Vitamin D, Proteinuria, Diabetic Nephropathy, and Progression of CKD

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Although the endocrine effects of vitamin D are widely recognized, somewhat less appreciated is that vitamin D may serve paracrine functions through local activation by 1-α-hydroxylation and thus maintain immunity, vascular function, cardiomyocyte health, and abrogate inflammation and insulin resistance. In the kidney, vitamin D may be important for maintaining podocyte health, preventing epithelial-to-mesenchymal transformation, and suppressing renin gene expression and inflammation. Replacement with pharmacologic dosages of vitamin D receptor agonists (VDRA) in animal models of kidney disease consistently show reduction in albuminuria, abrogation of glomerulosclerosis, glomerulomegalgy, and glomerular inflammation, effects that may be independent of BP and parathyroid hormone, but the effects of VDRA in preventing tubulointerstitial fibrosis and preventing the progression of kidney failure in these animal models are less clear. Emerging evidence in patients with chronic kidney disease (CKD) show that vitamin D can reduce proteinuria or albuminuria even in the presence of angiotensin-converting enzyme inhibition. In addition to reducing proteinuria, VDRA may reduce insulin resistance, BP, and inflammation and preserve podocyte loss providing biologic plausibility to the notion that the use of VDRA may be associated with salubrious outcomes in patients with diabetic nephropathy. Patients with CKD have a very high prevalence of deficiency of 25-hydroxyvitamin D. Whether pharmacologic dosages of vitamin D instead of VDRA in patients with CKD can overcome the paracrine and endocrine functions of this vitamin remains unknown. To demonstrate the putative benefits of native vitamin D and VDRA among patients with CKD, randomized, controlled trials are needed.

In 1978, a study published in The Lancet reported 18 patients who had advanced chronic kidney disease (CKD), creatinine clearance of <35 ml/min, and presence of renal osteodystrophy and were treated with either vitamin D₃ (4000 IU/d) or 1,25-dihydroxyvitamin D [1,25(OH)₂D; 1 μg/d] along with 500 mg of calcium after a 6-mo observation period (1). In the group treated with 1,25(OH)₂D, seven of eight patients developed hypercalcemia that necessitated a reduction in dosage. The percentage fall in creatinine clearance was greater during treatment than before treatment in all patients who were on 1,25(OH)₂D (P < 0.01) and in seven of nine patients on vitamin D₃ treatment (although the within group change here was NS). The authors concluded that deterioration of renal function was a major limitation of the clinical use of 1,25(OH)₂D and vitamin D₃ in nondialized patients with CKD.

Although the conclusions made by this important clinical study were strong, evaluating the state-of-the-art science in 1978, >30 yr later informs us that the dosage of 1,25(OH)₂D was rather high, the sample size too small, and the follow-up period of 6 mo too short to provide reliable information about the rates of progression of CKD in vitamin D–treated patients. Existing data seem to point to a salubrious effect of vitamin D receptor agonists (VDRA) in animals with kidney disease, and emerging evidence points to a similar benefit in patients with CKD. The evolution of science of VDRA in animal models of kidney disease and patients with CKD is the subject of this review.

The effects of vitamin D on the bone and elsewhere can be evaluated at the molecular level by examining knockout mice and are reviewed by Bouillon et al. (2). Absence of a functional vitamin D receptor (VDR) or its ligand, 25-hydroxyvitamin D-1 [25(OH)D₁] α-hydroxylase (CYP27B1), in mice creates a bone and growth plate phenotype that mimics severe vitamin D deficiency. VDR rescue in the intestine restores a normal bone and growth plate phenotype. Although the VDR is nearly ubiquitously expressed and almost all cells respond to 1,25(OH)₂D exposure, the function of active vitamin D and its receptor is not fully overlapping. For example, VDR-deficient mice but not vitamin D or 1-α-hydroxylase-deficient mice develop total alopecia as is also seen in humans. The immune system of VDR–or vitamin D–deficient mice shows increased sensitivity to autoimmune diseases such as inflammatory bowel disease or type 1 diabetes after exposure to predisposing factors. Although VDR-deficient mice do not have a spontaneous increase in cancer, they are more prone to oncogene- or chemocarcinogen-induced tumors. They also develop high renin hypertension, cardiac hypertrophy, and increased...
thrombogenicity. Vitamin D deficiency in humans is associated with increased prevalence of diseases, as predicted by the VDR null phenotype.

Animal Studies
Tian et al. (3) reviewed the mechanisms of salutary effects of vitamin D on the kidney. The noncalcemic effects of VDRA include vascular effects, immunomodulatory effects, anti-inflammatory effects, suppression of the renin-angiotensin system (RAS), and effects on glucose homeostasis, which are discussed further.

