An Insight Into The Impact of Vitamin D on Cardiovascular Outcomes in CKD

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Abstract

Patients with chronic kidney disease (CKD) experience excess cardiovascular morbidity and mortality that is unexplained by traditional cardiovascular risk factors. Vitamin D deficiency is highly prevalent in CKD and is associated with increased cardiovascular mortality in both the general population and in CKD patients. Vitamin D supplementation is a reasonably safe and simple intervention and meta-analyses of observational studies have suggested that vitamin D supplementation in CKD improves cardiovascular mortality. However, randomised controlled trials (RCTs) examining the impact of vitamin D supplementation in improving surrogate markers of cardiovascular structure and function remain inconclusive. This review investigates the impact of vitamin D supplementation on surrogate end-points and cardiovascular events from trials in CKD; and discusses why results have been heterogenous, particularly critiquing the effect of different dosing regimens and the failure to take into account the implications of vitamin D supplementation in study participants with differing vitamin D binding protein genotypes.

<u>Main text</u>

Introduction

Patients with chronic kidney disease (CKD) experience excess cardiovascular morbidity and mortality, usually secondary to coronary heart disease and heart failure.[1, 2] Traditional cardiovascular risk factors often present in CKD are unable to explain this excess risk, hence the interest in non-traditional risk factors such as vitamin D deficiency.

Vitamin D has a number of extra-skeletal physiological roles, including immunomodulation and cardiovascular protection.[3] Associations between hypovitaminosis D and increased cardiovascular mortality has been demonstrated in both the general population and in CKD.[4] Nutritional sources of vitamin D, cholecalciferol (D_3) and ergocalciferol (D_2), undergo two hydroxylation steps.[3] The first occurs in the liver, forming 25-hydroxy vitamin D (25[OH]D), whilst the second occurs in the kidneys via the enzyme 1-α hydroxylase, forming active hormonal 1,25-dihydroxy vitamin D (1,25[OH]$_2$D).[3] Vitamin D deficiency is highly prevalent in CKD and there are several reasons for this.[5] As CKD progresses, increased functional loss of renal tissue decreases the availability and functionality of 1-α hydroxylase, thereby decreasing 1,25(OH)$_2$D levels.[6] Additionally, CKD patients receive less nutritional vitamin D, since they often undergo intensive dietary restriction and lack sun exposure due to reduced mobility.[6]

As cardiovascular mortality is the commonest, often premature cause of mortality among those with CKD, the nephrology community has long sought for interventions that could improve the cardiovascular death in these patients. Vitamin D supplementation is reasonably safe and a simple intervention that could potentially help in this regard. Indeed, the meta-analysis by Duranton et al. demonstrated that active vitamin D supplementation in CKD reduced the relative risk of cardiovascular mortality by 37%.[4] However, the studies

reviewed therein were of observational design. In recent years, the impact of vitamin D on cardiovascular outcomes have been extensively investigated through randomised controlled trials (RCTs). Unfortunately, the results have been contradictory. This review investigates the impact of vitamin D on surrogate endpoints and hard cardiovascular outcomes in CKD. It also discusses why the results of these trials have been heterogeneous, particularly critiquing the effect of different dosing regimens and individual human biology.

Abnormal Cardiovascular Structure and Function in Chronic Kidney Disease Patients

Given the excess cardiovascular morbidity and mortality CKD patients experience, it is not surprising that they demonstrate abnormal cardiovascular structure and function. About 75% of non-dialysis CKD patients display left ventricular hypertrophy (LVH), making it one of the most prominent cardiovascular abnormality in CKD.[8] The progression of LVH and change in systolic as well as diastolic functions have been shown to be closely related to the severity of kidney dysfunction.[8]

Endothelial dysfunction, arterial stiffness, and accelerated atherosclerosis are common in CKD.[7] Traditional and non-traditional risk factors promote the development of endothelial dysfunction, which eventually leads to the development of atheromas. Intimal and medial calcification contribute to vascular stiffness, especially in association with progressive atherosclerotic lesions.

Flow-mediated dilation (FMD), an indicator of endothelial function that measures endothelial-dependent vasodilation, predicts CVD events in CKD.[9] Nitroglycerin-mediated dilation (NMD) assesses endothelial-independent vasodilation which reflects vascular smooth muscle cell (SMC) function in response to an exogenous nitric oxide (NO) source. Pulse wave velocity (PWV) and aortic augmentation index (AIx) evaluate arterial stiffness. PWV is

an independent predictor of CVD and mortality.[9] Left atrial volume indexed to body surface area (LAVi) is a sensitive indicator of diastolic dysfunction.[10] Patients with increased LAVi have a higher risk for CVD events.[10] Left ventricular mass index (LVMI) and ejection fraction (EF) are also important markers. The impact of vitamin D on surrogate markers of these abnormalities in CKD have been investigated in both observational and interventional studies, including randomised controlled trials.

