simpson RU (1996). Immunochemical identification of the 1,25-dihydroxyvitamin D3 receptor protein in human heart. *Cell Biol Int* **20**: 621–624.

O'Connell TD, Berry JE, Jarvis AK, Somerman MJ, Simpson RU (1997). 1,25-Dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy. *Am J Physiol* **272**: H1751–H1758.

O'Herrin JK, Hullett DA, Heisey DM, Sollinger HW, Becker BN (2002). A retrospective evaluation of 1,25-dihydroxyvitamin D(3) and its potential effects on renal allograft function. *Am J Nephrol* **22**: 515–520.

Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D (2008). Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes Metab* **10**: 185–197.

Panichi V, Migliori M, Taccola D, Filippi C, De Nisco L, Giovannini L *et al*. (2001). Effects of 1,25(OH)2D3 in experimental mesangial proliferative nephritis in rats. *Kidney Int* **60**: 87–95.

Park CW, Oh YS, Shin YS, Kim CM, Kim YS, Kim SY *et al*. (1999). Intravenous calcitriol regresses myocardial hypertrophy in hemodialysis patients with secondary hyperparathyroidism. *Am J Kidney Dis* **33**: 73–81.

Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C (2001). Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* **86**: 1633–1637.

Pittas AG, Lau J, Hu FB, Dawson-Hughes B (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* **92**: 2017–2029.

Price PA, Nguyen TM, Williamson MK (2003). Biochemical characterization of the serum fetuin-mineral complex. *J Biol Chem* **278**: 22153–22160.

Raggi P, Kleerekoper M (2008). Contribution of bone and mineral abnormalities to cardiovascular disease in patients with chronic kidney disease. *Clin J Am Soc Nephrol* **3**: 836–843.

Rahman A, Hershey S, Ahmed S, Nibbelink K, Simpson RU (2007). Heart extracellular matrix gene expression profile in the vitamin D receptor knockout mice. *J Steroid Biochem Mol Biol* **103**: 416–419.

Rebsamen MC, Sun J, Norman AW, Liao JK (2002). 1alpha,25-dihydroxyvitamin D3 induces vascular smooth muscle cell migration via activation of phosphatidylinositol 3-kinase. *Circ Res* **91**: 17–24.

Resnick LM, Muller FB, Laragh JH (1986). Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Ann Intern Med* **105**: 649–654.

Richy F, Dukas L, Schacht E (2008). Differential effects of D-hormone analogs and native vitamin D on the risk of falls: a comparative meta-analysis. *Calcif Tissue Int* **82**: 102–107.

Ritter CS, Armbrecht HJ, Slatopolsky E, Brown AJ (2006). 25-Hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. *Kidney Int* **70**: 654–659.

Robinson BM, Joffe MM, Pisoni RL, Port FK, Feldman HI (2006). Revisiting survival differences by race and ethnicity among hemodialysis patients: the Dialysis Outcomes and Practice Patterns Study. *J Am Soc Nephrol* **17**: 2910–2918.

Rostand SG (1997). Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* **30**: 150–156.

Scharla S (2006). Relative value of plain vitamin D and of biologically active vitamin D in the prevention and treatment of osteoporosis. *Z Rheumatol* **65**: 391–394, 396–399.

Schwarz U, Amann K, Orth SR, Simonaviciene A, Wessels S, Ritz E (1998). Effect of 1,25 (OH)2 vitamin D3 on glomerulosclerosis in subtotally nephrectomized rats. *Kidney Int* **53**: 1696–1705.

Scragg R (1981). Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation. *Int J Epidemiol* **10**: 337–341.

Scragg R, Khaw KT, Murphy S (1995). Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. *Eur J Clin Nutr* **49**: 640–646.

Segersten U, Correa P, Hewison M, Hellman P, Dralle H, Carling T *et al*. (2002). 25-hydroxyvitamin D(3)-1alpha-hydroxylase expression in normal and pathological parathyroid glands. *J Clin Endocrinol Metab* **87**: 2967–2972.

Shinaberger CS, Kopple JD, Kovesdy CP, McAllister CJ, van Wyck D, Greenland S *et al*. (2008). Ratio of paricalcitol dosage to serum parathyroid hormone level and survival in maintenance hemodialysis patients. *Clin J Am Soc Nephrol* **3**: 1769–1776.

Shoben AB, Rudser KD, de Boer IH, Young B, Kestenbaum B (2008). Association of oral calcitriol with improved survival in nondialyzed CKD. *J Am Soc Nephrol* **19**: 1613–1619.

Shoji T, Shinohara K, Kimoto E, Emoto M, Tahara H, Koyama H *et al*. (2004). Lower risk for cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a haemodialysis population. *Nephrol Dial Transplant* **19**: 179–184.

Simpson RU, Hershey SH, Nibbelink KA (2007). Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. *J Steroid Biochem Mol Biol* **103**: 521–524.

Slatopolsky E, Finch J, Brown A (2003). New vitamin D analogs. *Kidney Int* **85** (Suppl.): S83–S87.

de Souza Santos R, Vianna LM (2005). Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. *Clin Chim Acta* **358**: 146–150.

Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD (2008). Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. *Diabet Med* **25**: 320–325.

Szeto CC, Chow KM, Kwan BC, Chung KY, Leung CB, Li PK (2008). Oral calcitriol for the treatment of persistent proteinuria in immunoglobulin A nephropathy: an uncontrolled trial. *Am J Kidney Dis* **51**: 724–731.

Talmor Y, Bernheim J, Klein O, Green J, Rashid G (2008a). Calcitriol blunts pro-atherosclerotic parameters through NFkappaB and p38 *in vitro*. *Eur J Clin Invest* **38**: 548–554.