Suppression of RAS
Seminal discoveries by Li et al. (4) demonstrated that vitamin D is a potent negative endocrine regulator of the RAS and works predominantly as a suppressor of renin biosynthesis (5). Mice that lack the vitamin D receptor have elevated production of renin and angiotensin II (AngII), leading to hypertension, cardiac hypertrophy, and increased water intake. Vitamin D repression of renin expression is independent of calcium metabolism, the volume- and salt-sensing mechanisms, and the AngII feedback regulation (4). In normal mice, vitamin D deficiency stimulates renin expression, whereas injection of 1,25(OH)2D reduces renin synthesis (4). In cell cultures, 1,25(OH)2D directly suppresses renin gene transcription by a VDR-dependent mechanism. Combined treatment of diabetic mice with losartan and paricalcitol completely prevents albuminuria, restores glomerular filtration barrier structure, and markedly reduces glomerulosclerosis (6). These beneficial effects are accompanied by blockade of intrarenal renin and AngII accumulation induced by hyperglycemia and losartan. These data demonstrate that inhibition of the RAS with combination of vitamin D analogs and RAS inhibitors effectively prevents renal injury in diabetic nephropathy. 1,25(OH)2D also suppresses hyperglycemia-induced activation of the RAS and TGF-β in mesangial and juxtamasellar cells (7). TGF-β activates interstitial fibroblasts and induces tubular epithelial-to-mesenchymal transition. Thus, by blocking TGF-β, VDRA have the potential to abrogate tubulointerstitial fibrosis. Taken together, these studies suggest that receptor-mediated vitamin D actions in part via the RAS may provide renoprotection in diabetic nephropathy.

Improvement in Insulin Resistance
Vitamin D deficiency has been linked with impaired glucose metabolism, an established risk factor for cardiovascular disease (8). Because vitamin D is often deficient and its metabolism substantially impaired in patients with CKD, absolute or functional vitamin D deficiency may be particularly important in these patients. Furthermore, impaired glucose metabolism is common in CKD. De Boer (8) concluded in a recent review that short-term studies on maintenance hemodialysis patients have shown that vitamin D treatment improves insulin secretion and sensitivity, but interventional studies on people without ESRD have yielded mixed results. Improved glucose metabolism is one potential mechanism through which vitamin D may exert beneficial cardiovascular effects in patients with CKD, but further research is needed.

Vascular Effects
Somjen et al. (9) were the first to report an enzymatically active 25(OH)D1 α-hydroxylase system in human vascular smooth muscle cells, which could be upregulated by parathyroid hormone (PTH) and inhibited by exogenous vitamin D. Emerging experimental data demonstrate important physiologic effects of vitamin D on factors that are protective for vascular health (10).

Immunomodulating Effects
Vitamin D seems to regulate innate immunity through effects on microbial recognition peptides such as Toll-like receptors (TLRs) (11). TLRs are membrane-spanning receptors that recognize structurally conserved molecules derived from microbes and activate immune responses. Mycobacterium tuberculosis infection leads to increased 1α-hydroxylase expression and VDR upregulation in monocyte/macrophages in response to activation by TLRs (12). Furthermore, macrophages can also synthesize antimicrobial peptides such as cathelicidin, an effect that is facilitated in the presence of 25(OH)D (13). In fact, it is now being recognized that autocrine production of 1,25(OH)2D might also be an important negative feedback mechanism to deactivate innate and inflammatory responses of activated macrophages (14).

In the kidney, vitamin D has similar immunomodulating effects. For example, in vitro, 1,25(OH)2D attenuates TNF-α-induced monocyte chemotactic protein 1 (MCP-1) expression by human proximal tubule cells (15). A synthetic VDRA, paricalcitol, inhibits renal inflammation by promoting VDR-mediated sequestration of NF-κB signaling (16). Although cell surface receptor–mediated, nongenomic pathways (17,18) are described, the potent antiproliferative, prodifferentiative, and immunomodulating activities (17,19,20) seem to be modulated via vitamin D receptor–dependent genomic effects; the latter seem to provide the biologic basis for salutary effects of VDRA in patients with kidney disease.

Animal Models of Kidney Disease and Benefits of VDRA
Substantial number of studies point out the salutary effects of VDRA in animals (Table 1). In aggregate there appears to be a consistent reduction in albuminuria, abrogation of glomerulosclerosis, glomerulomegaly and glomerular inflammation, effects that may be independent of BP and PTH. However, from these studies the effects of VDRA in preventing tubulointerstitial fibrosis and preventing the progression of kidney failure are less clear.