Vitamin D Supplementation and Endothelial Dysfunction

Table 1 summarises findings from RCTs investigating the impact of vitamin D supplementation on cardiovascular structure and function. The effect of vitamin D on improving endothelial dysfunction remains contradictory. Kumar et al. provides strong evidence that the correction of vitamin D deficiency improves FMD in pre-dialysis CKD patients.[9] These findings agree with results from the PENNY trial, where 12-week paricalcitol supplementation improved FMD in CKD stage 3-4.[11] However, unlike the study by Kumar and colleagues, improvements in NMD did not occur in the PENNY trial. Kumar et al. only recruited those with true vitamin D deficiency, which could explain the most impressive effects seen. In contrast, Thethi et al. did not demonstrate any improvements in both FMD and NMD with paricalcitol supplementation.[12] However, this trial recruited diabetic CKD patients only, whereas there was a limited number of diabetics in the PENNY trial and Kumar's group excluded diabetics entirely. The study by Kendrick and colleagues was also negative but they found no difference in the response of vitamin D on FMD by diabetes status.[13] Considering this, it is unclear if the effect of vitamin D supplementation varies by diabetes status. The authors discussed that their negative findings may be due to insufficient doses of D_3 and calcitriol, as optimal doses for CKD patients are unknown.[13]

Vitamin D Supplementation and Vascular Stiffness

Kumar's group also demonstrated that D_3 therapy improves PWV (Table 1).[9] This is concordant with the study by Levin et al., where calcifediol supplementation improved PWV.[14] However, PWV remain unchanged in the group receiving calcitriol in the latter study. Generally, those who achieved the highest serum levels of vitamin D following supplementation had the greatest improvement in PWV. Mose et al. investigated the impact of D_3 supplementation on cardiac function in dialysis patients.[15] However, their study was only powered to detect a difference in plasma brain natriuretic peptide (BNP) levels. The authors could not exclude that a larger sample size could reveal differences in other measures assessed, including PWV, AIx, LVMI, and EF. Similarly, Marckmann et al. did not demonstrate improvements in PWV or AIx with D_3 administration.[5] They too discussed that their study population was small and that their short intervention period of 8 weeks may not be enough to show improvements in the markers measured.[5]

Vitamin D Supplementation and Cardiac Structure and Function

The PRIMO (Paricalcitol Capsule Benefits in Renal Failure–Induced Cardiac Morbidity) trial demonstrated that paricalcitol supplementation over 48 weeks improved LAVi (Table 1).[10] Decreases in LAVi was accompanied by attenuation in the rise in BNP. However, LVMI, EF, and Doppler measurements of diastolic function did not significantly change. The discrepancy between LAVi and Doppler measurements in evaluating diastolic function can be explained by the small sample size, which may not be enough to account for the known variability of Doppler measurements.[10] Interestingly, a synergistic effect between paricalcitol and renin-angiotensin-aldosterone-system (RAAS) inhibition in decreasing LAVi

was found. The largest decline in LAVi occurred in patients receiving both paricalcitol and RAAS inhibitors.[10] In contrast, the study by Thadhani et al. failed to demonstrate improvements in LVMI, EF, or echocardiographic measures of diastolic function with paricalcitol therapy. However, paricalcitol attenuated the rise in BNP and reduced CVD hospitalisations.[10] Wang et al. also found a lower incidence of cardiovascular-related hospitalisations with paricalcitol supplementation.[16] Nonetheless, they too did not demonstrate improvements in LVMI, EF, or diastolic function with paricalcitol.

Dose of Vitamin D and Hypercalcaemia in Randomised Controlled Trials

The results of the randomised trials discussed above have been contradictory and inconclusive. This may be due to the heterogeneity in patient populations and dosing regimens. Doses have been kept low in studies, presumably to avoid hypercalcaemia, which was uncommon in all studies. Due to this, vitamin D levels did not rise to biologically significant levels. Additionally, some of the studies were of short duration. These factors may explain the lack of improvement in surrogate markers seen in certain studies.

