The expanding spectrum of biological actions of vitamin D

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Introduction

The vitamin D hormonal system was classically implicated in the regulation of calcium homeostasis and bone metabolism. However, it also has extra-mineral metabolism functions through activation of non-renal vitamin D receptor (VDR) [1]. Vitamin D deficiency is an increasingly recognized public health problem in the general population and in chronic inflammatory disorders such as chronic kidney disease (CKD) [2,3]. Newly uncovered actions of the vitamin D hormone system could underlie the impact of vitamin D deficiency and supplementation on prognosis in CKD and non-CKD patients.

We review emerging extra-mineral metabolism functions of vitamin D from a translational perspective, highlighting recent information on vitamin D deficiency, replacement therapy, pathogenesis, and outcomes in cardiovascular, inflammatory and renal disease.

Vitamin D

Vitamin D$_2$ (ergocalciferol) is obtained from certain food and, principally, from vitamin supplements. Vitamin D$_3$ (VD$_3$, cholecalciferol) is present in food and vitamin supplements, but is mainly generated by skin exposed to ultraviolet B radiation: 7- and 8-dehydrocholesterol are converted by photolysis to pre-VD$_3$ and then by thermal isomerization, to VD$_3$. This pro-hormone is converted to 25-hydroxycholecalciferol [25(OH)D] in the liver by 25-hydroxylase (CYP27B1), an activity highly regulated by parathyroid hormone (PTH) axis [4]. Vitamin D is slowly (∼5–7 days) activated to 25(OH)D, which has a half-life of ∼15 days, while the
Vitamin D deficiency in CKD

In CKD, there is a widespread calcitriol deficiency and a high prevalence of 25(OH)D deficiency. The latter is due to poor sunlight exposure in chronically ill patients, decreased skin synthesis of cholecalciferol in response to sunlight, dietary restriction of vitamin D-rich food, and urine loss of 25(OH)D and DBP in proteinuric nephropathies [20–22]. In addition, renal megalin decreases with CKD progression, thus reducing 25(OH)D tubular reabsorption [23]. Even in dialysis patients, 25(OH)D supplementation can normalize serum calcitriol. This was attributed to an impaired availability of 25(OH)D, and suggests that the reduction of 1-α-hydroxylase activity is not the only problem [24]. Recent CKD guidelines recommend monitoring and correcting 25(OH)D levels [8,25]. Calcitriol levels decline from the early stages of CKD, before any changes in serum calcium, phosphorus or PTH levels. This facilitates secondary hyperparathyroidism and metabolic bone disease, and is associated with higher rates of cardiovascular events [26–29].

25(OH)D deficiency is common in patients with nephrotic syndrome and normal renal function [30,31]. In nephrotic syndrome and sub-nephrotic range proteinuria, there was an inverse relationship between serum 25(OH)D and proteinuria, attributed to urinary loss of 25(OH)D and DBP [32–34]. In NHANES III, low serum 25(OH)D levels were associated with an increased prevalence of albuminuria. This association was independent of age, sex, ethnicity, smoking status, body mass index and kidney function, and persisted for serum 25(OH)D <24.1 ng/mL in the presence of hypertension and diabetes [35].

In CKD, fibroblast growth factor-23 (FGF-23) could contribute to further reduce 25(OH)D and 1,25(OH)D levels [36–38]. Even in early stages of CKD, renal retention of phosphorus may contribute the impaired production of 1,25(OH)D directly, and by increasing FGF-23 levels. FGF-23 from osteocytes activates the FGFR1 receptor in the presence of Klotho co-receptor to induce phosphaturia, suppress renal 1-α-hydroxylase activity, suppress PTH production and increase 1,25(OH)D or 25(OH)D catabolism by activating 24-hydroxylase [1,39]. Thus, FGF-23 could contribute to both 25(OH)D and 1,25(OH)D deficiency in CKD. In advanced CKD, the phosphaturic effect of FGF-23 is limited by the low glomerular filtration rate (GFR). However, 1-α-hydroxylase activity is still suppressed, and serum phosphorus remains high despite high FGF-23 levels [40]. Calcium deprivation promotes 25(OH)D depletion [41]. This is thought to result from increased PTH, which promotes the conversion of 25(OH)D to 1,25(OH)D. 1,25(OH)D itself stimulates hepatic inactivation of 25(OH)D [41] by an enzyme later identified as 24-hydroxylase, leading to 24,25(OH)D synthesis [22]. Current knowledge of basic physiology of vitamin D (Figure 1) and of its deficiency in CKD has improved the management of mineral metabolism by dietary phosphorus restriction, use of phosphorus binders, vitamin D analogues and calcimimetics [42,43]. However, further studies are needed to improve our understanding of the PTH/FGF-23–klotho/vitamin D axis.

Vitamin D has actions beyond calcium–phosphorus–PTH axis regulation. These actions depend on renal half-life of 1,25(OH)D is 4–7 h. Thus, cautious 25(OH)D dosing and timing are required to avoid toxic serum 25(OH)D levels causing hypercalcemia [1]. Calcitriol regulates calcium in the gut and kidney. In the gut, calcitriol promotes active cellular calcium uptake and transport by inducing apical calcium channels (TRPV6 and TRPV5), cytosolic calcium-binding protein (CaBP or calbindin), ATPase, Ca2+/ATPase pump, and Na+/Ca2+ exchanger [1]. In the renal tubule, calcitriol controls its own homeostasis (suppression of 1-α-hydroxylase and stimulation of 24-hydroxylase), potentiates the effects of PTH on calcium reabsorption, and induces transepithelial calcium transport by TRPV5, calbindin, and Ca2+/ATPase pump [1,5]. Additionally, calcitriol increases bone osteoclastic activity and suppresses PTH release [1–4] (Figure 1).

