# Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and VDR activation

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**A normal vitamin D status is essential for human health. Vitamin D deficiency is a recognized risk factor for all-cause mortality in normal individuals and in chronic kidney disease (CKD) patients. The link between vitamin D deficiency and death is a defective activation of the vitamin D receptor (VDR) by 1,25-dihydroxyvitamin D (calcitriol, the vitamin D hormone) to induce/repress genes that maintain mineral homeostasis and skeletal integrity, and prevent secondary hyperparathyroidism, hypertension, immune disorders, and renal and cardiovascular (CV) damage. The kidney is the main site for the conversion of 25-hydroxyvitamin D (25D) to circulating calcitriol, and therefore essential for the health benefits of endocrine VDR activation. The kidney is also essential for the uptake of 25D from the glomerular ultrafiltrate for its recycling to the circulation to maintain serum 25D levels, extrarenal calcitriol synthesis, and the prosurvival benefits of autocrine/paracrine VDR activation. Indeed, both calcitriol and vitamin D deficiency increase progressively in the course of CKD, and associate directly with accelerated disease progression and death. Therefore, the safe correction of calcitriol and vitamin D deficiency/insufficiency is becoming a high priority among nephrologists. This review updates the pathophysiology behind 25D and calcitriol deficiency and impaired VDR activation in CKD, the adequacy of current recommendations for vitamin D supplementation, and potential markers of the efficacy of therapy to prevent or slow the development of renal and CV lesions unrelated to parathyroid hormone suppression, a knowledge required for the design of trials to obtain evidence-based recommendations for vitamin D and calcitriol replacement at all stages of CKD.**

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## VDR ACTIVATION AND HUMAN HEALTH

The health benefits of a normal vitamin D status go far beyond the prevention of disorders in mineral homeostasis, bone remodeling, or the development of secondary hyperparathyroidism (SHPT), with important renal and cardiovascular (CV) protective actions that contribute to the strong association between vitamin D deficiency and all-cause mortality in the general population (Figure 1).[1,2] Most vitamin D actions require the activation of the vitamin D receptor (VDR) by 1,25-dihydroxyvitamin D, also called calcitriol or 1,25D, the most active native vitamin D metabolite and a potent steroid hormone.[3] Similar to other steroid hormones, calcitriol binding to the VDR is the initial step to activate the VDR to induce or repress the expression of genes that maintain mineral homeostasis and skeletal health, as well as immune, renal, and CV function. Indeed, VDR-regulated gene products tightly coordinate intestinal calcium and phosphate absorption, bone metabolism, renal calcium and phosphate reabsorption, and parathyroid function to prevent or slow the development of SHPT, phosphate retention, and soft-tissue calcifications, and also inhibit or attenuate the activation of the renin–angiotensin system, systemic inflammation, and proteinuria, three important contributors to renal and CV damage and enhanced mortality rates.[4]

The kidney is the main site for the conversion of 25-hydroxyvitamin D (25D) to circulating calcitriol. In fact, in the course of chronic kidney disease (CKD), progressive reductions in serum calcitriol and endocrine VDR activation are important contributors to the development of SHPT, vascular calcifications, and high mortality rates.[5] Therefore, calcitriol replacement with either calcitriol or its analogs has been the therapy of choice to prevent or slow the progression of SHPT and its associated adverse effects.[5] Large retrospective trials in hemodialysis patients have suggested that calcitriol replacement confers a survival advantage unrelated