The studies by Kumar et al.,[9] Kendrick et al.,[13] and Marckmann et al.,[5] all used D_3 as one of their study interventions in investigating the effect of vitamin D on endothelial dysfunction and/or vascular stiffness (Table 1). The total dose of D_3 over the study period used by Kumar and colleagues was the highest amongst the three (600,000 IU), with 25(OH)D levels increasing by 24.91 ng/ml. This study was the only one that demonstrated improvements in all parameters measured. Importantly, no patient developed severe hypercalcaemia. Contrast this with the studies by Kendrick et al. and Marckmann et al., who administered less total dose of D_3 over the study duration, at 420,000 IU and 320,000 IU, respectively. They showed no change in the surrogate markers investigated. Notably, only 1

of 58 patients in Kendrick's D_3 group developed hypercalcaemia.[13] 5 of 25 patients in Marckmann's intervention group developed hypercalcaemia, but only 1 case was severe.[5] The intervention group in their study achieved the highest levels of 25(OH)D at a median of 62 ng/ml, but there was no improvement in vascular stiffness.[5] Their intervention lasted only 8 weeks, and it is possible that prolonged therapy with vitamin D could have improved certain markers.

Paricalcitol therapy was utilised in the studies by Zoccali et al. and Thethi et al. (Table 1).[11, 12] With a dose of 2 mcg/day used by Zoccali's group, improvements in endothelial function was demonstrated. Thethi's group used a dose of 1 mcg/day, and failed to find a change. The incidence of hypercalcaemia was not discussed in their study, but only 2 of 44 patients in Zoccali's intervention group developed frank hypercalcaemia (>2.75 mmol/L).

Paricalcitol was also used by Tamez et al.,[10] Thadhani et al.,[17] and Wang et al.,[16] but here it was to assess its effects on cardiac structure and function. Using a dose of 2 mcg/day, Tamez' study (post-hoc analysis of PRIMO trial) demonstrated improvements in LAVi, but not in other markers measured. However, LAVi is of more prognostic value compared to left ventricular morphology and function in patients with CKD.[10] Thadhani and colleagues utilised the same dose over the same study duration, but improvements in cardiac structure and function were not shown. This is akin to the results reported by Wang's group, who only used a dose of 1 mcg/day. The incidence of hypercalcaemia (serum calcium >2.62 mmol/L). in the study by Thadhani et al. was 22.6% It was 43.4% in the study by Wang's group (>2.55 mmol/L). The majority of participants in Wang's study were taking calcium-based phosphate binders. Upon stopping the binders, hypercalcaemia resolved without needing to adjust the paricalcitol dose.[16]

The importance of greater doses was evident in the trials evaluating endothelial dysfunction and vascular stiffness. This is less clear with the trials assessing cardiac structure and function, but Tamez and colleagues showed that a dose of at least 2 mcg/day of paricalcitol was needed to elicit any sort of improvement. Longer study durations may be needed to demonstrate improvements, as reductions in LAVi was further augmented between the 24th and 48th week in the study by Tamez' group.[10] It is important to stress that frank hypercalcaemia was uncommon in most studies. Considering this, increasing the doses and prolonging therapy may be important in uncovering improvements in surrogate markers assessed.

Another consideration to be made is the effect of different vitamin D formulations used on circulating 25(OH)D levels. 25(OH)D is the only vitamin D metabolite used to determine vitamin D sufficiency. $1,25(OH)_2D$ is not an ideal measure for vitamin D status as the circulating half life of it is only 4-6 hours (compared to 2-3 weeks for 25[OH]D) and its circulating levels are a thousand fold less than 25(OH)D (c). Furthermore, vitamin D deficiency leads to decreased intestinal calcium absorption, causing increased production and secretion of parathyroid hormone (PTH) (c). This subsequently increases the renal production of $1,25(OH)_2D$. Hence, those with vitamin D insufficiency and deficiency have normal or elevated $1,25(OH)_2D$, making it a useless measure of vitamin D status (c). However, different vitamin D formulations have variable effects on circulating 25(OH)D. For example, 25(OH)D levels increased greatly with calcifediol supplementation in the study by Levin et al., but as expected decreased with calcitriol supplementation (Table 1). Thus, different formulations limit the ability to evaluate improvements in overall vitamin D status when it is defined by the level of circulating 25(OH)D.

