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Article

Cholecalciferol, Calcitriol, and Vascular Function in CKD: A Randomized, Double-Blind Trial

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Abstract

Background and objectives High circulating vitamin D levels are associated with lower cardiovascular mortality in CKD, possibly by modifying endothelial function. We examined the effect of calcitriol versus cholecalciferol supplementation on vascular endothelial function in patients with CKD.

Design, setting, participants, & measurements We performed a prospective, double-blind, randomized trial of 128 adult patients with eGFR=15–44 ml/min per 1.73 $m^2$and serum 25-hydroxyvitamin D level <30 ng/ml at the University of Colorado. Participants were randomly assigned to oral cholecalciferol (2000 IU daily) or calcitriol (0.5 μg) daily for 6 months. The primary end point was change in brachial artery flow-mediated dilation. Secondary end points included changes in circulating markers of mineral metabolism and circulating and cellular markers of inflammation.

Results One hundred and fifteen patients completed the study. The mean (SD) age and eGFR of participants were 58±12 years old and 33.0±10.2 ml/min per 1.73 m^2, respectively. There were no significant differences between groups at baseline. After 6 months, neither calcitriol nor cholecalciferol treatment resulted in a significant improvement in flow-mediated dilation (mean±SD percentage flow-mediated dilation; calcitriol: baseline 4.8±3.1%, end of study 5.1±3.6%; cholecalciferol: baseline 5.2±5.2%, end of study 4.7±3.6%); 25-hydroxyvitamin D levels increased significantly in the cholecalciferol group compared with the calcitriol group (cholecalciferol: 11.0±9.5 ng/ml; calcitriol: −0.8±4.8 ng/ml; $P<0.001$). Parathyroid hormone levels decreased significantly in the calcitriol group compared with the cholecalciferol group (median [interquartile range]; calcitriol: −22.1 [−48.7–3.5] pg/ml; cholecalciferol: −0.3 [−22.6–16.9] pg/ml; $P=0.004$).

Conclusions Six months of therapy with calcitriol or cholecalciferol did not improve vascular endothelial function or improve inflammation in patients with CKD.

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Introduction

Multiple studies have shown that there is widespread vitamin D deficiency across the spectrum of CKD, and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] synthesis decreases with progressive kidney function decline (1–3). Results of several observational studies have found that both low 25-hydroxyvitamin D [25(OH)D] and 1,25$(OH)_2$D levels are associated with hypertension, cardiovascular disease, and all-cause mortality in patients with and without CKD (4–6). Similarly, low 25(OH)D and 1,25$(OH)_2$D levels are independently associated with vascular endothelial dysfunction in patients with CKD (2). Administration of nutritional vitamin D and active vitamin D analogs has anti-inflammatory effects, which could result in an improvement in endothelial dysfunction (4,7,8). A meta-analysis of randomized, controlled trials on vitamin D supplementation has shown a mortality reduction in the general population; however, a limitation of this analysis is that the main conclusion was driven by one study (9). Observational data suggest that active vitamin D therapy improves survival and lowers cardiovascular events in patients with CKD (10,11). Nonetheless, there is a paucity of randomized studies in CKD showing that vitamin D replacement prevents or treats diseases (12). Furthermore, no randomized trials have examined the effect of activated versus nutritional vitamin D on arterial dysfunction, which is a major factor that contributes to all-cause mortality in patients with CKD.

We conducted a prospective, randomized, double-blind trial to examine the effect of supplementation with active vitamin D (calcitriol) versus nutritional vitamin D (cholecalciferol) on vascular endothelial function in patients with CKD, eGFR=15–44 ml/min per 1.73 m^2, and vitamin D deficiency. The primary aim was to determine the effect of vitamin D supplementation on brachial artery flow-mediated dilation (FMD). Additionally, we examined the effect of vitamin D on markers of systemic and vascular endothelial cell inflammation. We hypothesized that treatment with calcitriol would be more effective than cholecalciferol in improving vascular

endothelial function on the basis of our previous data, showing that only low $1,25(OH)_2D$ levels and not 25(OH)D levels were associated with cardiovascular events in patients with advanced CKD (13).