There is no definite consensus about which serum 25(OH)D level better defines vitamin D deficiency. Applying the criteria of 25(OH)D levels required to suppress PTH, deficiency is defined as 25(OH)D <20 ng/mL (50 nmol/L) and relative insufficiency 21–29 ng/mL [6–8]. A target ≥30 ng/mL (75 nmol/L) has been suggested to be desirable for optimal health and for cancer prevention [6,9–11]. Less than 9% of older US whites and a smaller fraction of Mexican American and African American adults had serum 25(OH)D ≥36 ng/mL [12]. More than a billion people in the world have 25(OH)D levels <30 ng/mL [2]. This situation affects 40–50% of all age groups and both sexes [2,13]. In the National Health and Nutrition Examination Survey (NHANES), prevalence of 25(OH)D <10 ng/mL increased from 2% to 6%, and prevalence of >30 ng/mL levels decreased from 45% to 23% in the past decade [14]. One-third of black pregnant women and 5% of white pregnant women in the USA had 25(OH)D levels <15 ng/mL [15]. In Spain, 49% of 2230 ambulatory patients had 25(OH)D <30 ng/mL, 25% <20 ng/mL and 6.2% <10 ng/mL [16]. Vitamin D is required for bone and muscle health, and severe 25(OH)D deficiency causes rickets in children, and osteomalacia and myopathy in adults [17–19].
and extra-renal \(1\alpha\)-hydroxylase activity, and are mediated by VDR in immune system cells, breast, colon, prostate, kidney, intestine, pancreas and other tissues [44].

Peripheral \(1\alpha\)-hydroxylation of 25(OH)D to 1,25(OH)\(_2\)D depends on substrate [25(OH)D] availability and the activity of local \(1\alpha\)-hydroxylase and 24-hydroxylase. Extra-renal \(1\alpha\)-hydroxylase is not regulated by PTH or FGF-23, but it is activated by inflammatory mediators. Local 1,25(OH)\(_2\)D activates the VDR in an autocrine or paracrine manner [45,46]. These features may better explain the impact of 25(OH)D deficiency on renal and cardiovascular outcomes, diabetes, glucose intolerance, metabolic syndrome, obesity, pre-eclampsia, and hypertension [47–53].

### Clinical studies on vitamin D deficiency

#### Vitamin D status and cardiovascular outcomes in non-CKD patients

Observational studies have demonstrated the relationship between 25(OH)D deficiency and cardiovascular outcomes in patients without CKD, including all-cause mortality, hypertension, cardiovascular disease, myocardial infarction, stroke, prevalence of peripheral arterial disease, and cancer mortality. This association was also shown in older population (Table 1) [28,47–58]. After confounder adjustment, a serum 25(OH)D level <20 ng/mL doubled the risk of pre-eclampsia, a predictor of chronic hypertension and CKD [53–56]. In critically ill patients, the prevalence of low 25(OH)D was high, and low 25(OH)D values were associated with adverse outcome [59]. However, additional research is needed on the potential contribution of confounding factor such as a sedentary lifestyle, special dietary needs, or co-existence of non-renal chronic inflammatory disorders to both the lower 25(OH)D values and worse outcomes.

### Vitamin D replacement and outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>n</th>
<th>Age (years)</th>
<th>Follow-up (years)</th>
<th>Serum 25(OH)D (ng/mL)</th>
<th>Outcome</th>
<th>RR/OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsia [52]</td>
<td>Post-menopausal women</td>
<td>36 282</td>
<td>50–79</td>
<td>7 years</td>
<td>Ca 0.5 g/day+VD(_3) 400 IU vs Ca 0.5 g/day+placebo</td>
<td>Heart disease</td>
<td>1.0 (0.9–1.2)</td>
</tr>
<tr>
<td></td>
<td>German CHF patients</td>
<td>123</td>
<td>50–63</td>
<td>15 months</td>
<td>VD(_3) 50 µg/d+Ca 0.5 g/day vs Placebo+Ca 0.5 g/day</td>
<td>Stroke</td>
<td>0.95 (0.8–1.1)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Patient survival</td>
<td>85.7% vs 68.2%</td>
</tr>
<tr>
<td></td>
<td>Autier [62](^a)</td>
<td>57 311</td>
<td>47–92</td>
<td>5.7 years</td>
<td>VD(_3) or VD(_3)+calcium vs Placebo+calcium</td>
<td>All-cause mortality</td>
<td>0.93 (0.87–0.99)</td>
</tr>
</tbody>
</table>

AML, acute myocardial infarction; CI, confidence interval; CHF, congestive heart failure; CKD, chronic kidney disease; CV, cardiovascular; ND, no difference; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PAD, peripheral arterial disease; ProANP and ProBNP, propeptide atrial and brain natriuretic peptide; RCT, randomized clinical trials; RR, relative risk; TNF-\(\alpha\), tumour necrosis factor-alpha; VD\(_2\), ergocalciferol; VD\(_3\), cholecalciferol.

\(^a\)Corresponded to Z-standardized values.

\(^b\)Corresponded to a meta-analysis.

\(^c\)Lowest vs highest quartile.

\(^d\)To convert to nmol/L multiply 2.496.