Human Studies
Vitamin D Deficiency in Patients with CKD
As renal function declines, serum levels of 1,25(OH)2D progressively decrease, leading to a vitamin D–deficient state (28). Despite presence of adequate 25(OH)D, many patients with CKD will have low serum levels of 1,25(OH)2D, which underlies the functional vitamin D deficiency in these patients. Some-
<table>
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<th>Reference</th>
<th>Model</th>
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<tr>
<td>Schwarz et al., 1998 (21)</td>
<td>Subtotal nephrectomy with and without parathyroidectomy</td>
<td>1,25(OH)₂D</td>
<td>Glomerular volume and glomerulosclerosis were less in treated animals. Albuminuria was 21% that of control animals. No effect on vascular and tubulointerstitial sclerosis, systolic BP, or serum creatinine. Cell proliferation was less. TGF-β expression in vessels and tubulointerstitium was less. Effects were independent of PTH.</td>
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<td>Makibayashi et al., 2001 (22)</td>
<td>Anti-Thy1 GN</td>
<td>22-Oxa-calcitriol or 1,25(OH)₂D</td>
<td>Less mesangial cell proliferation, less glomerulosclerosis, less albuminuria at day 8. Reduced type I and IV collagens and α-smooth muscle actin mRNA expression. TGF-β expression in glomeruli reduced.</td>
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<tr>
<td>Panichi et al., 2001 (23)</td>
<td>Anti-Thy1 GN</td>
<td>1,25(OH)₂D</td>
<td>Proteinuria was 16% at day 7, and urinary IL-6 was significantly less compared with control animals. Decreased glomerular inflammation (neutrophil, monocyte), glomerular size, and glomerular cell proliferation. Creatinine at day 14 not statistically different from control group.</td>
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<tr>
<td>Hirata et al., 2002 (24)</td>
<td>Subtotal nephrectomy</td>
<td>22-Oxa-calcitriol</td>
<td>Albuminuria less (approximately 25% of controls at 8 wk); prevented increases in serum creatinine and BUN; and reduced glomerular cell number, volume, and sclerosis at 8 wk. β2-Microglobulin or NAG levels were not affected.</td>
</tr>
<tr>
<td>Kuhlmann et al., 2003 (25)</td>
<td>Subtotal nephrectomy</td>
<td>1,25(OH)₂D</td>
<td>Albuminuria less, glomerular volume less, more podocytes, less podocyte injury, less podocyte hypertrophy.</td>
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<tr>
<td>Tan et al., 2006 (26)</td>
<td>Obstructive nephropathy</td>
<td>Paricalcitol</td>
<td>Reduced renal interstitial fibrosis, suppressed renal TGF-β1 and its type I receptor expression, restored vitamin D receptor abundance, and inhibited cell proliferation and apoptosis. Paricalcitol also blocked directly the EMT.</td>
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<tr>
<td>Freundlich et al., 2008 (27)</td>
<td>Subtotal nephrectomy</td>
<td>Paricalcitol</td>
<td>Glomerular and tubulointerstitial damage, hypertension, proteinuria, and the deterioration of renal function were improved as a result of decrease in angiotensinogen, renin, renin receptor, and vascular endothelial growth factor mRNA levels in the remnant kidney by 30 to 50% with paricalcitol treatment.</td>
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<tr>
<td>Zhang et al., 2008 (7)</td>
<td>Diabetic VDR knockout mice</td>
<td>1,25(OH)₂D</td>
<td>Diabetic VDR knockout mice developed more severe albuminuria and glomerulosclerosis as a result of increased glomerular basement membrane thickening and podocyte effacement. In receptor knockout mice, increased renin, angiotensinogen, TGF-β, and connective tissue growth factor accompanied the more severe renal injury.</td>
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*a*1,25(OH)₂D, 1,25-dihydroxyvitamin D; BUN, blood urea nitrogen; EMT, epithelial-to-mesenchymal transition; GN, glomerulonephritis; PTH, parathyroid hormone; VDR, vitamin D receptor.
what less commonly recognized is the high prevalence of nutritional vitamin D deficiency, as determined by low 25(OH)D concentrations in these patients. Thus, “absolute” vitamin D deficiency is also common in patients with CKD. In 43 patients with CKD spanning an estimated GFR (eGFR) range of 11 to 111 ml/min per 1.73 m², Gonzalez et al. (29) reported vitamin D deficiency as defined by a serum 25(OH)D level <30 ng/ml in 86% of the patients. Possible explanations for this high prevalence of nutritional D deficiency in patients are reduced sun-light exposure (e.g., in the elderly, the sick, dark-skinned people, those who wear a veil for cultural reasons), losses of vitamin D–binding protein in proteinuric states [25(OH)D is lost in the urine bound to vitamin D–binding protein] and a compromised endogenous synthesis of vitamin D from the skin in the uremic state. It seems that increased removal of 25(OH)D via nonrenal pathways may also play a role in causing a low 25(OH)D level. In fact, it is believed that the vast majority of 25(OH)D is used by the nonrenal tissues. Because monocytes and macrophages express 1-α hydroxylase, it seems plausible that apparent nutritional vitamin D deficiency could be a manifestation of the inflammatory state in patients with CKD. In this context, VDRA administration may lead to reduction in the inflammatory state (30). Whether similar benefits can be realized with vitamin D administration needs to be studied in future trials. Endothelial cells can also rapidly induce 1-α hydroxylase activity in response to inflammatory cytokines and may serve to deplete the stores of 25(OH)D (31). In one small study, VDRA was unable to improve endothelial function, but a larger study with vitamin D is warranted.