Materials and Methods

Study Population

Participants were recruited from CKD clinics at the University of Colorado between October of 2011 and March of 2015. Eligibility criteria included age between 18 and 80 years old, eGFR using the four-variable Modified Diet in Renal Disease (MDRD) equation between 15 and 44 ml/min per 1.73 m^2, vitamin D deficiency or insufficiency defined as a 25(OH)D level <30 ng/ml, serum calcium <10.2 mg/dl, serum phosphate <4.6 mg/dl, albumin >3.0 g/dl, body mass index <40 kg/m^2, and no use of active vitamin D analogs within 30 days of randomization. All participants had to be on a stable antihypertensive, lipid-lowering, and diabetic regimen for at least 1 month before randomization. For women, participants could not be pregnant, breastfeeding, or unwilling to use birth control. Exclusion criteria included significant comorbid conditions that would lead the investigator to conclude that life expectancy was <1 year, history of liver disease, nephrotic-range proteinuria (>3.5 g/d), and expectation of undergoing living related kidney transplant in the next 6 months. The study protocol was approved by the Colorado Multiple Institutional Review Board. All patients provided written informed consent before study entry. All authors declare their adherence to the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT01384539).

Study Design

Participants who met eligibility criteria and provided informed consent were randomized 1:1 to receive oral calcitriol capsules or cholecalciferol capsules. A placebo group was not included in the study due to a protection of human subjects concern by the National Institutes of Health Scientific Review Group of withholding vitamin D supplementation from subjects with CKD and vitamin D deficiency. A statistician generated the randomization schedule. Study medication was compounded by Pharmacy Compounding Specialties (Dallas, TX) into capsules that were identical in size, shape, and color. An independent laboratory confirmed the content of each product after it was compounded. Study investigators, coordinators, and FMD image readers were blinded to treatment allocation.

Participants randomized to calcitriol started at 0.25 μg daily, with a protocol-specified dose increase to 0.5 μg daily after 1 month if there were no episodes of hypercalcemia. Participants remained on 0.5 μg daily for the remaining 5 months of the study. The dose of calcitriol needed for noncalcemic effects of vitamin D is unknown, and therefore, we chose dosing on the basis of the US Food and Drug Administration–recommended initiation and maintenance dose for treatment of secondary hyperparathyroidism in CKD. Participants randomized to cholecalciferol started at 4000 IU daily (loading dose), with a protocol-specified maintenance dose of 2000 IU daily after 1 month for the remaining 5 months of the study. This dosing regimen was on the basis of Kidney Disease Outcomes Quality Initiative (KDOQI) recommendations for treatment of vitamin D deficiency in CKD (KDOQI) (14).

Study End Points

The primary end point was change in brachial artery FMD over 6 months. Prespecified secondary end points included changes in C-reactive protein (CRP) and IL-6 levels over 6 months (7,8,15). Other prespecified secondary biochemical end points included change in serum 25(OH)D, $1,25(OH)_2D$, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), calcium, and phosphorus over 6 months.

Brachial Artery FMD and Endothelium-Independent Dilation. All vascular measures were performed in the morning at least 4 hours after ingestion of food and tobacco and 24 hours after ingestion of alcohol or caffeine or performing exercise by trained ultrasonagraphers at the University of Colorado Denver Clinical and Translational Research Center. Vascular measures were performed at baseline and the end of the study (6 months). FMD and endothelium-independent dilation were determined using high-resolution ultrasonography (GE Vivid 7 Dimension) as previously described by Celermajer *et al.* (16) and more recently, our group (17–19). Electrocardiogram-gated end diastolic ultrasound images were acquired during baseline and FMD conditions. For FMD, reactive hyperemia was produced by inflating a pediatric forearm cuff around the forearm to 250 mmHg for 5 minutes followed by rapid deflation. Conduit artery endothelium-independent dilation was determined by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg). A commercially available software package (Vascular Analysis Tools 5.8.1; Medical Imaging Applications, LLC, Iowa City, IA) was used to concurrently acquire and analyze electrocardiogram-gated brachial artery diameters. FMD was expressed as percentage change from baseline diameter. The FMD images were analyzed by two independent experienced readers who were blinded to treatment assignment. The inter-rate reliability between the two readers was 0.86.

Laboratory Measurements. Fasting blood samples were collected at baseline and end of study. Serum 25(OH)D and $1,25(OH)_2D$ were analyzed using immunoaffinity extraction and liquid chromatography-tandem mass spectrometry. Serum PTH was analyzed using the Beckman-Coulter DxI Analyzer. FGF23 was analyzed using the Kainos Human FGF-23 ELISA Kit. Serum high-sensitivity CRP was measured on a Beckman Coulter Analyzer. IL-6 was measured by ELISA (R&D Systems). Vitamin D binding globulin was measured using trypsin digestion-liquid chromatography-tandem mass spectrometry. eGFR was calculated at baseline and end of study using the MDRD equation (20). Spot urine samples were collected at baseline and end of study for measurement of urine albumin and creatinine.

