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Randomized controlled trial of cholecalciferol supplementation in chronic kidney disease patients with hypovitaminosis D

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Abstract

Background. Hypovitaminosis D is common in chronic kidney disease (CKD). Effects of 25-hydroxyvitamin D replenishment in CKD are not well described.

Methods. An 8-week randomized, placebo-controlled, double-blind parallel intervention study was conducted in haemodialysis (HD) and non-HD CKD patients. Treatment consisted of 40 000 IU of cholecalciferol orally per week. Plasma 25-hydroxyvitamin D (25-OHD), plasma 1,25-dihydroxyvitamin D (1,25-diOHD), plasma parathyroid hormone (PTH), serum phosphate, ionized serum calcium and serum fibroblast growth factor 23 (FGF-23) were analysed. We also investigated biomarkers related to cardiovascular disease (plasma D-dimer, plasma fibrinogen, plasma von Willebrand factor antigen and activity, plasma interleukin 6, plasma C-reactive protein, blood pressure, aortic augmentation index, aortic pulse wave velocity and 24-h urinary protein loss). Objective and subjective health variables were assessed (muscle function tests, visual analogue scores and Health Assessment Questionnaire).

Results. Fifty-two CKD patients with 25-OHD <50 nmol/L at screening were included. Cholecalciferol supplementation led to a significant increase to a median of 155 nmol/L 25-OHD (interquartile range 137–173 nmol/L) in treated patients ($n = 25$, $P < 0.001$). In non-HD patients, we saw a significant increase in 1,25-diOHD ($n = 13$, $P < 0.01$) and a lowering of PTH ($n = 13$, $P < 0.001$). This was not observed in HD patients. Cholecalciferol supplementation caused a significant increase in serum calcium and FGF-23.

Conclusions. 25-OHD replenishment was effectively obtained with the employed cholecalciferol dosing. In non-HD patients, it had favourable effects on 1,25-diOHD and PTH. Vitamin D-supplemented patients must be monitored for hypercalcaemia. The present study could not identify significant pleiotropic effects of 25-OHD replenishment.

Keywords: cholecalciferol; chronic kidney disease; FGF-23; pleiotropic effects; vitamin D

Introduction

Studies have demonstrated that hypovitaminosis D [plasma concentration of 25-hydroxylated vitamin D_2/D_3 (25-OHD or calcidiol) <75 nmol/L] is highly prevalent among patients with chronic kidney disease (CKD) [1–3]. Plasma 25-OHD is the substrate of the mitochondrial P450 1-α hydroxylase enzyme, CYP27B1, that converts 25-OHD to the biologically highly active 1,25-dihydroxy-vitamin D (1,25-diOHD or calcitriol). CKD is associated with an impaired renal 1-α hydroxylase activity [4, 5]. This is the primary reason for the low systemic 1,25-diOHD concentrations seen in CKD patients but hypovitaminosis D clearly aggravates this problem [6].

Numerous extra-renal cells possess their own 1-α hydroxylase activity, and their functions depend on the local production and autocrine and paracrine influence of 1,25-diOHD rather than on the delivery of pre-formed 1,25-diOHD from renal 1-α hydroxylase activity or 1,25-diOHD supplementation [7–9]. Cunningham *et al.* [6] estimated that >85% of plasma 25-OHD is used by target tissues for such an autocrine/paracrine activation to 1,25-diOHD. Accordingly, the availability of sufficient 25-OHD might be necessary for normal muscle function or effective immune response [10–13]. Also, hypovitaminosis D has been implicated as a factor contributing to cardiovascular disease in CKD patients and in the general population [11, 12, 14, 15].

Current treatment guidelines for CKD patients recommend that hypovitaminosis D should be corrected [16]. However, there are limited data behind these recommendations. We therefore conducted a placebo-controlled randomized trial to investigate the impact of vitamin D_3 supplementation in CKD patients with hypovitaminosis D. The present paper describes the effects on vitamin D status, parathyroid hormone (PTH) concentration and fibroblast growth factor 23 (FGF-23). Furthermore, we investigated the effect on biomarkers related to cardiovascular disease as described in the literature recently, like D-dimer, fibrinogen, von Willebrand factor antigen, interleukin 6 (IL6), C-reactive protein (CRP), aortic augmentation index and aortic pulse wave velocity (PWV) [17]. The impact of cholecalciferol supplementation on muscle function and subjective health variables was also assessed.

Materials and methods

The study was conducted at the Department of Nephrology at Odense University Hospital in Denmark (latitude 55° north). The protocol was in accordance with the ethical standards of the Declaration of Helsinki and was approved by the regional ethics committee (reference number: S-20090061) and by the Danish Medicines Agency (EudraCT: 2008-006438-82). The study was reported to the Danish Data Protection Agency (reference number: 11-88-37-29) and ClinicalTrials.gov (Identifier: NCT00968877).