Meta-analyses of observational studies have shown an association between vitamin D supplementation and improvement in all-cause mortality, cardiovascular mortality, and all clinically significant end-points.[4, 18] However, existing RCTs do not provide the same reassurance that vitamin D supplementation improves these hard end-points (Tables 2-4).[5, 16, 17, 19-32] There seems to be no difference in the occurrence of events when comparing those treated with vitamin D derivatives and controls. Nevertheless, it is important to realise that these studies were not designed to assess the impact of vitamin D therapy on hard-end points. Only one of the RCTs listed mortality as an a priori primary or secondary outcome.[20] Furthermore, the intervention duration and dosing regimens in individual trials differed substantially. As with the RCTs evaluating the impact of vitamin D supplementation on surrogate end-points, doses and the duration of treatment in certain studies may not have been enough to demonstrate differences. Thus, existing RCTs remain inconclusive about the effect of vitamin D supplementation on mortality and adverse cardiovascular outcomes in patients with CKD. This agrees with the meta-analysis of RCTs by Mann et al., who found no significant treatment effect of oral vitamin D on these end-points (b). However, they too cited that the heterogeneity between trials did not allow for appropriate evaluation of the impact of vitamin D therapy on cardiovascular risk and mortality (b).

The SIMPLIFIED trial (ISRCTN15087616) is the only ongoing RCT evaluating the impact of vitamin D supplementation on hard end-points. Study participants are randomised either to D_3 therapy with a dose of 60,000 IU to be taken fortnightly or to receive normal standard care for five and a half years. The primary outcome measure is all-cause mortality which will be determined from seven years after the start of the study. The secondary

outcomes of the study include hospitalisation-requiring composite cardiovascular events. This study is only recruiting adults with dialysis-requiring end stage renal disease.

More large-sized RCTs like the SIMPLIFIED trial that specify hard end-points as the primary or secondary outcome are needed to assess any potential benefit with vitamin D supplementation. Additionally, it is important that doses are high enough and the intervention lasts long enough before any conclusion can be drawn on the impact of supplementation on outcomes.

Vitamin D and Human Biology

Vitamin D mediates its effects on target cells by binding to the vitamin D receptor (VDR).[5, 33] Vitamin D is bound to the VDR, which forms a liganded complex with the retinoid X receptor (RXR) after internalization.[33] This complex then binds to vitamin D responsive elements in the promoter regions of vitamin D target genes, enabling the recruitment of transcriptional co-activators.[33] Important co-activators include p160 and steroid receptor activators (SRC-1, SRC-2, SRC-3). Together, they lead to changes in gene expression.[33] Genes regulated by vitamin D include but are not limited to RANKL, LRP5, CYP24A1, TRPV6, ENNP1, ENNP3, PDLIM2, GLUL, and SLC1A1.[33] VDR expression is almost ubiquitous. It is found in the endothelium, vascular SMCs, and cardiomyocytes.[10, 34] These cells can also locally convert circulating $25(OH)D$ to $1,25(OH)_2D$, as they carry local $1-\alpha$ hydroxylase, and hence making active vitamin D available for local action when CKD patients are supplemented with $25(OH)D$.[34] $1,25(OH)D$ is about 1000 times more potent in binding and mediating the local actions requiring far lower concentrations for VDR activation.[3]

Balanced production of endothelium-derived relaxing and contracting factors (EDRF and EDCF) ensures normal endothelial function. Imbalances in the production of these factors is associated with endothelial dysfunction.[33] Vitamin D increases endothelial NO synthase expression, which is a synthesising enzyme of the EDRF NO.[9, 33] In addition to maintaining endothelial function, an equally critical role of NO is to regulate LV (left ventricular) diastolic function.[11] The coronary artery endothelium releases NO, which accelerates LV relaxation and increases LV distensibility.[11] Thus, VDR activation is also important in increasing left ventricular (LV) lusitropy, hence critical for LV diastolic function.[11]

The RAAS is important in controlling vascular tone and volume homeostasis. Elevated activity in the setting of CVD is initially protective, as it allows the maintenance of cardiac output. However, it eventually leads to hypertension and cardiac hypertrophy, causing cardiac insufficiency. Vitamin D directly blocks renin expression by inhibiting the activity of the cyclic AMP response element in the renin gene promoter, thus suppressing the RAAS.[35] Furthermore, mice with cardiomyocyte-selective deletion of VDR develop cardiac hypertrophy independent of changes in the RAAS, suggesting that it may have a direct anti-hypertrophic effect on the heart.[36]

Vitamin D may also be important to maintain the extracellular matrix integrity of the heart. Increased matrix metalloproteinase (MMP)-2 and MMP-9 and decreased tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 activity has been demonstrated in VDR knockout mice.[37] This contributes to both systolic and diastolic dysfunction, as it is associated with increased fibrotic lesions.[10, 37]

It is important to discuss that the effect of vitamin D on vascular stiffness and structure may be non-linear, thus it can be both detrimental and protective to the vasculature. The anti-inflammatory effects of vitamin D is protective against atherosclerosis: it inhibits

the release of inflammatory cytokines and blocks foam cell formation (a). However, vitamin D also induces vascular SMC osteogenic differentiation, which promotes MMP production leading to vascular remodeling (a). High doses of vitamin D may promote calcification, though the development of atherosclerosis and vascular stiffness also occurs in the setting of deficiency.