Endothelial Cell Protein Expression. Vascular endothelial cells were collected as previously described (17–19,21). Briefly, an intravenous catheter was placed into the antecubital vein, and a J wire was advanced into the vein approximately 4 cm beyond the tip of the catheter and withdrawn. Cells were recovered by washing and centrifugation, fixed with 3.7% formaldehyde, and plated on slides. Nonspecific binding sites were blocked with 5% donkey serum, and cells were incubated

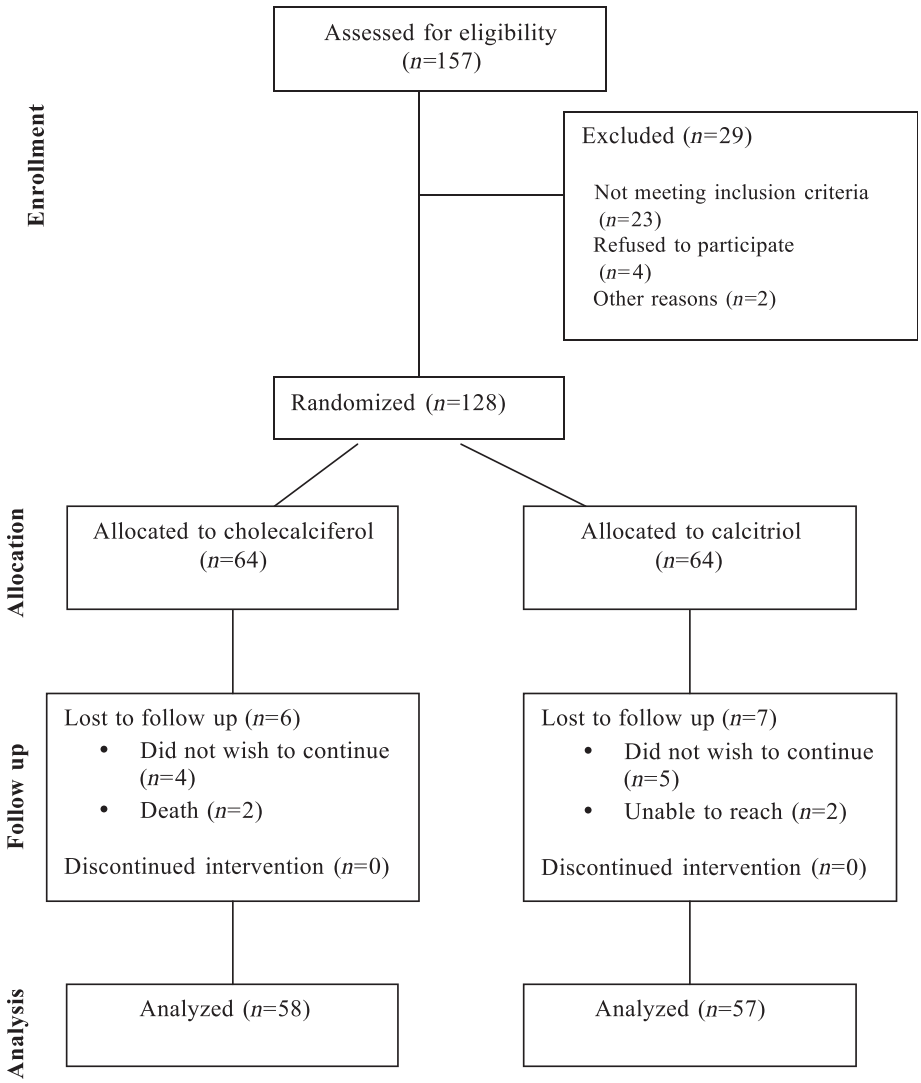

Figure 1. | **Consort diagram.**

with mAbs for NFκB (Novus). Slides were scanned to identify endothelial cells (positive staining of VE Cadherin), and nuclear integrity was confirmed by positive 4′,6′-diamidino-2-phenylindole hydrochloride staining. Endothelial cell images were captured, and the intensity of staining was analyzed using NIS Elements AR Software (Laboratory Imaging). Total NFκB was normalized to a human umbilical vein endothelial cell (HUVEC) control. Values are reported as ratios of endothelial cell expression of NFκB to HUVEC expression of NFκB.

Patient Safety. Participants had monthly safety visits, which included vital signs, serum calcium and phosphorus measurements, and adverse event assessment. A pill count for compliance was performed at months 3 and 6. If serum calcium was >10.5 mg/dl or phosphorus was >4.6 mg/dl, the measurement was repeated within 1 week. If the calcium level remained >10.5 mg/dl, the dose of the study medication was decreased. If serum phosphorus remained >4.6 mg/dl, the patient was first placed on a low-phosphate diet and then started on phosphate binders if phosphorus remained elevated. Sevelamer use was not allowed due to data showing an effect of sevelamer on FMD (22).