Talmor Y, Golan E, Benchetrit S, Bernheim J, Klein O, Green J *et al*. (2008b). Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. *Am J Physiol Renal Physiol* **294**: F1059–F1064.

Tan X, Li Y, Liu Y (2006). Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. *J Am Soc Nephrol* **17**: 3382–3393.

Tan X, Wen X, Liu Y (2008). Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF-kappaB signaling. *J Am Soc Nephrol* **19**: 1741–1752.

Taura S, Taura M, Kamio A, Kummerow FA (1979). Vitamin D-induced coronary atherosclerosis in normolipemic swine: comparison with human disease. *Tohoku J Exp Med* **129**: 9–16.

Taylor AV, Wise PH (1998). Vitamin D replacement in Asians with diabetes may increase insulin resistance. *Postgrad Med J* **74**: 365–366.

Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R (2003).

Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* **349**: 446–456.

Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernan MA, Camargo CA *et al.* (2005). Activated injectable vitamin D and hemodialysis survival: a historical cohort study. *J Am Soc Nephrol* **16**: 1115–1125.

Tentori F, Hunt WC, Stidley CA, Rohrscheib MR, Bedrick EJ, Meyer KB *et al.* (2006). Mortality risk among hemodialysis patients receiving different vitamin D analogs. *Kidney Int* **70**: 1858–1865.

Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D *et al.* (2002). Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *Qjm* **95**: 787–796.

Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF (1998). Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. *J Biol Chem* **273**: 30427–30434.

Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R (2003). Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab* **88**: 4623–4632.

Vervloet MG, Twisk JW (2008). Mortality reduction by vitamin D receptor activation in end-stage renal disease: a commentary on the robustness of current data. *Nephrol Dial Transplant*

Wang L, Manson JE, Buring JE, Lee IM, Sesso HD (2008a). Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. *Hypertension* **51**: 1073–1079.

Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K *et al.* (2008b). Vitamin D deficiency and risk of cardiovascular disease. *Circulation* **117**: 503–511.

Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R *et al.* (1997). Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* **96**: 1755–1760.

Webb AR, Kline L, Holick MF (1988). Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* **67**: 373–378.

Weishaar RE, Simpson RU (1987a). Involvement of vitamin D3 with cardiovascular function. II. Direct and indirect effects. *Am J Physiol* **253**: E675–E683.

Weishaar RE, Simpson RU (1987b). Vitamin D3 and cardiovascular function in rats. *J Clin Invest* **79**: 1706–1712.

Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H *et al.* (2007). Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* **72**: 1004–1013.

Wolf M, Betancourt J, Chang Y, Shah A, Teng M, Tamez H *et al.* (2008). Impact of activated vitamin D and race on survival among hemodialysis patients. *J Am Soc Nephrol* **19**: 1379–1388.

Wolisi GO, Moe SM (2005). The role of vitamin D in vascular calcification in chronic kidney disease. *Semin Dial* **18**: 307–314.

Wong MS, Delansorne R, Man RY, Vanhoutte PM (2008). Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol* **295**: H289–96.

Wu-Wong JR, Nakane M, Ma J, Ruan X, Kroeger PE (2006a). Effects of Vitamin D analogs on gene expression profiling in human coronary artery smooth muscle cells. *Atherosclerosis* **186**: 20–28.

Wu-Wong JR, Noonan W, Ma J, Dixon D, Nakane M, Bolin AL *et al.* (2006b). Role of phosphorus and vitamin D analogs in the pathogenesis of vascular calcification. *J Pharmacol Exp Ther* **318**: 90–98.

Wu-Wong JR, Tian J, Nakane M, Ma J, Fey TA, Kroeger P *et al.* (2006c). Cardiovascular disease in chronic kidney failure: the role of VDR activators. *Curr Opin Investig Drugs* **7**: 206–213.

Wu-Wong JR, Nakane M, Ma J, Ruan X, Kroeger PE (2007). VDR-mediated gene expression patterns in resting human coronary artery smooth muscle cells. *J Cell Biochem* **100**: 1395–1405.

Wu J, Garami M, Cao L, Li Q, Gardner DG (1995). 1,25(OH)2D3 suppresses expression and secretion of atrial natriuretic peptide from cardiac myocytes. *Am J Physiol* **268**: E1108–E1113.

Wu J, Garami M, Cheng T, Gardner DG (1996). 1,25(OH)2 vitamin D3, and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes. *J Clin Invest* **97**: 1577–1588.

Xiang W, Kong J, Chen S, Cao LP, Qiao G, Zheng W *et al.* (2005). Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* **288**: E125–E132.

Yuan W, Pan W, Kong J, Zheng W, Szeto FL, Wong KE *et al.* (2007). 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. *J Biol Chem* **282**: 29821–29830.

Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG (2003). Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *Faseb J* **17**: 509–511.

Zhang Z, Sun L, Wang Y, Ning G, Minto AW, Kong J *et al.* (2008a). Renoprotective role of the vitamin D receptor in diabetic nephropathy. *Kidney Int* **73**: 163–171.

Zhang Z, Zhang Y, Ning G, Deb DK, Kong J, Li YC (2008b). Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. *Proc Natl Acad Sci USA* **105**: 15896–15901.

Zhou C, Lu F, Cao K, Xu D, Goltzman D, Miao D (2008). Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int* **74**: 170–179.

Zhu Y, Mahon BD, Froicu M, Cantorna MT (2005). Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol* **35**: 217–224.

Zipitis CS, Akobeng AK (2008). Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* **93**: 512–517.

Zisman AL, Hristova M, Ho LT, Sprague SM (2007). Impact of ergocalciferol treatment of vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. *Am J Nephrol* **27**: 36–43.

Zittermann A, Schleithoff SS, Koerfer R (2005). Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br J Nutr* **94**: 483–492.