The vitamin D binding protein (DBP) functions as the primary carrier protein for all vitamin D metabolites in serum, binding 85-90% of total circulating 25(OH)D.[38] The non-DBP fraction, also referred to as bioavailable 25(OH)D, consists mainly of albumin-bound 25(OH)D and free 25(OH)D.[39] They make up 10-15% and less than 1% of total 25(OH)D, respectively.[39]

Studies have shown that DBP is highly polymorphic. Three phenotypic variants (*Gc1f, Gc1s, Gc2*) are commonly recognised and affect protein function, which has implications on vitamin D metabolism.[39] These variants are determined by two single-nucleotide polymorphisms (SNPs) in the coding region of the DBP gene (rs4588 and rs7041).[39] They determine serum DBP concentrations and cause changes in the amino acid sequence of DBP and appear to alter the binding affinity for vitamin D ligands.

The *Gc1f* phenotype has the highest affinity for vitamin D metabolites and *Gc2* the lowest.[40, 41] In the HANDLS study, *Gc1f* homozygous participants had the lowest DBP levels whilst *Gc1s* homozygous participants had the highest. *Gc2* homozygous had intermediate levels of DBP.[39] The prevalence of these polymorphisms differ between racial groups.[33, 39] The *Gc1f* form of DBP is much more common amongst Black and Asian populations, while Whites are more likely to carry the *Gc1s* form of DBP. The *Gc2* form is rare in Blacks but more frequent in people of Asian and European ancestry.

In the HANDLS study, racial differences in total 25(OH)D levels were apparent, with levels of 17.3 ± 0.3 ng/ml in Blacks vs. 25.5 ± 0.4 ng/ml in whites after multivariable

adjustment. DBP levels were also lower; 168 ± 3 mcg/ml in Blacks and 337 ± 5 mcg/ml in Whites. However, levels of bioavailable 25(OH)D were equivalent in Blacks and Whites. Vitamin D sufficiency has been determined at the level where total 25(OH)D causes calcium absorption to decline or PTH levels to increase.[42, 43] The exact level of total 25(OH)D where these changes occur is controversial, as experimental data are inconclusive.[20, 21] 77-96% of Black participants in the HANDLS study would be classified as vitamin D deficient if current guidelines are used (threshold for sufficiency of 20 or 30 ng/ml).[39] However, identifying them as vitamin D deficient would conflict with the observations of higher bone-mineral density, higher calcium levels, and only slightly higher PTH levels compared to the White study participants. Although levels of total 25(OH)D are low in Blacks, their low levels of DBP seems to be protective against the manifestations of vitamin D deficiency.[39] The bio-availability of other lipophilic hormones are influenced by the concentration of their carrier proteins. For example, lower total thyroid hormone levels are required for sufficiency when the concentration of thyroxine-binding globulin is low or undetectable.[44] Indeed, mice without DBP have low levels of 25(OH)D but do not demonstrate signs of vitamin D deficiency.[45] Considering these findings altogether, low total 25(OH)D may not indicate true vitamin D deficiency when DBP levels are low. Thus, bioavailable 25(OH)D may be a more appropriate marker for vitamin D sufficiency. Importantly, in the HANDLS study, genetic variants independently explained 79.4% of the variation in DBP levels after adjustment for other factors.[39] After genetic variants were accounted for, race explained <0.1% of the variation in DBP levels.[39]

The effect of vitamin D supplementation on total and bioavailable 25(OH)D levels in persons with different DBP genotypes is unclear. Studies evaluating the utility of vitamin D supplementation on soft and hard cardiovascular end-points in CKD patients have not taken this into account. Studies have hypothesized that correcting vitamin D deficiency would

improve cardiovascular outcomes. However, sufficiency has been determined using 25(OH)D levels. Participants who have been recruited with low 25(OH)D levels may not have been truly vitamin D deficient when considering DBP levels. Hence, it is possible that further vitamin D supplementation would provide no further benefit in certain patients. Thus, may partly explain the lack of positive findings in RCTs. It would be useful for future RCTs to consider the implications of different DBP genotypes in recruited participants.