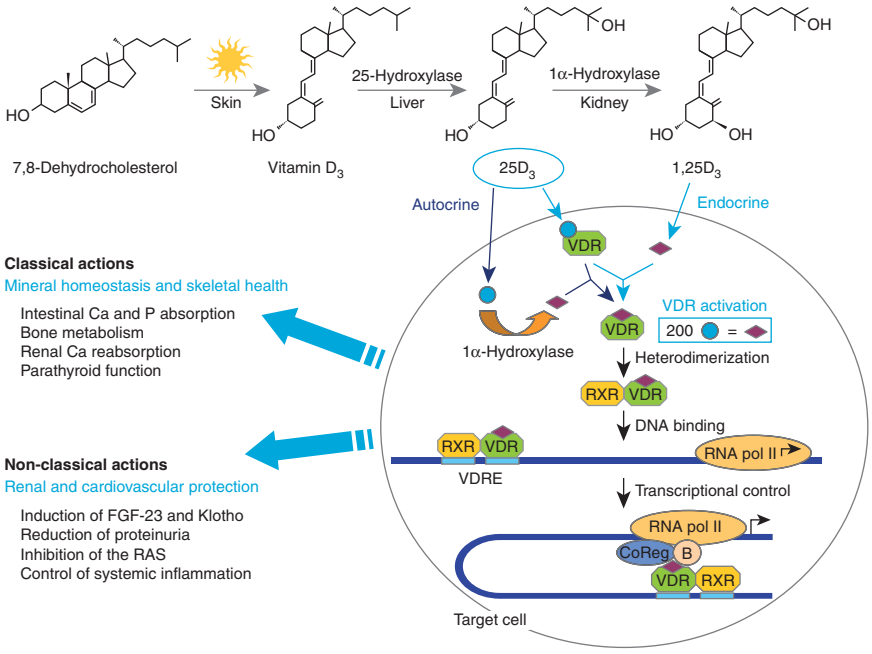


**Figure 1 | Health benefits of vitamin D receptor (VDR) activation.** Vitamin D actions require vitamin D activation to its hormonal form, 1,25-dihydroxyvitamin D (calcitriol; 1,25D), by renal and extrarenal 1-hydroxylases to bind and activate the VDR in an endocrine and/or autocrine manner, respectively. The precursor of 1,25D, 25-hydroxyvitamin D (25D), can also bind and activate the VDR with a much lower potency. Upon activation, ligand-bound VDR functions as a transcription factor to regulate the expression of genes involved in vitamin D maintenance of mineral homeostasis, skeletal health, and renal and cardiovascular protection. B, basal transcription factor; Ca, calcium; CoReg, coregulator molecule; FGF-23, fibroblast growth factor-23; P, phosphorus; RAS, renin–angiotensin system; RXR, retinoid x receptor; VDRE, vitamin D-responsive element; $25D_3$, 25-hydroxyvitamin $D_3$; $1,25D_3$, 1,25-dihydroxyvitamin $D_3$.

to its efficacy (or the lack of it) to suppress parathyroid hormone (PTH).[6] These studies have led to the identification of the most critical renal and cardioprotective properties of selective VDR activation in preclinical and small clinical trials, including the inhibition of the renin–angiotensin system, proteinuria, and systemic inflammation (reviewed in ref. 4). However, the initial enthusiasm to start calcitriol replacement earlier or to prolong its use to improve outcomes independently of its efficacy to suppress PTH was diminished after the reports from the Cochrane Group[7,8] of the lack of evidence from adequately powered prospective studies in support of the survival effects of VDR activation in predialysis and dialysis patients.

An important consideration that could improve the outcomes of exclusive calcitriol replacement is the maintenance of a normal vitamin D status: the kidney is not unique in the capacity to produce calcitriol, nor is endocrine VDR activation the only contributor to calcitriol prosurvival properties. Indeed, in the general population with normal renal function and normal serum calcitriol levels, vitamin D deficiency causes SHPT, bone loss, and strongly associates with a higher risk for hypertension, proteinuria, CV lesions, and higher rates of CV mortality.[9] The contribution of non-renal cells to systemic calcitriol levels is negligible in individuals with normal kidney function, which underscores the critical role of autocrine/paracrine VDR activation by calcitriol synthesized locally in the cell-specific prosurvival actions of a normal vitamin D status. In CKD patients, vitamin D deficiency is also a strong predictor of accelerated renal disease progression and death.[10] In view of the striking incidence of vitamin D deficiency/insufficiency among CKD patients, the safe correction of vitamin D and calcitriol deficiency/insufficiency is becoming a high priority for nephrologists. The implementation of vitamin D and/or calcitriol replacement that safely improves the prosurvival actions of selective VDR activation beyond PTH suppression at all stages of CKD requires an update of our understanding of the pathogenesis of calcitriol and vitamin D deficiency/insufficiency and impaired VDR activation.