<table>
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<tr>
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<th>Outcome</th>
<th>RR/OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dobnig [28]</td>
<td>Elders</td>
<td>3258</td>
<td>62</td>
<td>7.7</td>
<td>&lt;13.3</td>
<td>All-cause mortality</td>
<td>1.5 (1.2–2.0)</td>
</tr>
<tr>
<td>Wang [47]</td>
<td>Healthy volunteers</td>
<td>1739</td>
<td>59</td>
<td>5.4</td>
<td>&lt;15</td>
<td>CV mortality</td>
<td>1.8 (1.3–2.6)</td>
</tr>
<tr>
<td>Giovannucci [48]</td>
<td>Healthy volunteers</td>
<td>18 225</td>
<td>40–75</td>
<td>10</td>
<td>&lt;15</td>
<td>CV disease</td>
<td>1.6 (1.1–2.4)</td>
</tr>
<tr>
<td>Pilz [49]</td>
<td>CV symptoms</td>
<td>3316</td>
<td>54.8–76.0</td>
<td>7.8</td>
<td>0.19–0.50(^e)</td>
<td>Fatal stroke</td>
<td>0.7 (0.5–0.9)</td>
</tr>
<tr>
<td>Melamed [50]</td>
<td>NHANES</td>
<td>4839</td>
<td>56.2</td>
<td>No follow-up</td>
<td>&lt;17.8</td>
<td>PAD</td>
<td>1.8 (1.2–2.7)</td>
</tr>
<tr>
<td>Bodnar [51]</td>
<td>Pregnant women</td>
<td>274</td>
<td>14–44</td>
<td>Pregnancy &lt;20</td>
<td>20</td>
<td>Pre-eclampsia</td>
<td>2.4 (1.1–5.4)</td>
</tr>
<tr>
<td>Forman [53]</td>
<td>Healthy volunteers</td>
<td>1811</td>
<td>40–75</td>
<td>4–8</td>
<td>&lt;15</td>
<td>Hypertension</td>
<td>3.2 (1.4–7.3)</td>
</tr>
<tr>
<td>Melamed [57]</td>
<td>NHANES</td>
<td>13 331</td>
<td>41–47</td>
<td>6–12</td>
<td>&lt;17.8</td>
<td>All-cause mortality</td>
<td>1.26 (1.08–1.46)</td>
</tr>
<tr>
<td>Senba [58]</td>
<td>Older Italians</td>
<td>1006</td>
<td>≥65</td>
<td>6.5</td>
<td>&lt;10.5 vs &gt;26.5(^c)</td>
<td>All-cause mortality</td>
<td>2.1 (1.22–3.64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CV mortality</td>
<td>2.64 (1.14–4.79)</td>
</tr>
</tbody>
</table>

Vitamin D replacement and outcomes

[Table 1. Principal studies on vitamin D status and replacement and major clinical outcomes in non-CKD patients]

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### Vitamin D status and outcomes

<table>
<thead>
<tr>
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<th>Outcome</th>
<th>RR/OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang [69]</td>
<td>PD, Asian</td>
<td></td>
<td>55 ± 12</td>
<td>3</td>
<td>&gt;18.3</td>
<td>Fatal and non-fatal CV events</td>
<td>0.6–0.8</td>
</tr>
<tr>
<td>Melamed [70]</td>
<td>NHANES III</td>
<td>13 328</td>
<td>44.3 ± 0.5</td>
<td>9.1</td>
<td>&lt;15</td>
<td>ESRD</td>
<td>2.6 (1.0–7.1)</td>
</tr>
<tr>
<td>Ravani [72]</td>
<td>CKD 2–5</td>
<td>168</td>
<td>70.1 ± 11.9</td>
<td>4</td>
<td>≥15</td>
<td>Death</td>
<td>0.61 (0.36–1.0)</td>
</tr>
<tr>
<td>Mehrorat [74]</td>
<td>CKD</td>
<td>3011</td>
<td>55.4 ± 0.9</td>
<td>9</td>
<td>CV mortality</td>
<td>ESRD/Death</td>
<td>0.48 (0.26–0.90)</td>
</tr>
<tr>
<td></td>
<td>NHANES III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death+ESRD</td>
<td>0.56 (0.39–0.82)</td>
</tr>
</tbody>
</table>