Study population

All CKD patients attending our outpatient clinics in summer (June–August) 2009 had their vitamin D status screened. Patients were invited to participate in the study if they were adult (aged >18 years) and if plasma 25-OHD was <50 nmol/L at screening. Exclusion criteria were supplementary intake of a total of >10 000 IU ergocalciferol or cholecalciferol within the last 3 months, hypercalcaemia, severe hyperphosphataemia (P-phosphate >2.2 mmol/L at two consecutive measurements >1 week apart), sarcoidosis, malignant disease, psychotic disorder, alcohol or drug abuse, pregnancy, breastfeeding, current participation in other clinical studies, poor understanding of the Danish language, allergy towards soy protein, fertile women not using safe contraception and oestrogen use. During the 8-week intervention period in autumn (September till November), participants were excluded if they met any of the exclusion criteria or if they developed acute illness leading to hospital admission, they initiated dialysis therapy, were kidney transplanted or died.

Of 418 patients investigated during the screening period, 305 patients (73%) showed 25-OHD concentrations >50 nmol/L. A total of 79 of the 418 screened patients met the inclusion criteria and were invited to participate (Figure 1). Fifty-four study participants gave their written consent after oral and written information about the study.

One patient from the cholecalciferol-treated group was excluded from all analyses due to inadvertent major changes in medication directly related to calcium and vitamin D metabolism by a physician not involved in the study. One patient from the placebo group was excluded from all analysis due to a violation of inclusion parameters (severe hyperphosphataemia before start of intervention). Baseline characteristics of the remaining patients are presented in Tables 1 and 2.

Three patients had to be excluded from the study during the intervention period due to acute severe illness leading to admission at the intensive care unit ($n = 2$, one from each group) or death ($n = 1$, patient from placebo-treated group).

Study design

The study was an 8-week double-blind, placebo-controlled, randomized trial with two parallel arms. Patients were subdivided according to gender and CKD status (conservatively treated CKD patients, haemodialysis (HD) patients or transplanted patients). Random allocation to cholecalciferol and placebo treatment was undertaken in consecutive pairs of two within each of these six subgroups (balanced and stratified randomization) by third part (study manager at the local pharmacy at Odense University Hospital) using a computer-generated list of random numbers. Investigators and participants were both blinded to treatment until study completion.

Treatment arms

The treated group received one capsule containing 40 000 IU of vitamin D_3 (corresponding to two capsules of Dekristol® from MIBE, Brehna, Germany) weekly for 8 weeks. Eight capsules were provided in a container. The control group also received eight capsules in a container and was similarly instructed to take one capsule weekly for 8 weeks. Their capsules contained lactose. The capsules and containers of the two groups looked identical and were both prepared by the local pharmacy at Odense University Hospital. Capsules had to be consumed on the same day of each week. HD patients consumed their capsules immediately after an HD session.

The compliance in HD patients was 100% since the capsules were consumed under the supervision of the HD nurse in charge. The self-reported compliance of the non-HD patients was also 100%; in addition, 20 of 24 non-HD patients returned the empty medication container at the end of the study. The good compliance reported is in accordance with the increase of 25-OHD in all patients in the intervention group.

Blood and urine samplings and analyses

All analyses were performed at the local laboratories of the participating departments according to the current laboratory standards. Ionized

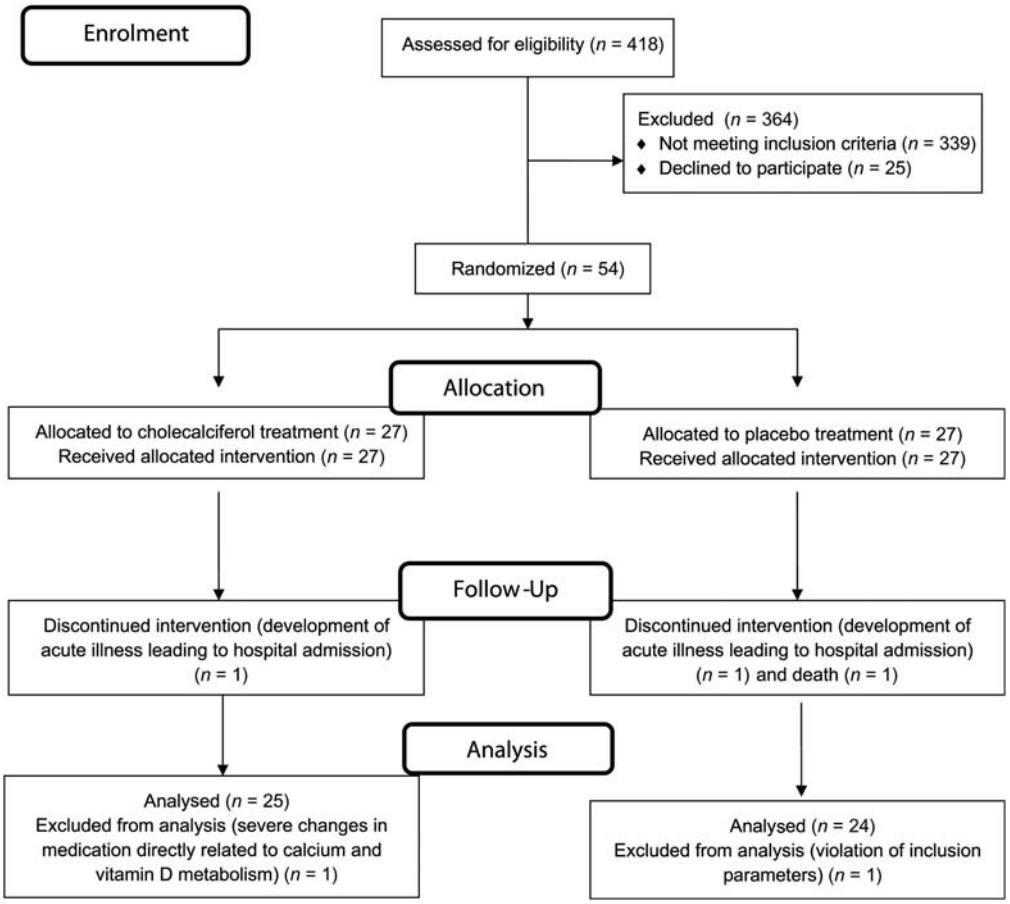

Fig. 1. Consort flow diagram showing the numbers of participants who were randomly assigned, received intended treatment and were analysed in the treated group and placebo group.