**Vitamin D and Cohort Studies in CKD**

In 15,068 adults who participated in the Third National Health and Nutrition Examination Survey (NHANES III), de Boer et al. (32) reported a stepwise increase in the prevalence of albuminuria with decreasing quartiles of 25(OH)D concentration. After multivariate adjustment including region and season of measurement and eGFR, relative risks for albuminuria by decreasing quartile of vitamin D concentration were significantly increased to 1.14, 1.22, and 1.37 in successive quartiles. In the NHANES survey, 25(OH)D deficiency and eGFR both were independently related to insulin resistance (33) as well as several other cardiovascular risk factors, such as hypertension, diabetes, obesity, and high serum triglyceride levels (34). Zehnder et al. (15) measured vitamin D metabolites, markers of inflammation and gene expression, in 174 patients with a variety of kidney diseases. Urinary MCP-1 and renal macrophage infiltration each were inversely correlated with serum 1,25(OH)₂D levels. Higher levels of 25(OH)D were also associated with lower inflammation. Patients with acute renal inflammation had a significant increase in urinary and kidney MCP-1, macrophage infiltration, and macrophage and renal epithelial 1-α hydroxylase (CYP27B1) expression but significantly lower levels of serum 1,25(OH)₂D in comparison with patients with chronic ischemic disease despite similar levels of renal damage. Thus, decreased serum vitamin D metabolites and activation of the paracrine/autocrine vitamin D system are important correlates of inflammation in patients with CKD. London et al. (35) reported that 25(OH)D as well as its active metabolite 1,25(OH)₂D is directly associated with endothelial function and inversely with arterial calcifications in patients with CKD. Taken together, it seems that 25(OH)D deficiency in patients with CKD is associated with worse risk factors (albuminuria, insulin resistance, inflammation, hypertension, dyslipidemia, and endothelial dysfunction) for progression of kidney disease.

The presence of vitamin D deficiency is associated with incident cardiovascular disease in the general population (36). Because risk factors for cardiovascular and kidney disease by and large overlap, it is possible that cardiovascular and renal benefits may accrue in patients with CKD. In fact, several studies of patients who had CKD and were not on dialysis supported the presence of a salutary effect of vitamin D. In an Italian cohort, Ravani et al. (37) found that 25(OH)D plasma concentration was an independent inverse predictor of disease progression and death in patients with stages 2 through 5 CKD after multivariate adjustment. Shoben et al. (38) reported that oral calcitriol use was associated with lower mortality in nondialysis US veterans with CKD. After multivariate adjustment including eGFR and baseline levels of PTH, calcium, and phosphorous, oral calcitriol use was associated with a 26% lower risk for death and a 20% lower risk for death or dialysis. The association of calcitriol with improved survival was not statistically different across baseline PTH levels, although calcitriol use was associated with a greater risk for hypercalcemia. In another cohort of US veterans with CKD, Kovesdy et al. (39) reported that the incidence rate ratios for mortality and combined death and dialysis initiation were significantly lower in patients who were treated with calcitriol. Treatment with calcitriol was also associated with a trend toward a lower incidence of dialysis.

It remains that neither cross-sectional nor longitudinal studies can prove a cause-and-effect relationship between use of VDRA and outcomes. Randomized trials are needed.