### Vitamin D replacement and outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Key</th>
<th>Age (years)</th>
<th>Follow-up</th>
<th>Comparation</th>
<th>Outcome</th>
<th>RR/OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tentori [65]</td>
<td>Incident HD</td>
<td>7731</td>
<td>48.5</td>
<td>5.75 years</td>
<td>Paricalcitol vs calcitriol</td>
<td>All-cause mortality</td>
<td>0.95 (0.79–1.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Doxercalcefor vs calcitriol</td>
<td>All-cause mortality</td>
<td>0.95 (0.77–1.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paricalcitol vs oxercalcefor</td>
<td>All-cause mortality</td>
<td>1.0 (0.82–1.12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None received vs any</td>
<td>All-cause mortality</td>
<td>1.20 (1.10–1.32)</td>
</tr>
<tr>
<td>Teng [66]</td>
<td>Prevalent HD</td>
<td>67 399</td>
<td>61.0</td>
<td>3 years</td>
<td>Paricalcitol vs calcitriol</td>
<td>All-cause mortality</td>
<td>0.80 (0.76–0.83)</td>
</tr>
<tr>
<td>Teng [67]</td>
<td>Incident HD</td>
<td>51 037</td>
<td>61.5</td>
<td>2 years</td>
<td>Injectable calcitriol or agonists (paricalcitol or doxercalcefor) vs never users</td>
<td>All-cause mortality</td>
<td>0.80 (0.76–0.83)</td>
</tr>
<tr>
<td>Naves-Diaz [68]</td>
<td>Incident and prevalent HD</td>
<td>16 004</td>
<td>58.4</td>
<td>16 months</td>
<td>Calcitriol or alfalcaldex vs non-users</td>
<td>Patient survival</td>
<td>0.55 (0.49–0.63)</td>
</tr>
<tr>
<td>Shoben [71]</td>
<td>CKD 3–4</td>
<td>1418</td>
<td>69 ± 10.3</td>
<td>1.9 years</td>
<td>Users vs non-users calcitriol</td>
<td>Mortality</td>
<td>0.76 (0.58–0.99)</td>
</tr>
<tr>
<td></td>
<td>CKD 3–4</td>
<td>1418</td>
<td>69 ± 10.3</td>
<td>1.9 years</td>
<td>None received vs any</td>
<td>Mortality+dialysis</td>
<td>0.80 (0.64–1.01)</td>
</tr>
<tr>
<td>Agarwal [77]</td>
<td>CKD 3–4</td>
<td>220</td>
<td>62.2</td>
<td>6 months</td>
<td>Paricalcitol (9.5 ug/week) vs placebo</td>
<td>Proteinuria reduction</td>
<td>3.2 (1.5–6.9)</td>
</tr>
<tr>
<td>Szeto [78]</td>
<td>IgA nephropathy</td>
<td>10</td>
<td>43.1 ± 9.9</td>
<td>3 months</td>
<td>Oral calcitriol (1.0 ug/week)</td>
<td>Proteinuria</td>
<td>1.9–1.5 g/g</td>
</tr>
<tr>
<td>Fishbane [79]</td>
<td>Proteinuric</td>
<td>61</td>
<td>57.8</td>
<td>6 months</td>
<td>Paricalcitol (1 ug/day) or placebo</td>
<td>Proteinuria reduction</td>
<td>57.1% vs 25.9%</td>
</tr>
<tr>
<td>Alborzi [80]</td>
<td>CKD 2–4</td>
<td>24</td>
<td>56–82</td>
<td>1 month</td>
<td>Paricalcitol (0, 1 and 2 ug/day)</td>
<td>24-h albuminuria</td>
<td>[35%, 48%, 46%]</td>
</tr>
<tr>
<td>VITAL [82]</td>
<td>Diabetic</td>
<td>281</td>
<td>64.9 ± 10.4</td>
<td>6 months</td>
<td>Paricalcitol (0, 1 and 2 ug/day)</td>
<td>Albuminuria P=0.015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CKD 2–4</td>
<td>281</td>
<td>64.9 ± 10.4</td>
<td>6 months</td>
<td>Paricalcitol (0, 1 and 2 ug/day)</td>
<td>Albuminuria P=0.053</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; CKD, chronic kidney disease; CRP, C-reactive protein; CV, cardiovascular; ESRD, end-stage renal disease; HD, haemodialysis; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RR, relative risk; VDRs, VDR activators.

aFor decrease of 10% in proteinuria.
bFor decrease of 24-h albuminuria evaluated as % geometric mean change.
cFor decrease of urinary albumin–creatinine ratio evaluated as % geometric mean change.
dTo convert to nmol/L multiply by 2.496.
mortality despite a relatively short follow-up [61]. In a recent meta-analysis of 57 311 patients from 18 randomized clinical trials, the relative risk for all-cause mortality was 0.93 (95% CI 0.87–0.99) in vitamin D-supplemented patients [62]. However, the primary end points in most studies were conditions associated with higher mortality such as clinical fractures, falls or bone mineral density. A randomized, multicentre study in post-menopausal women failed to show a benefit of 1000 mg/day calcium carbonate plus 400 IU/day VD₃ on the secondary outcome risk of heart disease or cerebrovascular events [52]. A possible explanation for the lack of impact on cardiovascular outcomes is the lower than expected prevalence of serum 25(OH)D <30 ng/mL. Serum 25(OH)D was not analysed as an interaction factor to the major outcomes. As the impact of therapy on 25(OH)D levels was not assessed, it is unclear whether the relatively low dose of VD₃ improved serum 25(OH)D.

**Vitamin D deficiency: therapy and cardiorenal outcomes in CKD and dialysis patients**

**Observational studies.** Vitamin D deficiency is prevalent and especially in chronic haemodialysis patients. Our group found 25(OH)D <20 ng/mL in 83% and <10 ng/mL in 25% of haemodialysis patients. No patient had levels >40 ng/mL [63]. The levels of 25(OH)D were lower in patients evaluated in February than in patients evaluated in December (13.4±5.2 vs 17.4±6.1 ng/mL, P<0.002), highlighting the progressive decrease of 25(OH)D stores during winter. Serum 1,25(OH)D was low in all patients.

Nutritional vitamin D deficiency is a potential risk factor for vascular disease and adverse outcomes of dialysis patients. However, in most observational studies, the focus of research was calcitriol or paricalcitol therapy (Table 2) [64–69]. In a historic cohort study of 67 399 haemodialysis outpatients, parenteral paricalcitol therapy was associated with 16% lower risk of death at 2 years versus parenteral calcitriol [70]. In a historical cohort of haemodialysis patients, parenteral paricalcitol was associated with 20% higher survival [67]. More recently, in a large historical cohort of 16 000 South American patients, oral calcitriol (<1 μg/day) was associated with reduced all-cause, cardiovascular, infectious diseases and cancer mortality [68]. In a prospective cohort study of peritoneal dialysis patients followed up for 3 years, the lowest 25(OH)D levels were associated with increased risk of cardiovascular events, although this effect was influenced by the degree of left ventricular hypertrophy and diastolic dysfunction [69]. Studies in patients with more preserved renal function (not on dialysis) are scarce. The impact of low 25(OH)D levels on CKD progression should be studied. In NHANES III, serum 25(OH)D <15 ng/mL was associated with the development of end-stage renal disease (ESRD) even after adjustment for several risk factors [70]. Non-dialysis CKD patients could benefit from calcitriol or paricalcitol therapy. In 1418 patients with CKD stages 3–4 and hyperparathyroidism followed up for 1.9 years, the use of oral calcitriol was associated with a 26% lower risk of death or admission to dialysis, but it was also associated with an increased risk of hypercalcaemia [71]. In 168 incident patients with CKD stages 2–4 followed up for a mean of 4 years, the basal 25(OH)D level was an independent predictor of progression to ESRD and death when adjusted for relevant variables [72]. In 140 CKD patients, including 10 in dialysis, 25(OH)D <16.7 ng/mL influenced the risk of death after adjustment for several cardiovascular risk factors [73]. Serum albumin was the only variable significantly correlated with serum 25(OH)D, but unfortunately, proteinuria was not evaluated by multivariable regression. In the largest study of the relationship between serum 25(OH)D and death in CKD, levels <15 ng/mL versus >30 ng/mL were associated with ~50% higher cardiovascular and all-cause mortality [74]. In these studies, the high mortality in CKD patients with 25(OH)D deficiency cannot be exclusively attributed to cardiovascular events. Similar to the general population, serious infections or cancer is a condition associated with 25(OH)D deficiency [60,75]. In summary, 25(OH)D deficiency is associated with undesirable clinical outcomes in CKD patients. However, due to the observational design of most studies, randomized clinical trials are necessary to improve the quality of evidence on the survival advantage offered by vitamin D therapy in patients with advanced CKD [76].