calcium was used to judge patients' calcium balance. The procedures are described in detail in Supplementary Material online.

Muscle function tests and subjective health indicators

For the assessment of upper extremities, the sum of kilograms lifted in 30 s with the dominant arm was recorded. For the assessment of the lower extremities, the number of times patients managed to stand up from the sitting position in 30 s was recorded.

Subjective health parameters were assessed from the visual analogue scores (Scale 0–10). The Health Assessment Questionnaire (HAQ) scores were obtained as published [18]. Further details are given in Supplementary Material online.

Blood pressure, pulse wave analyses and PWV

Blood pressure (BP) measurements were performed with an automated device (Omron M6; Omron HealthCare Europe B.V., Hoofddorp, The Netherlands). An applanation tonometer (Millar; SPT-301B, Houston, TX) was used for determinations of aortic PWV and aortic augmentation index (AIx@75) as published [19]. The procedures are described in detail in Supplementary Material online.

Statistics

Continuous data are reported as median (1–3 interquartile range). Mann–Whitney's *U*-test and Wilcoxon's matched pairs test were used to compare between-group and within-group differences of continuous variables, respectively. Frequency counts were calculated for categorical variables. Differences in categorical variables between groups were analysed by Fisher's exact test. Non-parametric bivariate correlation analysis (Spearman) was performed for selected variables. Analyses were performed with GraphPad prism software (version 5.0, GraphPad Software, San Diego, CA). All statistical tests were two-sided. Two-sided P-values <0.05 were considered to indicate statistical significance.

In our testing of treatment effects, we compared all cholecalciferol-treated patients with all placebo-treated patients, but we also did separate analyses for the subgroup of HD patients and the subgroup of non-HD patients.

With 50 study participants and the risk of Type I error (α) set at 5%, the study possesses a statistical power (1-β) of 80% to detect a 50% treatment difference in PTH in a two-sided test.

Results

Baseline data of 52 participants are presented in Tables 1 and 2. Twenty-seven patients were in regular HD treatment. Their median dialysis vintage was 32 months (range 4–158 months) and there was no significant difference between the cholecalciferol and the placebo-treated group. Four CKD patients with functioning kidney graft were included in each allocation group. Median time after transplantation was 92 months (range 11–244 months). Use of calcium supplements, phosphate binders, alphacalcidol and calcimimetics was not changed throughout the study period in patients included in the final analyses, except for one patient who stopped sevelamer hydrochloride treatment.

Table 1. Baseline characteristics of patients according to treatment group (one capsule of 40 000 IU vitamin D_3 or placebo weekly for 8 weeks)[a]

Parameter	Cholecalciferol ($n = 26$)	Placebo ($n = 26$)
Age, years	71 (62–78)	68 (59–76)
Gender, male/female	19/7	20/6
Body mass index, kg/m^2	25.9 (22.1–29.7)	24.6 (22.0–27.3)
Systolic BP, mmHg	134 (125–158)	135 (122–160)
Diastolic BP, mmHg	72 (67–79)	72 (68–84)
Treatment modality		
Regular HD therapy, *n* (%)	13 (50)	14 (54)
Functioning kidney graft, *n* (%)	4 (15)	4 (15)
Conservative treatment, *n* (%)	9 (35)	8 (31)
Underlying kidney disease, *n* (%)		
Diabetic nephropathy	4 (15)	3 (12)
Nephrosclerosis	7 (27)	10 (38)
Others	15 (58)	13 (50)
Disease prevalence, *n* (%)		
Diabetes	8 (31)	10 (38)
Hypertension	23 (88)	18 (69)
Coronary artery disease/cardiac insufficiency	10 (38)	13 (50)
Non-study medication, *n* (%)		
Calcium (supplement or phosphate binder)	12 (46)	14 (54)
Calcium-free phosphate binder	8 (31)	11 (42)
Cinacalcet	1 (4)	3 (12)
Alphacalcidol	12 (46)	8 (31)
ACE inhibitor/AT receptor antagonist	15 (58)	12 (46)
Erythropoietin analogue	15 (58)	14 (54)
Other variables		
B-haemoglobin, mmol/L	7.4 (7.0–8.2)	7.9 (7.6–8.1)
eGFR, mL/min/1.73 m^2	32 (20–54)[b]	29 (19–54)[c]

[a]Figures are medians (interquartile ranges) or numbers (percentages). ACE, angiotensin-converting enzyme; AT, angiotensin; eGFR, estimated glomerular filtration rate.
[b]$n = 13$ for eGFR determination in all non-HD patients.
[c]$n = 12$ for eGFR determination in all non-HD patients.