Statistical Analyses. Baseline characteristics are reported as mean±SD or when appropriate, median (interquartile range [IQR]) for continuous variables and number and percentage for categorical variables. Paired *t* tests and two-sample *t* tests were used to compare normally distributed variables between study groups, whereas signed rank tests and Wilcoxon rank sum tests were used for skewed variables. Efficacy analyses were conducted in all randomized patients who completed measures at baseline and the end of the study (*n*=115). In secondary analyses, linear regression was used to adjust for covariates and their interaction with the intervention. All analyses were performed using SAS software, version 9.4 (SAS Institute Inc.).

On the basis of previous studies (2), we estimated that treatment with cholecalciferol would result in an average FMD of 5.87 compared with 7.40 with calcitriol, with a common SD of 2.9. A sample size of 58 in each group provided 80% power to detect a difference in means of −1.53

Table 1. Baseline characteristics of study participants according to treatment group

Characteristic	Calcitriol, *n*=64	Cholecalciferol, *n*=64
Age, yr	59±12	58±13
Men, *N* (%)	41 (64)	45 (70)
Race/ethnicity, *N* (%)		
Non-Hispanic white	23 (36)	23 (36)
Non-Hispanic black	14 (22)	18 (28)
Hispanic	23 (36)	16 (25)
Other	4 (6)	7 (11)
Diabetes, *N* (%)	32 (50)	26 (41)
Hypertension, *N* (%)	61 (95)	62 (97)
Coronary artery disease, *N* (%)	10 (16)	11 (17)
Smoking status, *N* (%)		
Never	36 (56)	33 (52)
Former	5 (8)	14 (22)
Current	21 (33)	17 (27)
Etiology of kidney disease, *N* (%)		
Diabetes	31 (48)	24 (38)
Hypertension	15 (23)	17 (27)
PKD	1 (2)	4 (6)
GN	9 (14)	6 (9)
Obstruction	3 (5)	2 (3)
Unknown	13 (20)	8 (13)
Medication use, *N* (%)		
ACE-I/ARB	42 (66)	43 (67)
Diuretics	44 (69)	40 (63)
Lipid-lowering agents	34 (53)	36 (56)
Body mass index, kg/m^2	32.0±5.5	30.4±5.4
eGFR, ml/min per 1.73 m^2	32.8±10.0	33.5±10.4
Systolic BP, mmHg	132±18	132±17
Calcium, mg/dl	9.0±0.4	9.1±0.4
Phosphorus, mg/dl	3.6±0.6	3.7±1.0
25(OH)$_2$D, ng/ml	21.7±7.7	23.0±7.6
1,25(OH)$_2$D, pg/ml	29.4±11.2	29.2±11.1
PTH, pg/ml, median [IQR]	93.8 [60.7–139.4]	99.5 [62.2–145.7]
FGF23, pg/ml, median [IQR]	86.0 [56.3–118.2]	89.0 [60.3–131.8]
C-reactive protein, mg/dl, median [IQR]	3.4 [1.5–6.7]	3.0 [1.2–7.9]
IL-6, mg/dl, median [IQR]	3.0 [1.8–4.2]	3.5 [1.8–4.7]
Urinary albumin-to-creatinine ratio, mg/g, median [IQR]	222.0 [39.7–846.8]	158.8 [29.2–585.6]

All values are expressed as means±SD unless otherwise specified. PKD, polycystic kidney disease; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; IQR, interquartile range; FGF23, fibroblast growth factor 23.

using a two-group *t* test with a 0.05 two-sided significance level. To account for potential dropout and experimental failure of about 10%, 64 patients were randomized to each group.

Results

Study Enrollment and Participants

Figure 1 shows the consort flow diagram of the study. One hundred and twenty-eight participants were enrolled into the study, with 64 participants randomly assigned to receive oral calcitriol treatment and 64 participants randomly assigned to receive oral cholecalciferol treatment. Thirteen patients did not complete the follow-up assessment, and thus, only 115 patients were included in the analysis. The compliance with study medication (defined as percentage number of pills taken per number of pills provided) was 91.3% in the calcitriol group and 94.8% in the cholecalciferol group.