**Randomized Trials of VDRA in Patients with CKD**

At least three clinical trials have evaluated the use of VDRA in patients who had CKD and were not on renal replacement therapy. In the first study, Agarwal et al. (40) reported reduction in proteinuria detected semiquantitatively by dipstick using an automated analysis in patients who had stages 3 and 4 CKD with secondary hyperparathyroidism and participated in three randomized, controlled trials of oral paricalcitol. In that post hoc analysis, reduction in dipstick proteinuria occurred in the face of the frequent use of agents that block the RAS. Although previous studies reported that dipstick proteinuria is a good predictor of spot urine protein/creatinine ratios, an obvious limitation of that study was the method of detection of proteinuria (automated dipstick versus spot urinary protein/creatinine or albumin/creatinine ratio).

Extending these findings, Alborzi et al. (30) reported a prospective pilot trial of 24 patients who had CKD (two patients stage 2, remaining stage 3) and were randomly allocated in a double-blind manner to three equal groups to receive 0, 1, or 2 µg of paricalcitol, a vitamin D analog, orally for 1 mo. At 1 mo, treatment/baseline ratio of high-sensitivity C-reactive protein
was 1.5 (95% confidence interval [CI] 1.1 to 2.1; \( P = 0.02 \)) with placebo, 0.8 (95% CI 0.3 to 1.9; \( P = 0.62 \)) with 1-\( \mu \)g dosage and 0.5 (95% CI 0.3 to 0.9; \( P = 0.03 \)) with 2-\( \mu \)g dosage of paricalcitol. At 1 mo, treatment/baseline ratio of 24-h albumin excretion rate was 1.35 (95% CI 1.08 to 1.69; \( P = 0.01 \)) with placebo, 0.52 (95% CI 0.40 to 0.69; \( P < 0.001 \)) with 1-\( \mu \)g dosage, and 0.54 (95% CI 0.35 to 0.83; \( P = 0.01 \)) with 2-\( \mu \)g dosage (\( P < 0.001 \) for between-group changes). No differences were observed in iothalmate clearance, flow-mediated dilation (a measure of endothelial function), 24-h ambulatory BP, or PTH with treatment or upon washout. The limitations of that trial include the small sample size and the limited duration of exposure to VDR activator. Furthermore, all patients had low 25(OH)D deficiency in that study; whether nutritional replacement could have improved albuminuria or inflammation remains unknown. That study therefore cannot provide definite answers to questions of the clinical use of paricalcitol for renal protection; however, it seems from that study that paricalcitol-induced reduction in albuminuria and inflammation may be mediated independent of its effects on hemodynamics or PTH suppression.

In an uncontrolled trial, reported from Hong Kong, 10 patients with biopsy-proven IgA nephropathy and persistent proteinuria despite angiotensin-converting enzyme inhibition and AngII receptor blockade were treated with 0.5 \( \mu \)g calcitriol twice weekly for 12 wk (41). After calcitriol treatment, there was a significant overall decrease in urine protein-creatinine ratio from 1.98 ± 0.74 to 1.48 ± 0.81 g/g (\( P = 0.007 \)) during the first 6 wk that persisted throughout the study period. No significant change in BP or renal function was noted. There was a simultaneous decrease in serum TGF-\( \beta \) level, and percentage of decrease in serum TGF-\( \beta \) level significantly correlated with percentage of change in proteinuria (Spearman \( r = 0.643; P = 0.02 \)).

Whether VDRA should be used to reduce the rate of progression of CKD cannot be answered by the studies discussed herein; larger trials are required. Lowering of albuminuria or proteinuria in these trials, however, occurred without changes in BP even when recorded by ambulatory BP monitoring, raising the notion that improvement in kidney disease conferred by the use of these drugs may occur via nonhemodynamic pathways.

**Future Directions**

Randomized, controlled trials with paricalcitol are under way to demonstrate whether paricalcitol will have antiproteinuric or cardioprotective effects in patients with CKD. In one such trial, reduction in albuminuria from baseline to 24 wk is the primary end point (ClinicalTrials.gov identifier NCT00421733), whereas, in another, the change in left ventricular mass index assessed by cardiac magnetic resonance imaging over 48 wk is the primary end point (ClinicalTrials.gov identifier NCT00497146). The Vitamin D Receptor Activator (Paricalcitol) in Albuminuria Lowering (VITAL) study has randomly assigned 282 patients with diabetes in a double-blind, multicenter trial to placebo or 1 or 2 \( \mu \)g of paricalcitol taken once daily in patients with eGFR between 15 and 90 ml/min per 1.73 m\(^2\) and urine albumin/creatinine concentration between 100 and 3000 mg/dl on average of three morning void specimen. All patients have to be on a stable dosage of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker and have reasonable control of BP and blood glucose to participate. Results are awaited. Each of these trials, conducted with patients with CKD, should provide better evidence (or lack thereof) for cardiovascular and renal protection.

**Disclosures**

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**References**