Bone mineral metabolism

Cholecalciferol supplementation was associated with a highly significant increase in plasma 25-OHD (Table 3). At Week 8, the median 25-OHD was 154.7 nmol/L (range 81.4–240.3) in the cholecalciferol-treated group ($n = 25$) versus 23.5 nmol/L (range 10.9–62.7) in the placebo-treated group ($n = 24$). The increase in 25-OHD was of similar magnitude in the cholecalciferol-treated subgroups of HD and non-HD patients. None of the cholecalciferol-treated patients had hypovitaminosis D at Week 8. In contrast, all placebo-treated patients had hypovitaminosis D at Week 8, and 23 of 24 patients had vitamin D deficiency (25-OHD <40 nmol/L). Four of the cholecalciferol-treated patients had 25-OHD concentrations >200 nmol/L (range 220–240 nmol/L) at Week 8.

Plasma 1,25-diOHD concentrations increased significantly in the cholecalciferol-treated group (Table 3). The rise in 1,25-diOHD was significantly higher in non-HD (+49 pmol/L) than in HD patients (+14 pmol/L) ($P < 0.01$ for between-group difference). In subgroup analyses, the 1,25-diOHD increase was only significant in non-HD patients compared to placebo-treated patients.

The 1,25-diOHD concentration that was obtained at the end of the study in the non-HD group treated with cholecalciferol was 105 pmol/L (60–148 pmol/L). In the HD group, the 1,25-diOHD concentration at the end of the study in patients treated with cholecalciferol was 47 pmol/L (33–77 pmol/L) and it was 59 pmol/L (40–49 pmol/L) in those HD patients with concomitant use of alphacalcidol. The 1,25-diOHD values in HD patients were significantly lower compared with non-HD patients ($P < 0.05$), even if they received alphacalcidol. It seems therefore unlikely that the absence of a significant response of 1, 25-diOHD concentrations to cholecalciferol treatment in the HD group resulted from a ceiling effect due to a concomitant use of alphacalcidol.

Plasma PTH was significantly lowered by cholecalciferol in the subgroup of non-HD patients (Table 3). Further dissection of data from this subgroup demonstrated that this PTH lowering was significant in both CKD Stages 1–3 ($n = 6$) and CKD Stages 4–5 patients ($n = 7$, $P < 0.01$ compared to placebo). The prevalence of hyperparathyroidism among the cholecalciferol-treated non-HD patients declined from 6 of 13 patients to 4 of 13 patients, whereas it increased from 6 of 12 patients to 9 of 10 patients in the control non-HD group (group difference at end of study, Fisher's test: $P < 0.01$). PTH changes were small and insignificant in HD patients.

Ionized calcium concentrations in serum increased significantly in the cholecalciferol-treated group compared with the placebo-treated group (Table 3). At Week 8, 5 of 25 individuals in the cholecalciferol-treated group had hypercalcaemia versus 0 of 24 patients in the control group ($P = 0.05$ for group difference). The hypercalcaemia was mild in two cases (ionized calcium 1.32 and 1.32 mmol/L; total calcium 2.61 and 2.53 mmol/L; one of them on calcium-based phosphate binder), moderate in two cases (ionized calcium 1.44 and 1.46 mmol/L; total calcium 2.48 and 2.70 mmol/L; both were HD patients and both were taking calcium-based phosphate binder and alphacalcidol) and severe in one non-HD case (ionized calcium 1.63 mmol/L; total calcium 3.11 mmol/L; he neither took calcium nor activated vitamin D).

Compared with placebo, cholecalciferol supplementation caused a small but significant increase in serum FGF-23 (Table 3).

Correlation analyses for markers of bone mineral metabolism

Non-parametric correlation analyses of changes from Week 0–8 in the whole patient group demonstrated that Δ25-OHD and Δ1,25-diOHD were significantly and positively associated (Table 4). Also, Δ25-OHD was significantly and inversely associated with changes in PTH. The association between Δ1,25-diOHD and PTH changes did not reach statistical significance in the whole patient group. Changes in serum ionized calcium correlated positively with Δ25-OHD and inversely with PTH changes.

Changes in FGF-23 were positively associated with Δ25-OHD. A strong highly significant positive association was seen between changes in serum phosphate and FGF-23. This association was positive and significant also in separate analyses of non-HD and HD patients ($P < 0.05$ and <0.01, respectively). In cholecalciferol-treated

Table 2. Baseline biochemical characteristics according to treatment group (cholecalciferol or placebo)[a]