**Clinical trials.** Clinical trials evaluated the impact of VDR activators on proteinuric CKD [77–80] (Table 2). In a double-blind, randomized, placebo-controlled trial of oral paricalcitol (mean dose 9.5 μg/week) in 220 patients with CKD stages 3–4 with secondary hyperparathyroidism, paricalcitol reduced dipstick proteinuria (51% vs 25% for placebo). However, proteinuria was not a primary outcome and was measured semi-quantitatively, and the follow-up was too short (24 weeks) [77]. In a small open-label prospective uncontrolled trial, 10 patients with IgA nephropathy and persistent proteinuria despite renin–angiotensin–aldosterone system (RAAS) blockade received calcitriol 0.5 μg, twice weekly for 12 weeks [78]. There was a modest antiproteinuric effect (urine protein–creatinine ratio decreased from 1.98±0.7 to 1.48±0.8 g/g in 6 weeks), without significant changes in blood pressure or renal function. A double-blind randomized study comparing paricalcitol 1 μg/day or placebo for 6 months in 61 patients with proteinuric kidney disease showed a significant reduction in proteinuria (~3% for controls and ~18% for paricalcitol) [79]. In a randomized double-blind trial, oral paricalcitol (1 or 2 μg/day for 1 month) reduced inflammation [high-sensitivity C-reactive protein (hs-CRP)] and albuminuria without changes in endothelial function (flow-mediated dilatation and vascular smooth muscle function), blood pressure, GFR, or PTH levels in 24 patients with CKD stages 2–3 and RAAS blockers [80]. The VITAL double-blind, randomized, placebo-controlled study evaluated the anti-albuminuric effect of paricalcitol 1 or 2 μg/day versus placebo in 281 patients with CKD stages 2–4, diabetic nephropathy, and standard therapy to control blood pressure and proteinuria [81]. There was only a trend towards reduced albuminuria in the primary outcome measure (albumin/creatinine ratio), but in additional outcome measures, 2 μg/day paricalcitol significantly but reversibly reduced 24-h albuminuria at
24 weeks [82]. Paricalcitol 2 μg/day reversibly reduced blood pressure (0 to −10 mmHg), a beneficial effect beyond action on proteinuria, but it also reduced the estimated GFR (0 to −6 mL/min/1.73 m²). This change could be real or related to potential actions of paricalcitol on tubular creatinine secretion. In this regard, oral calcitriol for 6 months decreased creatinine clearance by 22% but did not change inulin or para-aminomipurate clearance, suggesting changes in creatinine secretion [83]. The incidence of hypercalcaemia was not increased. In all trials involving paricalcitol for proteinuria, serum 25(OH)D levels were <30 ng/ml in most patients.

We still need formal large randomized clinical trials supporting the benefits of treating 25(OH)D and calcitriol deficiency on relevant clinical outcomes (cardiovascular events, admission to dialysis and mortality), and comparing the different pharmacological agents available.

A systematic review of 16 randomized clinical trials (894 patients with CKD without dialysis) concluded that several vitamin D compounds (calcitriol, alfacalcidol, doxercalciferol, paricalcitol and maxacalcitol) did not alter mortality or need for dialysis, but decreased serum PTH and increased serum phosphorus and calcium [84]. This review did not evaluate the changes in proteinuria or serum 25(OH)D with therapy and considered active and non-active compounds, and some trials were too short or did not evaluate cardiovascular outcomes.

1,25-Dihydroxyvitamin D and VDR: biology, extra-bone functions and therapeutic modulation

Basic molecular biology and genomic actions of VDR

The biological actions of calcitriol and its active synthetic analogues are mediated by the VDR which is an ubiquitous, ligand-activated transcription factor belonging to the superfamily of steroid/thyroid hormone receptors [85]. There are four steps for VDR control of gene transcription: (i) ligand binding in the C-terminal portion of VDR (ligand-binding domain, LBD), (ii) heterodimerization with retinoid X receptor (RXR) at the LBD and nuclear translocation, (iii) binding of VDR–RXR by the DNA-binding domain of VDR to specific DNA sequences in the promoter region of target genes (VD response elements, VDREs), and (iv) recruitment of transcription mediator complexes, regulate DNA helicase and protein kinase activity. The main co-activators are the nuclear proteins or DRIPs (co-regulators or co-factors) and CREB-binding protein (CBP/p300) (Figure 2). The main co-activators are the steroid receptor co-activator family (SRC-1, SRC-2 and SRC-3) and CREB-binding protein (CBP/p300) [1]. Co-activators modify chromatin via histone acetylation, recruit transcription mediator complexes, regulate ligand-dependent proteasomal degradation, and provide DNA helicase and protein kinase activity. The main co-repressors are NcoR-1, NcoR-2 and Hairless which deacetylase histone lysine residues, compact chromatin, and silence genes (chromatin remodelling) [86,88]. The VDR co-modulator NCoA62/Skip may have a bifunctional role, promoting activation or repression, depending on the relative levels of NcoR and CBP/300 [89]. Calcitriol itself could modulate the transcriptional activity through selective induction of TIF2 (a p160 co-activator) and/or SMRT (co-repressor) [90]. The final balance between co-activators and co-repressors determines the control of gene transcription in the presence of physiological and pathological stimuli.