Conclusion

Meta-analyses of observational studies have suggested that vitamin D supplementation in CKD patients improves mortality, including cardiovascular mortality.[4, 18] However, RCTs evaluating the utility of vitamin D supplementation in improving surrogate markers of cardiovascular structure and function remain inconclusive. In many cases, the interventional dose has been kept low in fear of developing hypercalcaemia, which is uncommon. Thus, vitamin D levels in study participants did not rise significantly, which may explain the negative findings. Furthermore, the use of different vitamin D formulations limit the ability to compare improvements in overall vitamin D status between studies. Meanwhile, there are no completed RCTs that are appropriately designed to evaluate the impact of vitamin D supplementation on hard end-points in CKD. The SIMPLIFIED trial is projected to finish in 2025 and will hopefully provide an improved understanding of the role of vitamin D supplementation in improving all-cause and cardiovascular mortality in CKD patients.

Currently, the implications of vitamin D supplementation in patients with differing DBP genotypes is unclear. Indeed, previous trials have not taken this into account. More research is needed to further clarify the role of DBP in enabling true vitamin D sufficiency.

Also, further research to understand the mechanism by which vitamin D effects the cardiovascular system is needed.

With these considerations in mind, the nephrology community may be able to find the right dose for the right patient to improve cardiovascular morbidity and mortality in CKD the population.

Acknowledgements

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Tables

Legend: *n* number of participants, *CKD* chronic kidney disease, *D₃* cholecalciferol, *FMD* flow-mediated dilation, *NMD* nitroglycerin-mediated dilation, *PWV* pulse wave velocity, *LAVi* left atrial volume index
†(95% confidence interval)
‡median (interquartile range)
§Calcium levels of study is unreliable

Table 1. Summary of randomised controlled trials examining impact of vitamin D on cardiovascular structure and function

Study	n	Population	Intervention	Duration	Changes in surrogate markers	Changes in 25(OH)D (ng/ml) and Calcium (mmol/L)
Kumar et al.[9]	120	CKD 3-4	D_3 300,000 IU x 2 (at baseline and 8 weeks) Placebo	16 weeks	**Improvements in FMD, NMD, PWV**	**25(OH)D**: 13.40 ± 4.42 (baseline), changed by 24.91 (21.77-28.06)† by week 16 **Calcium**: 2.25 ± 0.18 to 2.30 (2.23-2.36)†
Zoccali et al.[11]	89	CKD 3-4	Paricalcitol 2 mcg/day Placebo	12 weeks	**Improvements in FMD** **No changes in NMD**	**25(OH)D**: increased by 2 (0.4-3.6) **Calcium**: increased by 0.07 (0.03-0.11)
Thethi et al.[12]	60	CKD 3-4 (diabetic)	Paricalcitol 1 mcg/day Placebo	12 weeks	**No changes in FMD, NMD**	**1,25(OH)₂D**: 45.0 (27.0–91.0)‡ pg/ml (baseline) **Calcium**: not reported
Kendrick et al.[13]	128	CKD	Calcitriol 0.25 mcg/day for month 1, increased to 0.5 mcg/day for months 2-6 if episodes of hypercalcaemia do not occur	6 months	**No changes in FMD, NMD**	**Calcitriol group 25(OH)D**: 21.7 ± 7.7 to 21.2 ± 7.5 **D₃ group 25(OH)D**: 23.0 ± 7.6 to 34.8 ±

			D$_3$ 4000 IU/day for month 1, 2000 IU/day for months 2-6			9.3 **Calcitriol group calcium**: 2.25 ± 0.1 to 2.27 ± 0.15 **D$_3$ group calcium**: 2.27 ± 0.1 to 2.25 ± 0.1
Levin et al.[14]	119	CKD 3B-4	Calcitriol (1,25[OH]$_2$D) 0.5 mcg x 3/week Calcifediol (25[OH]D) 5000 IU x 3/week Placebo	6 months	**Improvements in PWV** in calcifediol group	**Calcitriol group 25(OH)D**: 26.6 ± 10.8 to 23.8 ± 8.9 **Calcifediol group 25(OH)D**: 25.8 ± 9.6 to 94.1 ± 51.9 **Calcitriol group calcium**: 2.32 ± 0.12 to 2.35 ± 0.12 **Calcifediol group calcium**: 2.30 ± 0.12 to 2.32 ± 0.12
Mose et al.[15]	64	CKD (dialysis)	D$_3$ 3000 IU/day Placebo	6 months	**No changes in PWV, AIx, LVMI, EF**	**25(OH)D**: 11.2 (8-19.2)‡ to 33.6 (26-50)‡ **Calcium**§
Marckmann et al.[5]	54	CKD	D$_3$ 40,000 IU/week Placebo	8 weeks	**No changes in PWV, AIx**	**25(OH)D**: 9.6 (6.8–16.4)‡ to 62 (32.4–96)‡ **Calcium**: 2.27 (2.21–2.27)‡ (baseline), changed by 0.05 (-0.07-0.22)‡ by week 8