Parameter	Cholecalciferol all, $n = 26$	Placebo all, $n = 26$	Cholecalciferol non-HD, $n = 13$	Placebo non-HD, $n = 12$	Cholecalciferol HD, $n = 13$	Placebo HD, $n = 14$
P-25-OHD, nmol/L	23.8 (17.2–41.4)	33.1 (23.7–42.8)	39.3 (17.6–50.2)	28.6 (19.4–37.8)	20.7 (16.3–28.9)[b]	35.9 (25.5–45.9)
P-1,25-diOHD, pmol/L	49 (33–80)	54 (21–69)	66 (41–94)	67 (54–78)	35 (24–60)	22 (15–57)
P-PTH, pmol/L	11.3 (3.9–31.8)	17.1 (10.4–29.8)	8.4 (4.5–15.7)	12.7 (7.2–21.4)	18.0 (2.6–54.9)	23.8 (14.8–40.7)
S-phosphate, mmol/L	1.25 (1.04–1.67)	1.41 (1.05–1.71)	1.09 (0.94–1.25)	1.05 (0.98–1.26)	1.61 (1.21–1.84)	1.61 (1.44–2.16)
S-calcium ion, mmol/L	1.22 (1.15–1.25)	1.23 (1.20–1.27)	1.21 (1.16–1.23)	1.23 (1.21–1.24)	1.24 (1.14–1.26)	1.23 (1.19–1.28)
S-calcium total, mmol/L	2.27 (2.21–2.27)	2.34 (2.26–2.41)	2.25 (2.20–2.36)	2.27 (2.22–2.38)	2.27 (2.22–2.40)	2.38 (2.31–2.44)
S-FGF-23, pg/mL	185 (52–2637)	717 (116–6795)	54 (31–144)	90 (39–268)	2448 (203–5069)	3119 (1398–14 785)
P-fibrinogen, µmol/L	11.8 (9.7–14.7)	12.5 (10.2–14.7)	13.2 (10.6–14.7)	12.4 (9.7–14.6)	11.5 (9.0–12.6)	13.5 (10.6–15.5)
P-D-dimer, mg/L	0.74 (0.80–1.44)	0.60 (0.36–1.25)	0.82 (0.47–1.57)	0.54 (0.23–0.82)	0.68 (0.47–2.42)	0.98 (0.38–1.54)
P-CRP, mg/L	6.1 (2.0–18.5)	5.3 (1.4–12.6)	5.0 (2.0–15.8)	3.2 (1.4–9.5)	6.9 (1.7–23.6)	6.6 (3.6–17.1)
P-IL6, pg/mL	6.5 (3.1–9.8)	5.6 (3.2–8.4)	3.5 (2.5–8.5)	4.2 (1.9–8.1)	8.5 (5.3–12.7)	6.5 (3.4–10.7)
P-vWF:act, %	145 (102–179)	144 (105–196)	146 (94–207)	137 (105–170)	143 (110–171)	149 (82–324)
P-vWF:ag, %	183 (136–249)	182 (131–346)	190 (103–271)	170 (140–260)	175 (148–250)	204 (115–399)
Systolic BP, mmHg	134 (125–158)	135 (122–160)	140 (126–158)	147 (132–168)	130 (124–159)	129 (116–138)
Diastolic BP, mmHg	72 (67–79)	72 (68–84)	73 (68–80)	75 (68–89)	71 (59–79)	72 (67–82)
PWV, m/s	12.0 (9.0–13.9)[c]	10.0 (7.8–13.2)[d]	12.8 (8.6–14.5)[e]	11.0 (8.3–13.8)[f]	10.5 (9.0–13.3)[g]	9.0 (6.7–12.5)[e]
AIx@75, %	28 (22–31)[h]	26 (18–30)[d]	28 (16–31)[g]	26 (22–29)[f]	28 (23–33)[i]	23 (14–31)[e]
Urinary protein, g/day	n/a	n/a	0.3 (0.2–0.7)	0.5 (0.3–1.8)	n/a	n/a

[a]Figures are medians (interquartile ranges). non-HD, CKD patients not in HD therapy; HD, patients in HD therapy; 25-OHD, 25-hydroxyvitamin D; 1,25-diOHD, 1,25-dihydroxyvitamin D; vWF:act, von Willebrand factor activity; vWF:ag, von Willebrand factor antigen; AIx@75, aortic augmentation index.
[b]Significantly different from corresponding placebo group at $P < 0.01$.
[c]$n = 14$.
[d]$n = 17$.
[e]$n = 8$.
[f]$n = 9$.
[g]$n = 6$.
[h]$n = 13$.
[i]$n = 7$.

Table 3. Eight-week changes in markers of bone mineral metabolism according to treatment (cholecalciferol or placebo)[a]

Parameter	Cholecalciferol all, $n = 25$	Placebo all, $n = 24$	Cholecalciferol non-HD, $n = 13$	Placebo non-HD, $n = 11$	Cholecalciferol HD, $n = 12$	Placebo HD, $n = 13$
ΔP-25-OHD, nmol/L	117.8 (89.4–151.9)#	−9.8 (−20.7 to −1.4)	127.4 (104.9 to 155.2)#	−7.1 (−12.3 to 9.0)	114.9 (82.5 to 153.0)#	−10.4 (−21.4 to −6.5)
ΔP-1,25-diOHD, pmol/L	19 (10–50)#	−1 (−7 to 10)	49 (15 to 60)§	1 (−14 to 18)	14 (−4 to 19)	−1 (−6 to 4)
ΔP-PTH, pmol/L	−1.3 (−8.0 to 0.4)	0.8 (−4.7 to 7.0)	−3.1 (−6.9 to −0.8)#	4.3 (0.5 to 9.1)	−0.3 (−9.9 to 19.3)	−1.3 (−7.2 to 2.2)
ΔS-phosphate, mmol/L	0.00 (−0.10 to 0.19)	−0.07 (−0.32 to 0.14)	0.04 (−0.03 to 0.17)	0.05 (−0.07 to 0.17)	−0.04 (−0.20 to 0.26)	−0.18 (−0.51 to 0.03)
ΔS-calcium ion, mmol/L	0.01 (−0.04 to 0.08)*	−0.02 (−0.05 to 0.00)	0.01 (−0.04 to 0.06)	−0.02 (−0.05 to −0.02)	0.01 (−0.06 to 0.11)	−0.03 (−0.06 to 0.00)
ΔS-calcium total, mmol/L	0.05 (−0.07 to 0.22)§	−0.03 (−0.08 to −0.01)	0.06 (0.02 to 0.15)*	−0.03 (−0.04 to 0.00)	0.00 (−0.10 to 0.22)	−0.03 (−0.14 to 0.01)
ΔS-FGF-23, pg/mL	8 (−243 to 123)*	−50 (−1680 to 0)	13 (−7 to 153)	−10 (−34 to 15)	−60 (−2154 to 136)	−1089 (−3945 to −36)