## CRITICAL ROLE OF THE KIDNEY IN AUTOCRINE/PARACRINE VDR ACTIVATION

Until recently, our understanding of the pathogenesis of calcitriol deficiency in the course of CKD was the progressive loss of renal 1-hydroxylase with the reductions in functional renal mass, and the inhibition of remnant 1-hydroxylase activity by elevations in serum levels of uremic toxins, PTH fragments, and fibroblast growth factor (FGF)-23 (reviewed in ref. 11). However, defects in renal uptake of 25D in CKD are important contributors to systemic calcitriol deficiency, and also key determinants of 25D deficiency because renal proximal tubular cells obtain the 25D for its conversion to calcitriol not from the blood but from the glomerular ultrafiltrate.[12] Figure 2 shows that megalin, a multiligand receptor, mediates the active process of endocytosis of the filtered 25D bound to its carrier, the vitamin D-binding

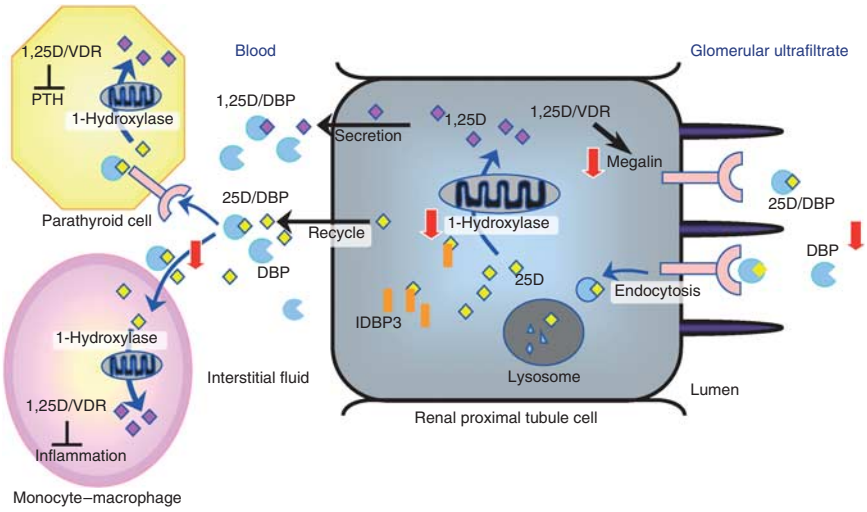


**Figure 2 | Critical role of normal kidney function in vitamin D receptor (VDR) activation.** The endocytic receptor megalin in renal proximal tubular cells is required for the uptake of 25-hydroxyvitamin D (25D; ◆) bound to its carrier, the vitamin D-binding protein (DBP; ●) from the glomerular ultrafiltrate. Once inside proximal tubular cells, 25D is either delivered to renal 1-hydroxylase for its conversion to 1,25-dihydroxyvitamin D (calcitriol; 1,25D; ◆), to maintain 1,25D production and renal and endocrine VDR activation, or recycled back to the blood to maintain serum 25D levels and to supply extrarenal 1-hydroxylases for its conversion to 1,25D for autocrine VDR activation. Chronic kidney disease (red arrows) reduces not only renal 1-hydroxylase and the amount of filtered 25D but also renal megalin content and the uptake of 25D by monocytes–macrophages, markedly impairing both endocrine and autocrine VDR activation. IDBP3, intracellular vitamin D binding protein 3; PTH, parathyroid hormone.

protein, from the glomerular ultrafiltrate. Once inside proximal tubular cells, 25D is either delivered to 1-hydroxylase for its conversion to calcitriol or recycled back to the circulation to maintain normal serum 25D and the appropriate supply of substrate to non-renal cells expressing 1-hydroxylase for local calcitriol production and autocrine VDR activation. The key contribution of megalin-mediated recycling of 25D to maintain normal serum levels of 25D was conclusively demonstrated in the megalin-null mouse, in which severe vitamin D deficiency occurs in spite of normal renal function.[12] Importantly, renal megalin is induced by calcitriol.[13] Therefore, vitamin D deficiency/insufficiency in normal individuals results in reduced 25D levels in the glomerular ultrafiltrate in spite of normal glomerular filtration rates, causing a vicious cycle for progressive deterioration of renal calcitriol synthesis and megalin expression. The latter can account for the strong association between vitamin D deficiency and albuminuria in normal individuals,[14] as megalin also mediates urinary albumin reabsorption.