25(OH)D binds nearly 100 times less avidly to VDR than calcitriol [1,91]. Since 25(OH)D levels are 1000 times higher than 1,25(OH)D concentration, VDR activation by 25(OH)D is likely. In addition, even in CKD patients with very low renal 1-α-hydroxylase, 25(OH)D supplementation increases serum 1,25(OH)D probably through non-renal 1-α-hydroxylases [92] (Figure 3).

Non-genomic actions of VDR

Calcitriol has rapid, non-genomic actions, such as activation of kinases, phosphatases, phosphoinositide metabolism, cytosolic calcium levels and cyclic GMP signalling pathways. The contribution of non-genomic actions to the pathophysiology of the vitamin D endocrine system remains controversial. One potential role is modulation of VDR genomic actions by phosphorylation of VDR co-regulators that influence VDR transcriptional activity. Uraemic conditions may also interfere with interactions between 1,25(OH)D–VDR–RXR, co-regulators and signalling pathways [93,94].

Experimental studies in renal and vascular pathology: potential therapeutic modulation of VDR activation

The main endocrine effect of 1,25(OH)D in the kidney is a tight control of its own homeostasis, by suppressing 1-α-hydroxylase, stimulating 24-hydroxylase and inducing megalin expression in proximal tubular cells [86,95]. Increased megalin expression could favour megalin-mediated protein uptake in renal tubules and thus, reduce proteinuria [81,86]. In addition, VDR activation has antiproliferative, pro-differentiation and immunomodulator actions [1], which may modulate the development of osteoporosis, autoimmune, inflammatory and infectious diseases. The VDR axis also contributes to blood pressure...

Fig. 2. Activation and regulation of gene expression of VDR by 1,25(OH)D. The first step is the formation of 1,25(OH)D/VDR or, with less affinity (dotted arrow), 25(OH)D/VDR complex. VDR then heterodimerizes with RXR. This new complex binds to VDRE of nuclear DNA to regulate (influenced by co-activators or co-repressors) gene transactivation or gene transrepression. These genomic actions are responsible for vitamin D biological actions. Non-genomic 1,25(OH)D/VDR actions appear to involve the same VDR when associated with caveolin close to the inner layer of the plasma membrane (not shown). They activate signalling pathways that modulate the activity of proteins involved in genomic actions and other functions. 1,25(OH)D itself could modulate transcriptional activity through TIF2 and/or SMRT (dotted arrow). DRIPs, VDR interaction nuclear proteins; IDBP, intracellular VD binding protein; NcoA62/Skip, co-modulator; NcoR-1 and NcoR-2, co-repressors 1 and 2, respectively, to the chromatin remodelling; pCAF, CREB-associated factor; RXR, retinoic X receptor; SMRT, co-repressor; SRC, steroid receptor co-activator; VDR, vitamin D receptor; VDRE, vitamin D response elements.
Non-classical biological actions of 1,25(OH)D–VDR interaction. Non-calciotropic consequences of VDR activation that may contribute to the impact of vitamin D deficiency on outcome in patients with and without CKD. ANP, atrial natriuretic peptide; AT II, angiotensin II; AT1R, angiotensin I receptor; Bcl2 (B-cell lymphoma 2 protein) and Bax (Bcl2-associated X protein) are proteins of Bcl2 family that protect from and promote apoptosis, respectively; CEBP/β, transcription factor that regulates growth and differentiation and suppresses cyclin D1 signature; EGFR, epidermal growth factor receptor; γ-IFN, gamma interferon; p21 and p27 are genes that control cellular proliferation; GDNF, glial cell line-derived neurotrophic factor; GMCSF, granulocyte–macrophage colony-stimulating factor; NF-κB, nuclear factor κ B; RAAS, renin–angiotensin–aldosterone system; RR, renin receptor; TGF-α, transforming growth factor-α; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