Baseline characteristics of participants by study group are shown in Table 1. The mean age and eGFR of participants were 58±12 years old and 33.0±10.2 ml/min per 1.73 m^2, respectively. Over 50% of participants were men, 45% had a history of diabetes, and over 90% had a history of hypertension. Serum 25(OH)D increased significantly in the cholecalciferol group compared with the calcitriol group, indicating that cholecalciferol administration had an effect on circulating 25(OH)D levels (cholecalciferol: 11.0±9.5 ng/ml; calcitriol: −0.8±4.8 ng/ml; $P<0.001$). There was no change in serum 1,25(OH)$_2$D in either group (calcitriol: 0.4±12.8 pg/ml; cholecalciferol: −1.0±10.3 pg/ml; $P=0.44$). Calcitriol significantly reduced vitamin D binding globulin from baseline to the end of the study (−12.1±40.9 μg/ml; $P=0.04$), whereas there was no significant change in the cholecalciferol group

Table 2. Change from baseline in vascular function according to treatment group

Parameter	Calcitriol, *n*=57	Cholecalciferol, *n*=58	*P* Value
FMD, %			
Baseline	4.8±3.1	5.2±5.2	
End of study	5.1±3.6	4.7±3.6	
Percentage change from baseline	0.3±3.5	−0.5±4.2	0.63
Brachial artery dilation to NTG, %			
Baseline	16.5±9.2	16.1±7.9	
End of study	16.8±9.9	15.7±8.6	
Percentage change from baseline	0.3±5.7	−0.4±5.9	0.75
Systolic BP, mmHg			
Baseline	131.6±18.1	131.9±16.8	
End of study	129.4±15.8	130.1±12.3	
Percentage change from baseline	−2.4±16.5	−0.8±14.9	0.60
Diastolic BP, mmHg			
Baseline	78.0±12.7	79.8±13.1	
End of study	78.0±12.0	78.5±11.2	
Percentage change from baseline	−0.3±10.2	−0.5±10.1	0.90

Values are expressed as mean change from baseline ±SD. FMD, flow-mediated dilation; NTG, nitroglycerin.

(−6.9±44.0 μg/ml; *P*=0.29). However, there was no significant difference between groups in the change in vitamin D binding globulin (*P*=0.54).

Effect of Calcitriol and Cholecalciferol on Vascular Function

At baseline, FMD was similar between the two groups (Supplemental Figure 1, Table 1) (*P*=0.19). After 6 months of treatment, there was no significant change in FMD from baseline in either group and no significant difference in FMD between groups (Supplemental Figure 1, Table 2). Endothelium-independent vasodilation (brachial artery dilation in response to sublingual nitroglycerin) was unaffected by calcitriol or cholecalciferol (Table 2). The effect of vitamin D on FMD was not different across age, sex, race, diabetes status, or use of angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs; *P*>0.30 for all). There was no significant effect of vitamin D on FMD after adjustment for FGF23 (*P*>0.30 for both groups). There were no significant changes in systolic or diastolic BP between groups. (Table 2). When stratifying by baseline vitamin D levels, those with a 25(OH)D level <15 ng/ml, had less of a decline in FMD with calcitriol versus cholecalciferol treatment (−0.23% versus −4.64%; *P*=0.02).

Effect of Calcitriol and Cholecalciferol on Inflammatory Markers and Vascular Endothelial Cell Protein Expression of NFκB

Changes from baseline to the end of the study in secondary end points are shown in Table 3. Median (IQR) changes in serum levels of high-sensitivity CRP did not differ in calcitriol- and cholecalciferol-treated patients (calcitriol: 0.20 [−1.15–2.18] mg/dl; *P*=0.30; cholecalciferol: 0.20 [−1.98–1.85] mg/dl; *P*=0.90). There was no significant change in median (IQR) IL-6 levels in either group (calcitriol: 0.10 [−0.75–1.25] mg/dl; *P*=0.71; cholecalciferol: 0.12 [−1.84–1.64] mg/dl; *P*=0.99). There was no change in total vascular endothelial cell NFκB expression between treatments (calcitriol: −0.01±0.1; cholecalciferol: 0.03±0.1 units of ratio relative to HUVEC control; *P*=0.13) (Figure 2).