In CKD, progressive reductions in glomerular filtration rate and also in renal megalin expression[15] further aggravate the low renal uptake of 25D caused by vitamin D deficiency/insufficiency, decreasing calcitriol production by the remnant 1-hydroxylase and, consequently, renal autocrine VDR activation. Defective VDR activation of renal megalin in CKD patients may partially account not only for the degree of albuminuria, but also for phosphate retention in spite of high serum PTH. Indeed, a specific deletion of megalin expression in the kidney eliminates the phosphaturic response to PTH because megalin mediates the internalization of the sodium–phosphate cotransporter NaPi2a required for PTH to stop renal phosphate reabsorption.[16]

Low serum 25D levels are not the only impediment to maintain normal autocrine VDR activation in CKD (Figure 2). Peripheral blood mononuclear cells from patients in hemodialysis elicit *ex vivo* a markedly impaired uptake of 25D.[17] Thus, vitamin D deficiency contributes to the progressive reductions of serum calcitriol in the course of CKD by impairing both renal and extrarenal calcitriol production. The safe correction of vitamin D deficiency at early stages of CKD could help prevent/slow the loss of the renal and cardioprotective properties of endocrine and/or autocrine/paracrine VDR activation.

## CONSIDERATIONS FOR THE SAFE CORRECTION OF VITAMIN D DEFICIENCY IN CKD

Native vitamin D is available in two distinct forms: ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$). Studies in normal individuals have demonstrated that vitamin $D_2$ has a lower efficacy for a sustained correction of 25D levels compared with vitamin $D_3$.[18] Both vitamin D forms elicit similar elevations in serum vitamin D levels upon a single oral dose of 100,000 IU and a similar disappearance from the circulation. However, whereas vitamin $D_3$ dosage results in a sustained increase in serum $25D_3$ for 30 days, upon vitamin $D_2$ administration $25D_2$ levels decrease to baseline values within 2 weeks.[18] Importantly, switching the frequency for vitamin $D_2$ administration from bolus to daily can enhance its efficacy to sustain serum $25D_2$ levels similar to that of vitamin $D_3$.[19] $25D_2$ and $25D_3$ have the same potency in humans. An additional advantage of lower daily

doses of vitamin D is that the rate of conversion of vitamin $D_2$ or $D_3$ to $25D_{2/3}$ is much higher for doses below 4000 IU.[20] These studies, however, may not reflect the efficacy of a low daily dose of vitamin D to correct 25D deficiency in predialysis and dialysis patients because CKD may alter both the metabolism of vitamin $D_2$ and $D_3$ and the rate of conversion of vitamin D to 25D.

In Europe, 25D is available for oral administration; however, caution is warranted with dose frequency because there is no conversion required and also because the half-life of 25D is longer (15–18 days) than that of vitamin D (3–7 days), which could result in rapid accumulation to levels above the normal upper range (150 ng/ml).

Importantly, the radioimmunoassays and enzyme-linked immunosorbent assays available to measure $25D_{2/3}$ have a 100% cross-reactivity with 24,25-dihydroxyvitamin $D_{2/3}$, which could lead to an overestimation of the efficacy of vitamin D supplementation to correct serum 25D levels in CKD patients in whom $24,25(OH)_2D$ synthesis is induced by high serum FGF-23 or calcitriol replacement therapy (reviewed in ref. 11).

## SAFETY AND EFFICACY OF VITAMIN D SUPPLEMENTATION TO IMPROVE AUTOCRINE VDR ACTIVATION

The contribution of vitamin D deficiency to the low serum calcitriol of CKD patients was conclusively demonstrated in the 1980s. Administration of 25D can normalize the very low calcitriol levels of patients with a glomerular filtration rate below 25 ml/min undergoing hemodialysis,[21] and also the almost undetectable serum calcitriol of bilaterally nephrectomized patients.[22] These findings suggest that at earlier stages of CKD, vitamin D supplementation that effectively enhances serum 25D levels will increase the proportion of D-binding protein carrying filtered 25D and the levels of 25D available for renal and extrarenal calcitriol synthesis. The lower the glomerular filtration rate, remnant renal 1-hydroxylase, and megalin content, and the higher serum FGF-23 levels, the higher must be the serum 25D levels to normalize serum calcitriol from renal and extrarenal sources. In fact, whereas 4 ng/ml of 25D is sufficient to maintain normal serum calcitriol in individuals with normal kidney function,[23] the requirements increase to levels of 25D above 100 ng/ml in hemodialysis patients, and above 200 ng/ml in anephric patients, in whom extrarenal 1-hydroxylases are the only contributors to systemic calcitriol. The high levels of 25D required to normalize serum calcitriol may compromise rather than improve survival. In fact, in a group of normal women, serum 25D levels above 50 ng/ml start reversing the decline of mortality rates with the correction of vitamin D deficiency.[24]