Tamez et al.[10]	196	CKD	Paricalcitol 2 mcg/day Placebo	48 weeks	**Improvements in LAVi** **No changes in LVMI, EF**	**25(OH)D**: not reported **Calcium**: 2.40 (2.30-2.45)‡ (baseline), changed by 0.07 (0.05-0.10)† by week 48
Thadhani et al.[17]	227	CKD	Paricalcitol 2 mcg/day Placebo	48 weeks	**No changes in LVMI, EF, diastolic function**	**25(OH)D**: not reported **Calcium**: 2.40 (9.30-9.45)‡ (baseline), changed by 0.08 (0.05-0.11)† by week 48
Wang et al.[16]	60	CKD 3-5 (non-dialysis)	Paricalcitol 1 mcg/day Placebo	52 weeks	**No changes in LVMI, EF, diastolic function**	**25(OH)D**: not reported **Calcium**: 2.32 ± 0.10 to 2.39 ± 0.11

Legend: *n* number of participants, *CKD* chronic kidney disease, *HD* haemodialysis, *D₃* cholecalciferol, *D₂* ergocalciferol, *PTH* parathyroid hormone, *RCT* randomised controlled trial

Table 2. Summary of randomised controlled trials examining impact of vitamin D supplementation on all cause mortality

Study	Population	Intervention	Duration	n placebo	n treatment	Events in Placebo Group	Events in Treatment Group
Alvarez et al.[19]	CKD 2-3	D_3 50,000 IU/week for 12 weeks followed by 50,000 IU every other week for 40 weeks	52 weeks	24	22	1	1
Bhan et al.[20]	CKD (HD)	D_2 50,000 IU/week D_2 50,000 IU/month	12 weeks	36	36 (D_2 weekly) 33 (D_2 monthly)	5	3 (D_2 weekly)
Coburn et al.[21]	CKD 3-4	Doxercalciferol 1 mcg/day increased by 0.5 mcg/day every month depending on PTH levels with a maximum permitted dose of 5 mcg/day	24 weeks	28	27	1	0
Coyne et al.[22]	CKD 3-4	Three RCTs were discussed in this study: Study 1 & 2 dose: Paricalcitol 2 mcg x 3/week or Paricalcitol 4 mcg x 3/week depending on PTH levels Study 3 dose: Paricalcitol 1 mcg/day Doses in all 3 trials were adjusted accordingly throughout the trial duration depending on serum calcium, phosphorus, and PTH levels	24 weeks	113	107	1	2

de Zeeuw et al.[23]	CKD (diabetic)	Paricalcitol 1 mcg/day Paricalcitol 2 mcg/day	24 weeks	93	93 (1 mcg/day) 95 (2 mcg/day)	0	2
Delanaye et al.[24]	CKD (HD)	D_3 25,000 IU every 2 weeks	12 months	21	22	5	6
Frazao et al.[25]	CKD (HD)	Doxercalciferol 10 mcg x 3/week	8 weeks	67	71	2	1
Hamdy et al.[26]	CKD	Alfacalcidol 0.25 mcg/day (titrated according to serum calcium concentration)	2 years (or until patient required dialysis)	87	89	1	4
Hewitt et al.[27]	CKD (HD)	D_3 50,000 IU/week for 8 weeks, then 50,000 IU/month for 4 months	6 months	30	30	1	1
Li et al.[28]	CKD (HD)	D_3 50,000 IU/week	12 months	34	62	2	10
Marckmann et al.[5]	CKD	D_3 40,000 IU/week	8 weeks	27	27	1	0
Massart et al.[29]	CKD (HD)	D_3 25,000 IU/week	13 weeks	29	26	1	0
Memmos et al.[30]	CKD (HD)	D_3 0.25 mcg or 0.50 mcg	1 or 2 years	30	27	3	3
Merino et al.[31]	CKD (HD)	D_3 180,000 IU x 1	16 weeks	47	47	2	3
Thadhani et al.[17]	CKD	Paricalcitol 2 mcg/day	48 weeks	112	115	1	1
Wasse et al.[32]	CKD (HD)	D_3 200,000 IU/week	3 weeks	27	25	1	2
Wang et al.[16]	CKD 3-5 (non-dialysis)	Paricalcitol 1 mcg/day	52 weeks	30	30	0	0