Effect of Calcitriol and Cholecalciferol on Markers of Mineral Metabolism

In the calcitriol group, the change in serum calcium from baseline was statistically significant (0.15±0.5; *P*=0.04) (Table 3). There was no difference between the two groups in the change in serum calcium (*P*=0.48). There were no significant changes in serum phosphate in either group (calcitriol: 0.04±0.72 mg/dl; cholecalciferol: 0.1±0.7 mg/dl; *P*>0.40 for both). Calcitriol significantly reduced PTH levels during the follow-up period (median [IQR]: −22.1 [−48.7–3.5] pg/ml; *P*<0.001), whereas there was no significant change in PTH in the cholecalciferol group (median [IQR]: −0.3 [−22.6–16.9] pg/ml; *P*=0.74). Serum FGF23 levels increased significantly in both groups over the 6-month period (median [IQR]: calcitriol: 27.7 [4.7–80.7] pg/ml; *P*<0.001; cholecalciferol: 24.6 [−3.7–69.5] pg/ml; *P*<0.001), but there was no significant difference between groups (*P*=0.26). eGFR decreased slightly in both groups from baseline but was only significantly lower in the calcitriol group (calcitriol: −1.90±6.5 ml/min per 1.73 m^2; *P*=0.04; cholecalciferol: −1.20±5.5 ml/min per 1.73 m^2; *P*=0.10). However, there was no significant difference between groups in the change in eGFR (*P*=0.91).

Effect of Calcitriol and Cholecalciferol on Proteinuria

The urinary albumin-to-creatinine ratio decreased from baseline in the calcitriol group by 14.6% (*P*=0.02) and increased in the cholecalciferol group by 5.8% (*P*=0.35) (Table 2). The change in albumin-to-creatinine ratio between the two groups was significant (mean =−3.96; 95% confidence interval, −7.25 to −0.62; *P*=0.02). The between-group difference remained significant after adjustment for age, sex, race, systolic BP, and use of ACE-I/ARB.

Adverse Effects

Adverse events are shown in Table 4. There were no differences in adverse events between the two groups. There were two deaths during the study: both in the cholecalciferol group. One death was related to an accidental

Table 3. Changes in markers of mineral metabolism, inflammatory markers, and kidney function from baseline to 6 months

Parameter	Calcitriol, *n*=57			Cholecalciferol, *n*=58			Between-Group *P* Value
	Baseline	6 mo	*P* Value	Baseline	6 mo	*P* Value	
25(OH)D, ng/ml	21.7±7.7	21.2±7.5	0.20	23.0±7.6	34.8±9.3	<0.001	<0.001
1,25(OH)$_2$D, pg/ml	29.4±11.2	30.4±15.1	0.84	29.2±11.1	28.5±9.7	0.45	0.44
Vitamin D binding globulin, μg/ml	263±46	251±40	0.04	257±48	250±48	0.29	0.54
Calcium, mg/dl	9.0±0.4	9.1±0.6	0.04	9.1±0.4	9.0±0.4	0.55	0.48
Phosphorus, mg/dl	3.6±0.6	3.6±0.7	0.66	3.6±0.7	3.7±0.7	0.42	0.85
PTH, pg/ml	94 (61–139)	57 (32–109)	<0.001	99 (62–146)	102 (59–135)	0.74	0.004
FGF23, pg/ml	86.0 (56.3–118.2)	117.0 (85.5–164.5)	<0.001	89.0 (60.3–131.8)	117.1 (80.9–189.1)	<0.001	0.26
hs-CRP, mg/dl	3.4 (1.5–6.7)	3.9 (0.3–2.1)	0.30	3.0 (1.2–7.9)	3.1 (1.7–8.3)	0.84	0.69
IL-6, mg/dl	3.0 (1.8–4.2)	3.1 (1.8–5.1)	0.71	3.5 (1.8–4.7)	3.4 (2.0–5.2)	0.99	0.78
GFR, ml/min per 1.73 m^2	32.8±10.0	31.6±11.0	0.04	33.3±10.4	31.3±11.6	0.10	0.91
Urinary albumin-to-creatinine ratio, mg/g	255.2 (39.6–844.7)	131.4 (21.7–708.6)	0.02	166.1 (30.3–611.3)	355.6 (25.9–830.4)	0.35	0.02

Values are expressed as mean±SD or median (interquartile range). Comparisons were performed by using paired *t* tests (6 months versus baseline) and two-sample *t* tests (between groups) for all variables except PTH, FGF23, CRP, and IL-6, for which signed rank tests (6 months versus baseline) and rank sum tests (between groups) were performed. 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25 dihydroxyvitamin D; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; hs-CRP, high-sensitivity C-reactive protein.

narcotic overdose, and the other was sudden and unexpected. Five patients became hypercalcemic during the study: four in the calcitriol group and one in the cholecalciferol group. Twenty-nine patients developed a serum phosphate >4.6 mg/dl during the study. Fifteen patients developed a phosphate >5.0 mg/dl (ten in the calcitriol group and five in the cholecalciferol group), but no patient developed a sustained phosphate >5.5 mg/dl. More patients in the calcitriol group were started on phosphate binders (10.9% versus 6.3% in the cholecalciferol group).