**Fig. 3.** Non-classical biological actions of 1,25(OH)D–VDR interaction. Non-calciotropic consequences of VDR activation that may contribute to the impact of vitamin D deficiency on outcome in patients with and without CKD. ANP, atrial natriuretic peptide; AT II, angiotensin II; AT1R, angiotensin I receptor; Bcl2 (B-cell lymphoma 2 protein) and Bax (Bcl2-associated X protein) are proteins of Bcl2 family that protect from and promote apoptosis, respectively; CEBP/β, transcription factor that regulates growth and differentiation and suppresses cyclin D1 signature; EGFR, epidermal growth factor receptor; γ-IFN, gamma interferon; p21 and p27 are genes that control cellular proliferation; GDNF, glial cell line-derived neurotrophic factor; GMCSF, granulocyte–macrophage colony-stimulating factor; NF-κB, nuclear factor κ B; RAAS, renin–angiotensin–aldosterone system; RR, renin receptor; TGF-α, transforming growth factor-α; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.
and plasma volume homeostasis, cardiac function, and integrity of renal cells. A critical non-classical renal action is induction of type A natriuretic peptide receptor (NPR-A), a key regulator of urinary sodium [96]. VDR immunoregulatory and anti-inflammatory effects may regulate atherogenesis and kidney injury [97,98]. In cardiomyocytes, calcitriol inhibited cell proliferation and apoptosis, and enhanced cardiomyocyte formation [99]. In mesenchymal multi-potent cells, calcitriol activated the VDR and increased anti-fibrotic factors, such as bone morphogenetic protein (BMP) 2, 7 and matrix metalloproteinase 8, and decreased expression of TGF-β1, plasminogen activator inhibitor-1, and collagens I and III [100]. In this regard, calcitriol led to regression of left ventricular hypertrophy and improved survival [101]. Clinical studies have demonstrated that RAAS inhibition slows the progression to ESRD in proteinuric CKD [102–105]. In hypertensive patients, renin activity is inversely related with plasma calcitriol levels [106]. VDR knockout mice or mice with deficient calcitriol synthesis developed hypertension and increased renin and angiotensin II expression and left ventricular hypertrophy, while the VDR activation decreased renin expression and left ventricular hypertrophy [107–110]. In Dahl salt-sensitive rats, paricalcitol decreased left ventricular mass, increased fractional ejection shortening, and lowered myocardium brain and atrial natriuretic peptides, and renin expression [111]. In uraemic rats, paricalcitol decreased renin, renin receptor, angiotensin II, angiotensin AT1 receptor, blood pressure, proteinuria, glomerular sclerosis, tubulointerstitial injury, vascular endothelial growth factor (VEGF) and TGF-β1 [112]. VDR activation decreases inflammation and nuclear factor kappa B (NF-κB) activation in vivo and in cultured renal cells [113]. VDR activation is also nephroprotective in experimental diabetic nephropathy [114]. In patients with acute renal inflammation, decreased serum 1,25(OH)D and 25(OH)D were associated with increased renal inflammation (increased urinary macrophage chemoattractant protein-1) [115]. Yet, many aspects of the VDR–RAAS relationship remain poorly characterized, including the interaction between VDR and angiotensin AT2 receptor, a receptor mediating vasodilatation and inhibition of cell growth; the generation of angiotensin 1–7 (Mas receptor activator) from angiotensin II; or the interaction with angiotensin-converting enzyme 2, a cardiorenal protective enzyme [107,116].

The potential mechanisms involved in cardiovascular protection by calcitriol include anti-inflammation, anti-atherogenesis, inhibition of cardiac hypertrophy and proliferation of myocytes, and regulation of RAAS with preservation of renal function [117]. VDR activation by paricalcitol decreased the expression of genes involved in vascular cell growth, thrombogenicity, fibrinolysis and endothelial regeneration in human coronary artery vascular cells [118]. In humans, VDR activation reduced left ventricular hypertrophy in haemodialysis patients [119,120]. These findings provide a biological basis for the benefit afforded by VDR agonists in epidemiological studies. However, VDR activation may carry a risk of vascular calcification. Thus, while VD deficiency is associated with increased inflammation, reduced endothelial protecting factors and a pro-atherogenic status; VD overdose leads to hypercalcaemia, and increases metalloproteinases, vascular calcification, arterial stiffness and left ventricular hypertrophy [121]. Vascular calcification may be a consequence of excessive suppression of PTH by VDR activators leading to a dynamic bone disease [122]. In a cross-sectional study of dialysis children treated with daily oral alfacalcidol, there was a U-shaped distribution for vascular disease [123]. Patients with high and low 1,25(OH)D levels had greater carotid intima-media thickness and calcification scores than patients with normal levels. Furthermore, 1,25(OH)D levels showed a linear correlation with serum calcium–PO4 product. Inflammation, assessed as hs-CRP, was higher in patients with vascular calcification and inversely correlated with 1,25(OH)D levels [123].

Paricalcitol has a lower risk of vascular calcification than calcitriol [124,125]. In experimental CKD vascular calcification, calcitriol (20 ng/kg) and paricalcitol (100 ng/kg) (dosages just sufficient to decrease PTH levels) reduced neointimal vascular calcium content and aortic gene expression of osteoblastic activity markers [126]. However, a higher paricalcitol (400 ng/kg) dose increased calcification and expression of osteoblast transcription factors.

**VDR agonists as immunomodulatory agents in autoimmune diseases and transplantation**

VDR is expressed in most cells of the immune system, including macrophages and dendritic cells, and CD4+ and CD8+ T-lymphocytes. 1-α-Hydroxylase in macrophages, dendritic cells and skin keratinocytes is stimulated by cytokines but not by PTH, and is not inhibited by calcitriol [127]. In inflammatory/granulomatous disorders, macrophage 1-α-hydroxylase is not suppressed by the excessive calcitriol in the circulation. However, in kidney disease, peripheral blood monocyte 1-α-hydroxylase is exquisitely sensitive to feedback inhibition by physiological concentrations of serum 1,25(OH)D [128]. VDR activation inhibits Th1 cell, promotes Th2 cell development, and modulates the function of macrophages and dendritic cells, favouring self-tolerance and induction of regulatory CD4+CD25+ T-cells [129,130]. These properties are related to transrepression by 1,25(OH)D of inflammatory cytokines that may be related to interference with NF-κB activation [86].

Topical VDR agonists are effective in the clinical psoriasis [131]. Experimentally, VDR agonists prevent systemic lupus erythematosus (SLE) in MRL/lpr/lpr mice, allergic encephalomyelitis, collagen-induced arthritis, Lyme’s disease, inflammatory bowel illness and autoimmune diabetes in non-obese diabetic mice [132–135]. VDR agonists are synergistic with immunosuppressive agents such as cyclosporine A, mycophenolate mofetil and sirolimus in murine models of graft tolerance [136]. In experimental murine SLE, calcitriol improved kidney disease and survival, similar to high doses of steroids, findings that require confirmation [137–139].