Studies on the efficacy of 25D supplementation to improve autocrine VDR activation have been limited to PTH suppression. In transplant recipients, doses of vitamin D of 100,000 IU twice a month are necessary to control PTH. Importantly, the efficacy of the same dosage decreases when provided only monthly.[25] In CKD stages 3 and 4, only 50% of the patients in whom serum 25D levels increase above 35 ng/ml in response to 50,000 IU of ergocalciferol administered twice a month can suppress PTH.[26] Reductions in parathyroid megalin[27] may add to the impaired renal uptake of 25D to increase the requirements of vitamin D supplementation to effectively suppress PTH. In view of the adverse effects reported for 25D levels above 50 ng/ml in women with normal serum calcitriol,[24] transient increases in serum 25D above 50 ng/ml after a high bolus of 'inactive' vitamin D can exert detrimental effects on survival through direct activation of the VDR (Figure 1). These adverse effects may be overlooked if changes in serum phosphate and calcium levels are measured a month after dosage. The long-term beneficial or detrimental effects of prolonged supplementation with high bolus doses of vitamin D need to be tested in prospective trials.

Importantly, in a recent report in CKD stages 3 and 4, daily dosage of cholecalciferol of 4000 IU/day for a month, followed by 2000 IU for two additional months was sufficient to increase 25D levels above 37 ng/ml and to normalize serum calcitriol; however, it failed to reduce PTH, proteinuria, or blood pressure.[28] These findings support the higher conversion rates of vitamin D to 25D for low daily doses and demonstrate that the correction of serum calcitriol and/or 25D levels is inaccurate to monitor the efficacy of vitamin D supplementation to improve prosurvival actions of VDR activation unrelated to PTH suppression.

The correction of calcitriol deficiency can lower the amount of vitamin D supplementation required to improve extrarenal calcitriol synthesis and autocrine VDR activation. In hemodialysis patients, the correction of the low serum calcitriol levels through intravenous calcitriol administration corrects the impaired uptake of 25D by peripheral blood mononuclear cells.[17] Thus, in CKD patients, vitamin D supplementation corrects the impaired renal uptake of 25D to maintain renal calcitriol synthesis and serum 25D levels for extrarenal calcitriol production. In turn, calcitriol replacement to normalize serum calcitriol corrects the defective uptake of 25D by non-renal cells bearing 1-hydroxylase. Taken together, these findings support the benefits of combined vitamin D and calcitriol replacement. Indeed, preliminary studies in 5/6 nephrectomized rats demonstrated the benefits of combination therapy with 25D (at weekly doses sufficient to increase serum levels above 35 ng/ml) and paricalcitol (at a dose insufficient to suppress PTH), compared with monotherapy either to prevent or slow proteinuria and aortic calcifications.[29] These preclinical studies support dual benefits of vitamin D and calcitriol replacement: low doses of selective VDR activators may improve the renal and CV outcomes of vitamin D supplementation at early stages of CKD. In addition, vitamin D supplementation may enhance the renal and cardioprotective efficacy of low doses of paricalcitol. The latter suggests that the simple correction of vitamin D deficiency in vitamin D-deficient patients in the Selective Vitamin D Receptor (VDR) Activator for Albuminuria Lowering (VITAL) Study

might have increased the efficacy of 1 μg of paricalcitol to control proteinuria.[30] However, the design of safe trials to provide evidence-based recommendations for combined vitamin D and calcitriol replacement at all stages of kidney disease is a very difficult task. Not only can the high levels of 25D required to enhance autocrine VDR activation induce toxicity at non-hypercalcemic doses of calcitriol or selective VDR activators, but also the high levels of selective activators required to suppress PTH or induce renal and CV protection in tissues with reduced VDR compromise rather than enhance extrarenal calcitriol production by suppressing 1-hydroxylase expression,[17] and also by enhancing the degradation of 25D, calcitriol, and its analogs, thereby reducing both autocrine and paracrine VDR activation. The achievement of the appropriate balance of serum levels of 25D and selective VDR activators to maximize VDR activation requires the identification of accurate markers of renal and CV lesions.