Discussion

In this randomized, double-blind trial, we found that neither calcitriol nor cholecalciferol supplementation improved vascular endothelial function measured by FMD in patients with CKD (eGFR=15–44 ml/min per 1.73 m^2); however, both treatments were safe and well tolerated. Furthermore, treatment with either vitamin D formulation had no significant effects on other cardiovascular risk factors, including BP and circulating and cellular markers of inflammation. To our knowledge, this is the first interventional trial examining the effect of calcitriol versus cholecalciferol on vascular endothelial function in patients with CKD in a direct head to head comparison.

Endothelial dysfunction begins early in the course of CKD (23,24), and reduced FMD independently predicts a high risk for cardiovascular events (23,25,26). Because low vitamin D levels are associated with inflammation, activation of the renin-angiotensin-aldosterone system, oxidative stress and endothelial dysfunction, it is biologically plausible that treatment with vitamin D may reduce cardiovascular risk. In observational studies, treatment with active vitamin D is associated with improved survival (10,11). However, evidence from randomized clinical trials that vitamin D supplementation improves clinical outcomes is lacking. In the largest CKD study to date (*n*=227), treatment with paricalcitol for 48 weeks did not alter left ventricular mass index or improve diastolic dysfunction (27). Findings were similar in a smaller study of 60 patients randomized to paricalcitol or placebo for 52 weeks (28). Our data may partially explain the lack of benefit seen in previous studies, because vitamin D did not have an effect on FMD, markers of systemic or vascular endothelial cell inflammation, or BP, all of which are mechanisms involved in left ventricular mass index progression.

Data regarding the effect of vitamin D supplementation on vascular endothelial dysfunction are conflicting. Similar to our results, a randomized trial of 60 patients with CKD stage 3 or 4 and type 2 diabetes found that paricalcitol treatment for 3 months resulted in no improvement in FMD (29). However, contrary to our results, another randomized, placebo-controlled trial of 88 patients with CKD stage 3 or 4 (30) showed tht 12 weeks of paricalcitol improved FMD. The reason for the conflicting findings may be differences in the patient populations. In the first study, all of the patients had diabetes, whereas in the latter study, only a small percentage had diabetes. However, in our study, there was no difference in the response of vitamin D on FMD by diabetes status.

As expected, calcitriol significantly lowered PTH levels. Cholecalciferol had no effect on PTH levels, despite a significant increase in 25(OH)D levels. A recent meta-analysis found no

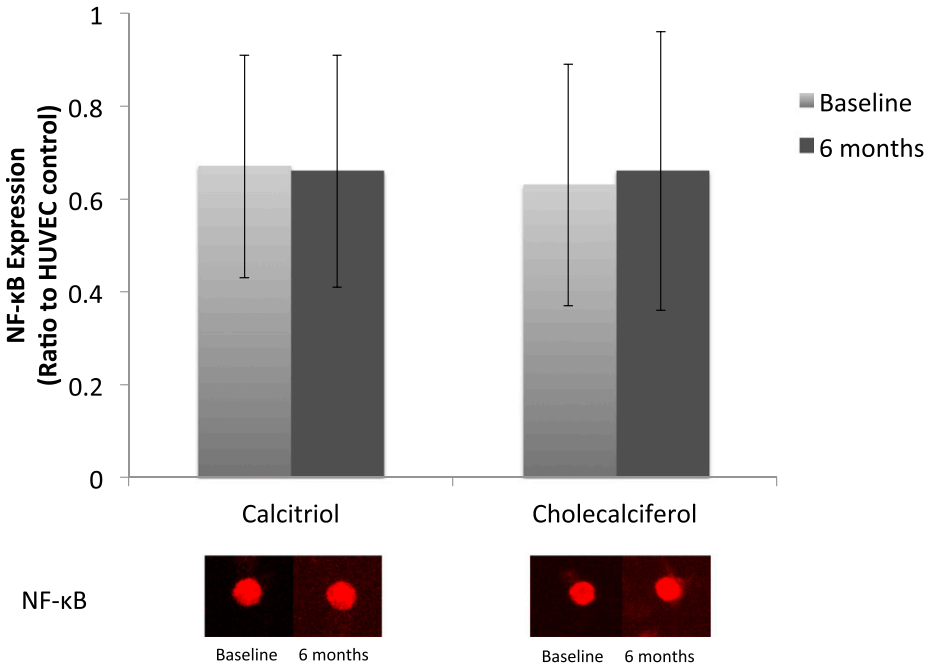

Figure 2. | **Venous endothelial protein expression of NFκB at baseline and 6 months.** Representative images are shown below the summary graph for NFκB. Values are mean±SD. HUVEC, human umbilical vein endothelial cell.

significant PTH-lowering effect of nutritional vitamin D (31). Additionally, two small trials comparing the effect of nutritional versus active vitamin D on PTH levels also found no lowering effect with nutritional vitamin D (32,33). Hence, data from head to head randomized trials do not support significant PTH lowering with nutritional vitamin D in patients with CKD, consistent with our findings.