VDR polymorphisms have been studied for susceptibility and severity of lupus [140]. Most studies are small and comprise mainly Asian populations. Some found associations between genotype BB, SLE and lupus nephritis...
Multiple factors contribute to 25(OH)D and 1,25(OH)D deficiency in CKD. Both 25(OH)D and 1,25(OH)D deficiency contribute to multiple pathophysiologic processes that may impact progression of CKD and its complications leading to increased morbidity and mortality. AMI, acute myocardial infarction; CKD, chronic kidney disease; DBP, VD-binding protein; ESRD, end-stage renal disease; FGF-23, fibroblastic growth factor 23; GFR, glomerular filtration rate; LV, left ventricular; PAD, peripheral arterial disease; RAAS, renin–angiotensin–aldosterone system.
mature cardiovascular disease (up to 50 times vs controls), panic patients [146,147]. SLE leads to a higher rate of pre
poor outcome groups, such as African American and His-
lupus disease activity, and its prevalence was higher in [144,145]. 25(OH)D deficiency is associated with higher
specific severe in cases of renal disease and photosensitivity
FokI VDR polymorphisms [142,143]. In lupus patients,
African American and Hispanic patients [146,147]. SLE leads to a higher rate of pre-
ants, such as African American and Hispanic patients [146,147]. SLE leads to a higher rate of pre-
In human kidney transplantation, the prevalence of 25 (OH)D and 1,25(OH)D deficiency is very high in the first
year post-transplant [153], and low 1,25(OH)D levels predict delayed graft function and poor outcomes, such as
cancer and death [154]. High-dose VDR activator is re-
quired to control hyperparathyroidism without apparent se-
vere adverse events [155]. However, clinical studies on VD status, immune function, and graft and patient survival are scarce. Clearly, we need randomized clinical trials to deter-
mine the target serum 25(OH)D levels that modulate the
immune system, enhance the activity of current immuno-
modulators, and especially improve cardiovascular and
overall prognosis of patients with chronic inflammatory
diseases, including kidney transplantation.

Vitamin D deficiency, VDR activation, glucose metabolism and metabolic syndrome

Experimental studies and clinical observations suggest an association between VD deficiency, abnormal glucose me-
tabolism, type 1 and type 2 diabetes mellitus (DM), and metabolic syndrome. Modulation of the immune system
may preserve long-term beta-cell function and prevent type
1 DM [1]. Calcitriol inhibits pancreatic β cell infamma-
tion and the onset of type 1 DM in non-obese diabetic-
prone mice, and reduces streptozotocin-induced diabetes
[156–158]. In humans, vitamin D supplementation reduces the risk of developing type 1 DM [159–161]. In healthy
individuals, there is an inverse correlation between serum
25(OH)D and glucose concentration or insulin resistance
[162,163]. In type 2 DM, cholecalciferol decreased plasma
glucose in obese Wistar rats [164]. In observational studies,
low 25(OH)D levels are associated with reduced insu-
lin sensitivity, increased risk of metabolic syndrome, and
type 2 DM [165–167]. Clinical trials suggest that com-
bined vitamin D and calcium supplementation may prevent
type 2 DM in high-risk populations such as people with
hypertension and CKD [168]. Nevertheless, there
were important limitations such as few subjects, short dur-
ation, and different formulations of vitamin D and calcium
supplementation.

Several potential mechanisms may account for the action
of vitamin D on glucose and insulin metabolism [162].
VDR stimulates insulin secretion, insulin receptor expres-
sion and insulin responsiveness [169–171], and oral chole-
calciferol increased insulin secretion post-glucose load in
type 2 DM patients [172]. Indirectly, VDR activation may
reverse the reduced insulin sensitivity associated with in-
creased PTH activity [173,174]. However, some studies
did not show consistent results [172,175,176]. In dialysis
patients, VDR activation may increase insulin secretion and sensitivity, and glucose uptake [172,177,178]. The spe-
cific role of vitamin D therapy (non-active or active analogues) in patients with and without vitamin D deficiency and metabolic syndrome or DM requires further study.

Additionally, calcitriol suppresses cell growth and reg-
ulates apoptosis, epidermal differentiation, anti-microbial
responses, and muscle and nervous system function
[179–184] (Figure 3). Different therapeutic applications
are being explored, including cancer therapy.

Conclusions

Vitamin D deficiency, defined as serum 25(OH)D levels
<20 ng/mL, is a common condition in the general popula-
tion and in chronic inflammatory disorders, especially in
CKD, which favours the development of metabolic bone
disease. Current clinical and experimental evidence
strongly suggest that vitamin D deficiency is a new risk fac-
tor for progression of kidney and cardiovascular disease.
This relationship is most evident in CKD. In CKD patients,
it is likely that therapy with nutritional vitamin D, calcitriol
and/or VDR activators improves cardiovascular outcomes
and patient survival. The widespread expression of VDR in
many organ systems constitutes the biological basis for
the pleiotropic and non-skeletal actions of vitamin D. These
properties include RAAS inhibition, endothelial protec-
tion, immune modulation and anti-inflammatory actions.
In this regard, vitamin D deficiency is associated with in-
sulin resistance, left ventricular hypertrophy, proteinuria,
atherogenicity, decreased thrombolysis, immune imbal-
cances, susceptibility to infections and perpetuation of in-
fammation (Figure 4). Studies on vitamin D and survival
have generated controversy because of the observational
design or short follow-up in clinical trials. Further clinical
trials should more accurately define the precise therapeutic
agent, dose, timing, monitoring parameters and indications
of vitamin D therapy. Meanwhile, simple and relatively in-
expensive therapeutic measures, such as supplementing
ergocalciferol or cholecalciferol to normalize serum 25
(OH)D, seem warranted. Studies addressing the biological
actions of VDR activators should be performed in patients
with optimized 25(OH)D levels. Otherwise, it is difficult
to conclude that, in vivo, they have intrinsic properties
different from natural vitamin D.

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