## TACE ACTIVATION CAUSES RENAL AND CV DAMAGE

Increases in renal levels and activity of tumor necrosis factor-α (TNFα)-converting enzyme (TACE, also called ADAM17) and TACE-mediated release of transforming growth factor-α are the cause of proteinuria, glomerular sclerosis, mononuclear cell infiltration, tubular hyperplasia, and fibrosis upon nephron reduction, or prolonged exposure to angiotensin II in mice (Figure 3).[31] Similar increases in TACE and transforming growth factor-α occur in fibrotic and inflammatory human kidney disease.[32] Upon the initial mechanism triggering renal TACE activation, TACE-mediated release to the circulation of the profibrotic and proinflammatory cytokines soluble TNFα, soluble intercellular cell adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1 from their transmembrane precursors causes systemic inflammation and aggravates renal and CV damage (reviewed in ref. 29). Increases in TACE and the release of TNFα are sufficient to explain the high risk of CV mortality in CKD. TNFα is a well-recognized cause of systemic inflammation, atherosclerosis, and vascular calcifications.[33] More significantly, polymorphisms of TACE that mildly elevate serum TNFα are associated with a higher risk of death from CV disease in normal individuals.[34] Furthermore, increases in serum sICAM-1 and sVCAM-1 levels in transplant recipients are better indicators than proteinuria of poor prognosis.[35] The accuracy of the prevention/attenuation of increases in renal and monocyte TACE and/or serum markers of renal TACE activation to monitor the efficacy of therapy was tested in rat CKD and in hemodialysis patients. In rats, the higher efficacy over monotherapy of the 25D and paricalcitol combination to prevent or slow proteinuria and aortic calcification was associated with its higher potency to reduce TACE expression in the kidney and in monocyte/macrophages.[29] Studies in hemodialysis patients demonstrated a threefold enhancement of TACE expression in peripheral monocytes and in serum levels of TNFα, ICAM-1, and VCAM-1 compared with normal controls, and the efficacy of the combination of vitamin D supplementation and paricalcitol to synergize with anti-renin–angiotensin system therapy to reduce TACE expression and activity.[36] Thus, TACE expression in monocytes and serum markers of tissue TACE activation have the potential to help monitor the severity of renal and CV lesions and systemic inflammation and its changes with therapeutic interventions with anti-renin–angiotensin system and/or vitamin D therapy.

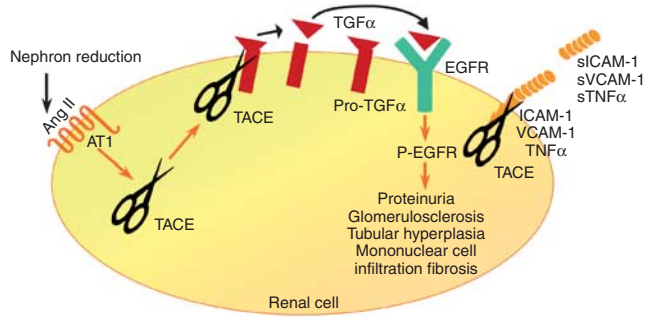


**Figure 3 | Renal tumor necrosis factor-α (TNFα)-converting enzyme (TACE) activation in chronic kidney disease contributes to renal and cardiovascular (CV) lesions.** Renal lesions upon nephron reduction and/or activation of the renin–angiotensin system involve angiotensin II (Ang II) binding to its receptor (AT1) to induce the activation of TACE and TACE-mediated release of transforming growth factor-α (TGFα) from its transmembrane precursor (pro-TGFα) to activate its receptor, the epidermal growth factor receptor (EGFR). Once activated in the kidney, TACE-mediated release of the profibrotic, proinflammatory cytokines TNFα, and intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) into the circulation causes systemic inflammation, CV lesions, and CV mortality. P-EGFR, tyrosine-phosphorylated EGFR; sICAM-1, soluble ICAM-1; sVCAM-1, soluble VCAM-1; sTNFα, soluble TNFα.

In summary, normal kidney function is essential to maintain not only serum calcitriol levels but also serum 25D levels, and thus the health benefits of both endocrine and autocrine VDR activation. CKD induces severe abnormalities to the complex regulation of renal and extrarenal calcitriol synthesis and catabolism that impede providing simple safe recommendations to improve clinical outcomes at all stages of CKD. Furthermore, the design of the much-needed clinical trials for safe and evidence-based recommendations also requires previous testing of the accuracy of available markers of renal and CV lesions unrelated to PTH suppression to monitor the efficacy of therapy. All tools to address this overwhelming challenge for nephrologists are at hand.

## DISCLOSURE

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