[a]Cholecalciferol treatment consisted of oral supplementation of 40 000 IU vitamin D_3 every week for 8 weeks. Figures are medians (interquartile ranges). non-HD, CKD patients not in HD therapy; HD, patients in HD therapy; 25-OHD, 25-hydroxyvitamin D; 1,25-diOHD, 1,25-dihydroxyvitamin D.
*Significantly different from corresponding placebo group at $P < 0.05$.
§Significantly different from corresponding placebo group at $P < 0.01$.
#Significantly different from corresponding placebo group at $P < 0.001$.

patients, Δ1,25-diOHD was strongly and negatively associated with baseline FGF-23 ($n = 25$, Spearman's rho = −0.67, P = 0.0002).

Muscle function and general health

Cholecalciferol supplementation had no significant effect on muscle function (Chair test and Deltoideus test), visual

analogue scores for muscle pain, bone pain, itching and appetite or on HAQ scores (baseline data are shown in the Supplementary Table S1; changes of parameters after 8 weeks of cholecalciferol treatment are shown in Supplementary Table S2).

Endothelial and inflammatory markers, BP, aortic augmentation index (AIx@75) and aortic PWV

Cholecalciferol supplementation for 8 weeks had no significant impact on plasma concentrations of biomarkers related to cardiovascular disease. Neither markers of endothelial dysfunction and increased coagulation [von-Willebrand factor (vWF) antigen and activity], hypercoagulation (D-dimer and fibrinogen) and inflammation (IL6 and CRP) nor functional markers of cardiovascular risk (BP, AIx@75 and PWV) changed (Table 5).

Urinary data

There were no significant effects of cholecalciferol supplementation on 24-h urinary loss of protein in non-HD patients, hence those patients with maintained diuresis (Table 5).

Discussion

Most of the previously published intervention studies on vitamin D_2 or D_3 supplementation in CKD were either single-armed, unblinded, uncontrolled or used historical controls [20–32]; some randomized controlled trials involved pre-dialytic patients [33–35]. The present randomized, double-blind and placebo-controlled intervention trial investigated the effects of cholecalciferol supplementation in CKD patients with inadequate vitamin D stores (hypovitaminosis D). It was one of the basic ideas of the study that pleiotropic, or non-classical, effects of vitamin D could result from extra-renal 1-α hydroxylase activity in target tissues independent from renal 1-α hydroxylase activity.

Cholecalciferol-treated patients ingested one capsule of 40 000 IU D_3 weekly for 8 weeks. This dosing was inspired by kidney disease outcomes quality initiative guidelines, where an oral dose of 50 000 IU D_2/D_3 weekly for 4–12 weeks is recommended in mild to severe vitamin D deficiency [36]. Our results demonstrate that the chosen supplementation dose is sufficient to correct hypovitaminosis D, even in subjects with severe vitamin D deficiency and uraemia. At the end of study, cholecalciferol-treated patients were fully vitamin D replenished and had a plasma 25-OHD concentration 6-fold higher than controls, which on average were vitamin D deficient with a median 25-OHD concentration of 23.5 nmol/L. A reduction of liver CYP450 isoforms and 25-hydroxylation of vitamin D by a PTH-dependent mechanism has been shown in uraemia [37]. The cholecalciferol supplementation in our study obviously overcame such mechanism sufficiently.

The cholecalciferol supplementation caused a significant increase in 1,25-diOHD. The increase was statistically significant in non-HD patients, whereas in HD patients, the increase was smaller and insignificant. In agreement with our findings, others have reported significant 1,25-diOHD increments in non-HD CKD patients [22]. The 1,25-diOHD increase observed in our study in HD patients was not significant although it was in other studies [25, 27, 31]. In a classical study, even anephric patients were shown to respond to 25-OHD supplementation with 1,25-diOHD increments due to extra-renal 1,25-diOHD production, but supra-physiological concentrations (∼2.5 times the upper normal range) of 25-OHD were necessary to achieve an effect in those patients [7].