FGF23 levels increased significantly in both groups, and there was no difference in the change in FGF23 levels between groups. Because FGF23 is associated with endothelial dysfunction and inflammation in patients with CKD (33–35), the increase in FGF23 levels may have modified the effect of vitamin D on FMD. However, there was no significant effect of vitamin D on FMD after adjustment for FGF23. Other studies

Table 4. Adverse events according to treatment group

	Calcitriol, *n*=64	Cholecalciferol, *n*=64
Serious adverse events		
No. of hospitalizations, *N* (%)	13 (20)	13 (20)
Reasons for hospitalizations		
Infections	2 (15)	1 (8)
Congestive heart failure exacerbation	2 (15)	1 (8)
Volume overload not caused by congestive heart failure	0 (0)	3 (23)
Myocardial infarction/stroke	0 (0)	2 (15)
Angina	1 (8)	1 (8)
Shortness of breath	2 (15)	2 (15)
Pneumonia	0 (0)	1 (8)
Surgery	5 (38)	2 (15)
Pancreatitis	1 (8)	0 (0)
No. of deaths, *N* (%)	0 (0)	2 (3)
Deaths related to the study	0	0
Expected adverse events		
Hypercalcemia (calcium >10.5 mg/dl), *N* (%)	4 (6)	1 (2)
Hyperphosphatemia, *N* (%)		
Phosphate >4.6 mg/dl	15 (23)	14 (22)
Phosphate >5.0 mg/dl	10 (16)	5 (8)
Phosphate binders started	7 (11)	4 (6)

Adverse events are shown for all randomized patients.

have found a similar effect of active vitamin D analogs on FGF23 levels in patients with CKD (30,36). However, studies done with nutritional vitamin D have not found an increase in FGF23 levels in patients with CKD (37–39). Because we found no difference between the groups at the end of the study, it is possible that the increased FGF23 levels represent the natural course of FGF23 in patients with CKD over 6 months.

Vitamin D deficiency is associated with a higher risk of proteinuria and kidney disease progression in patients with CKD (13,40,41). We found that treatment with calcitriol but not treatment with cholecalciferol resulted in a significant decrease in albuminuria in patients with CKD and eGFR=15–44 ml/min per 1.73 m^2. The effect of calcitriol on albuminuria was independent of BP and use of ACE-I/ARB. Several other clinical trials have also shown that active vitamin D reduces proteinuria (42–45). The mechanism by which active vitamin D reduces proteinuria is not clear but may be due to the downregulation of the renin-angiotensin-aldosterone system (46).

Although those with the lowest vitamin D levels (<15 ng/ml) did have less of a decline with calcitriol than with cholecalciferol, there was still no significant improvement in FMD with calcitriol. Hence, there is currently no justification for prescribing vitamin D to improve vascular function in patients with CKD. Vitamin D supplementation may still be a good candidate to prevent and/or treat mineral and bone disease in patients with CKD. Current guidelines recommend using active vitamin D therapies to reduce PTH levels, and our data support this recommendation. Additionally, there may be a role for the use of active vitamin D as an antiproteinuric therapy in patients with CKD. Future studies are needed to determine whether vitamin D therapy slows kidney disease progression. Furthermore, whether combined treatment with nutritional and active vitamin D results in improved outcomes in patients with CKD needs to be explored.

Our study has limitations, including that study duration was only 6 months and that we were unable to determine the effect of vitamin D supplementation on hard clinical outcomes. Additionally, we did not have an untreated control group. Because the optimal 25(OH)D levels and doses of cholecalciferol and calcitriol in patients with CKD are unknown, it is possible that the doses that we used were not sufficient to result in changes in FMD. Additionally, we did not control for menstrual status, oral contraceptive use, or postmenopausal hormone therapy in women, and we did not collect information on duration of diabetes or hypertension. Our study also has several strengths, including that it was a double-blinded, randomized trial and that it was powered to detect a clinically significant different in FMD. In addition, we also included a detailed examination of markers of inflammation and mineral metabolism, including at the endothelial cell level. In conclusion, 6 months of treatment with calcitriol at a dose that significantly reduces secondary hyperparathyroidism or cholecalciferol at a dose that significantly increases 25(OH)D levels does not result in a significant improvement in vascular endothelial function in patients with CKD and eGFR=15–44 ml/min per 1.73 m^2.

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Disclosures

None.

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