Legend: *n* number of participants, *CKD* chronic kidney disease, *HD* haemodialysis, *D₃* cholecalciferol, *PTH* parathyroid hormone

Table 3. Summary of randomised controlled trials examining impact of vitamin D supplementation on cardiovascular mortality

Study	Population	Intervention	Duration	n placebo	n treatment	Events in Placebo Group	Events in Treatment Group
Coburn et al.[21]	CKD 3-4	Doxercalciferol 1 mcg/day increased by 0.5 mcg/day every month depending on PTH levels with a maximum permitted dose of 5 mcg/day	24 weeks	28	27	1	0
de Zeeuw et al.[23]	CKD (diabetic)	Paricalcitol 1 mcg/day Paricalcitol 2 mcg/day	24 weeks	93	93 (1 mcg/day) 95 (2 mcg/day)	0	1
Frazao et al.[25]	CKD (HD)	Doxercalciferol 10 mcg x 3/week	8 weeks	67	71	2	1
Hamdy et al.[26]	CKD	Alfacalcidol 0.25 mcg/day (titrated according to serum calcium concentration)	2 years (or until patient required dialysis)	87	89	1	4
Hewitt et al.[27]	CKD (HD)	D₃ 50,000 IU/week for 8 weeks, then 50,000 IU/month for 4 months	6 months	30	30	0	1
Massart et al.[29]	CKD (HD)	D₃ 25,000 IU/week	13 weeks	29	26	1	0
Merino et al.[31]	CKD (HD)	D₃ 180,000 x 1	16 weeks	47	47	0	1
Thadhani et al.[17]	CKD	Paricalcitol 2 mcg/day	48 weeks	112	115	1	1

Legend: *n* number of participants, *CKD* chronic kidney disease, *HD* haemodialysis, *D₃* cholecalciferol, *D₂* ergocalciferol, *PTH* parathyroid hormone, *RCT* randomised controlled trial

Table 4. Summary of randomised controlled trials examining impact of vitamin D supplementation on cardiovascular adverse events

Study	Population	Intervention	Duration	n placebo	n treatment	Events in Placebo Group	Events in Treatment Group
Bhan et al.[20]	CKD (HD)	D_2 50,000 IU/week D_2 50,000 IU/month	12 weeks	36	36 (D_2 weekly) 33 (D_2 monthly)	3	6 (D_2 weekly) 2 (D_2 monthly)
Coburn et al.[21]	CKD 3-4	Doxercalciferol 1 mcg/day increased by 0.5 mcg/day every month depending on PTH levels with a maximum permitted dose of 5 mcg/day	24 weeks	28	27	2	1
Coyne et al.[22]	CKD 3-4	Three RCTs were discussed in this study: Study 1 & 2 dose: Paricalcitol 2 mcg x 3/week or Paricalcitol 4 mcg x 3/week depending on PTH levels Study 3 dose: Paricalcitol 1 mcg/day Doses in all 3 trials were adjusted accordingly throughout the trial duration depending on serum calcium, phosphorus, and PTH levels	24 weeks	113	107	0	1

de Zeeuw et al.[23]	CKD (diabetic)	Paricalcitol 1 mcg/day Paricalcitol 2 mcg/day	24 weeks	93	93 (1 mcg/day) 95 (2 mcg/day)	1	5
Frazao et al.[25]	CKD (HD)	Doxercalciferol 10 mcg x 3/week	8 weeks	67	71	2	1
Hamdy et al.[26]	CKD	Alfacalcidol 0.25 mcg/day (titrated according to serum calcium concentration)	2 years (or until patient required dialysis)	87	89	2	1
Hewitt et al.[27]	CKD (HD)	D_3 50,000 IU/week for 8 weeks, then 50,000 IU/month for 4 months	6 months	30	30	0	1
Massart et al.[29]	CKD (HD)	D_3 25,000 IU/week	13 weeks	29	26	9	4
Thadhani et al.[17]	CKD	Paricalcitol 2 mcg/day	48 weeks	112	115	8	2
Wang et al.[16]	CKD 3-5 (non-dialysis)	Paricalcitol 1 mcg/day	52 weeks	30	30	3	0