In non-HD patients, cholecalciferol supplementation caused a fall in PTH, so that the prevalence of hyperparathyroidism was 4 of 13 patients at Week 8 in the cholecalciferol-treated versus 9 of 10 patients in the placebo-treated group. Other randomized studies of pre-dialytic CKD 3–5 patients also reported PTH declines with vitamin D_2 or D_3 supplementation [28, 30, 34]. In contrast, PTH fluctuations were small and insignificant in HD patients in the present study. The findings of previous vitamin D_2 or D_3 supplementation studies of HD patients have been inconsistent: some studies reported significant declines in PTH [25, 27, 32], whereas others observed no significant PTH changes [20, 26, 31]. The results of our study are in accordance with the notion that clinically reliable changes in PTH cannot be expected with 25-OHD replenishment in HD patients.

Proximal muscle pain, bone pain and muscle weakness are among the classical signs of severe vitamin D deficiency. We did not observe a significant effect of cholecalciferol supplementation on muscle or bone pain. Our

Table 4. Correlation matrix of 8-week changes (Δ) in markers of bone mineral metabolism of all study participants (both cholecalciferol and placebo-treated patients, $n = 49$)[a]

	ΔP-1,25-diOHD, pmol/L	ΔP-PTH, pmol/L	ΔS-Calcium ion, mmol/L	ΔS-Phosphate, mmol/L	ΔS-FGF-23, pg/mL
ΔP-25-OHD, nmol/L	0.53#	−0.37§	0.40§	0.17	0.28*
ΔP-1,25-diOHD, pmol/L		−0.26	0.22	0.12	0.27
ΔP-PTH, pmol/L			−0.38§	0.06	−0.03
ΔS-calcium ion, mmol/L				−0.08	0.13
ΔS-phosphate, mmol/L					0.71#

[a]Figures are Spearman's rho. 25-OHD, 25-hydroxycholecalciferol; 1,25-diOHD, 1,25-dihydroxycholecalciferol.
*$P < 0.05$.
§$P < 0.01$.
#$P < 0.001$.

Table 5. Eight-week changes in cardiovascular risk markers according to treatment[a]

Parameter	Cholecalciferol all, $n = 25$	Placebo all, $n = 24$	Cholecalciferol non-HD, $n = 13$	Placebo non-HD, $n = 11$	Cholecalciferol HD, $n = 12$	Placebo HD, $n = 13$
ΔP-fibrinogen, µmol/L	0.0 (−1.2 to 1.9)	−0.4 (−2.1 to 1.2)	−0.2 (−1.8 to 1.4)	−0.2 (−1.3 to 1.1)	0.5 (−0.7 to 2.8)	−0.6 (−2.6 to 1.8)
ΔP-D-dimer, mg/L	0.07 (−0.17 to 0.78)	0.04 (−0.11 to 0.18)	−0.05 (−0.25 to 1.84)	0.00 (−0.12 to 0.13)	0.16 (−0.13 to 0.53)	0.10 (−0.14 to 0.27)
ΔP-CRP, mg/L	−0.17 (−9.90 to 3.77)	−0.18 (−3.66 to 1.19)	−0.23 (−6.38 to 3.77)	−0.11 (−0.98 to 0.74)	0.10 (−13.46 to 5.23)	−1.73 (−6.45 to 1.52)
ΔP-IL6, pg/mL	0.1 (−0.8 to 1.2)	0.3 (−0.7 to 1.6)	−0.1 (−1.8 to 1.6)	0.2 (−0.3 to 1.5)	0.5 (−0.8 to 1.1)	0.5 (−0.8 to 1.8)
ΔP-vWF:act, %	1 (−20 to 16)	−4 (−16 to 15)	15 (0–30)	2 (−8 to 17)	−15 (−26 to 7)	−9 (−34 to 9)
ΔP-vWF:ag, %	6 (−21 to 27)	4 (−17 to 31)	12 (2–42)	8 (−23 to 31)	−8 (−36 to 13)	4 (−13 to 31)
ΔSystolic BP, mmHg	−2 (−8 to 11)	−3 (−8 to 9)	−3 (−12 to 9)	−3 (−8 to 9)	0 (−8 to 22)	−3 (−13 to 9)
ΔDiastolic BP, mmHg	0 (−4 to 9)	−2 (−7 to 6)	1 (−5 to 5)	−1 (−5 to 6)	−2 (−7 to 11)	−2 (−8 to 6)
ΔPWV, m/s	0.7 (−1.2 to 3.2)[b]	−0.3 (−0.7 to 0.8)[c]	0.9 (−0.6 to 3.7)[d]	0.0 (−0.8 to 0.7)[e]	−1.3 (−2.8 to 3.2)[f]	−0.5 (−0.7 to 0.8)[e]
ΔAIx@75, %	−1.5 (−5.0 to 3.0)[g]	−2.0 (−5.3 to 2.5)[h]	−3.0 (−5.5 to 0.5)[f]	−2.0 (−6.0 to 6.5)[d]	1.5 (−5.0 to 8.0)[i]	−2.0 (−5.0 to 1.0)[d]
ΔUrinary protein, g/day	n/a	n/a	−0.1 (−0.1 to 0.0)	0.0 (−0.1 to 0.8)	n/a	n/a

[a]Cholecalciferol treatment consisted of oral supplementation of 40 000 IU vitamin D_3 every week for 8 weeks. Figures are medians (interquartile ranges). non-HD, CKD patients not in HD therapy; HD, patients in HD therapy; vWF:act, von Willebrand factor activity; vWF:ag, von Willebrand factor antigen; AIx@75, aortic augmentation index.
[b]$n = 12$.
[c]$n = 16$.
[d]$n = 7$.
[e]$n = 8$.
[f]$n = 5$.
[g]$n = 9$.
[h]$n = 14$.
[i]$n = 4$.

study participants—most of them with moderate to severe hypovitaminosis D—had limited muscle and bone complaints at baseline. This could be one explanation for the absent effect of cholecalciferol on these parameters. We also performed clinical tests of muscle function, but the findings here were also negative. In contrast to our study, Shah *et al.* [20] reported reduced bone pain and muscle weakness already after 4 weeks of supplementation with vitamin D_2, 50 000 IU/week, in 23 peritoneal dialysis patients with severe vitamin D deficiency. To our knowledge, no further studies of CKD patients on this subject have been published. Based on our findings, we find it unlikely to expect marked improvements in muscle function with 25-OHD replenishment of CKD patients.

Cholecalciferol supplementation caused a small but significant rise in serum FGF-23. An indirect effect mediated by the induced increase in 1,25-diOHD is likely: earlier studies showed that 1,25-diOHD stimulates FGF-23 secretion [4, 38]. In HD patients, increasing FGF-23 was independently associated with an increased mortality [39]. Serum FGF-23 of deceased cases was almost twice as high as that of surviving controls. Similar results were obtained by Isakova *et al.* [40] in patients with CKD Stages 2–4. Accordingly, the observed 5–10% increase in FGF-23 with cholecalciferol supplementation observed in the present study might have a modest influence on mortality. We observed a highly significant inverse association between baseline FGF-23 and 1,25-diOHD increments among cholecalciferol-treated patients. This finding supports earlier reports of an inhibitory effect of FGF-23 on 1-hydroxylase activity.

In a recent uncontrolled trial of seven HD patients, Stubbs *et al.* [31] reported significant and favourable effects after 8 weeks of cholecalciferol supplementation on the concentration of inflammatory cytokines, including a 30% decline in IL6. In their study, the vitamin D supplementation caused a 4-fold increase in 25-OHD. These findings are in contrast to our data: we observed no impact of an even more effective cholecalciferol supplementation (6-fold increase in 25-OHD compared to controls) on IL6 and other markers of inflammation such as CRP, fibrinogen and D-dimer.

Cross-sectional studies of HD patients showed an independent association between 25-OHD and 1,25-diOHD on the one hand and PWV on the other hand [14]. These aspects were also investigated in the present study. We

observed no effect of 25-OHD replenishment on any of a number of biomarkers related to cardiovascular disease [17]. Neither markers of endothelial dysfunction, increased coagulation and inflammation nor functional markers of cardiovascular risk like BP, aortic augmentation index, PWV or proteinuria changed significantly.

The majority of vitamin D effects on target cells and tissues involve the vitamin D receptor (VDR) [13]. VDR expression is decreased in uraemia and uraemic high phosphataemic conditions [41, 42]. Also, VDR-mediated gene transcription is modulated by increased phosphate [43]. Mechanisms like these might be involved in the described failure of 25-OHD replenishment in our study. In a recent review by Dusso and Tokumoto [5], an exclusive 25-OHD supplementation to sustain calcitriol/VDR actions in advanced CKD was judged as being questionable since normalization of 1,25-OHD concentration is insufficient to compensate for low tissue VDR content in CKD.

Current treatment guidelines for CKD patients recommend correction of hypovitaminosis D. The implementation of this recommendation should be executed safely [44–47]. In our study, we gave vitamin D supplements in a dosage inspired by published guidelines (40 000 IU D_3 weekly for 8 weeks) [36]. We observed mild to moderate hypercalcaemia in 5 of 25 cholecalciferol-treated patients. Hypercalcaemia was very mild in two and moderate in two other patients. One patient developed more serious hypercalcaemia that required admission.

In conclusion, we find that the chosen supplementation dosage is effective concerning 25-hydroxyvitamin D replenishment but also that it is necessary to monitor for hypercalcaemia at regular intervals.

It is a limitation of our study that the study population was relatively small ($n = 52$). The intervention period of 8 weeks may have been too short to allow the complete presentation of all biochemical and pleiotropic effects of cholecalciferol supplementation. On the other hand, earlier smaller-sized studies reported significant effects of 25-OHD replenishment, e.g. on muscle function and IL6, already after 4–8 weeks [20, 31].

The strengths of the study are the randomized (balanced and stratified) and double-blinded design and the very distinct differences in 25-hydroxyvitamin D status that were achieved by the intervention. Both properties minimize the risk of misleading chance findings and increase the chance to identify the effects of 25-OHD replenishment.

In conclusion, the present study demonstrates that hypovitaminosis D can be corrected with weekly ingestion of 40 000 IU D_3 for 8 weeks. In non-HD patients, this replenishment is associated with desirable increases in circulating 1,25-diOHD, lowering of PTH and reduced prevalence of hyperparathyroidism. We were not able to demonstrate any beneficial effects of vitamin D3 supplementation in HD patients.

Except for an unwelcome rise of FGF-23, we were not able to show pleiotropic effects of cholecalciferol supplementation on the investigated markers of cardiovascular risk, inflammation, muscle function or subjective health parameters. For the further evaluation of 25-OHD supplementation in CKD patients, a randomized controlled follow-up study with hard endpoints is warranted.

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