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Rationale for Raising Current Clinical Practice Guideline Target for Serum 25-Hydroxyvitamin D in Chronic Kidney Disease

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Keywords
Chronic kidney disease · Secondary hyperparathyroidism · Vitamin D insufficiency · 25-Hydroxyvitamin D · Extended-release calcifediol · Bone turnover markers

Abstract
Background: Vitamin D repletion is recommended for secondary hyperparathyroidism (SHPT) and associated vitamin D insufficiency (VDI) in chronic kidney disease (CKD), but optimal levels of serum total 25-hydroxyvitamin D remain undefined. Clinical practice guidelines target sufficiency, whereas recent data indicate that higher levels are required to control the elevation of intact parathyroid hormone (iPTH) as CKD advances. This secondary analysis of 2 randomized controlled trials seeks to identify the minimum level of mean serum 25-hydroxyvitamin D required to control SHPT arising from VDI in stage 3 or 4 CKD. ***Methods:*** Adult subjects ($n = 429$) with SHPT, VDI, and stage 3 or 4 CKD were stratified by stage and treated daily with either extended-release calcifediol (ERC) or placebo in 2 identical, parallel, randomized, double-blind studies. After treatment for 26 weeks, all subjects were ranked by the level of serum total 25-hydroxyvitamin D and divided into quintiles in order to examine the relationships between the degree of vitamin D repletion and the associated changes in plasma iPTH, serum bone turnover markers, calcium, phosphorus, intact fibroblast growth factor 23 (FGF23) and vitamin D metabolites, estimated glomerular filtration rate (eGFR), and urine calcium:creatinine (Ca:Cr) ratio. ***Results:*** Progressive increases in serum 1,25-dihydroxyvitamin D and reductions in plasma iPTH and serum bone turnover markers were observed as mean posttreatment serum 25-hydroxyvitamin D rose from 13.9 ng/mL (in Quintile 1) to 92.5 ng/mL (in Quintile 5), irrespective of CKD stage. Mean serum calcium, phosphorus and FGF23, eGFR, and urine Ca:Cr ratio (collectively "safety parameters") did not significantly change from Quintile 1. Suppression of iPTH and bone turnover markers was not observed until serum 25-hydroxyvitamin D rose to at least 50.8 ng/mL (Quintile 3). ***Conclusion:*** ERC therapy produced exposure-dependent reductions in plasma iPTH and bone turnover markers only when mean serum total 25-hydroxyvitamin D reached at least 50.8 ng/mL, indicating that current targets for vitamin D repletion therapy in CKD are too low. Gradual elevation of mean serum 25-hydroxyvitamin D to 92.5 ng/mL was not associated with significant adverse changes in safety parameters.

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Introduction

Secondary hyperparathyroidism (SHPT) commonly develops with progressive chronic kidney disease (CKD) and is characterized by overproduction of intact parathyroid hormone (iPTH) and hypertrophy of the parathyroid glands [1]. It is associated with low serum total 25-hydroxyvitamin D, elevation of serum phosphorus and fibroblast growth factor 23 (FGF23), and decreased serum 1,25-dihydroxyvitamin D and calcium. Untreated, SHPT can lead to bone disease, increased fracture rates, vascular calcification, morbidity, and mortality [2].

There is a general agreement that vitamin D insufficiency (VDI) is more prevalent in CKD than in the general population [3, 4] and a modifiable risk factor for SHPT that should be corrected in CKD [5–7]. Supplementation with nutritional vitamin D (ergocalciferol or cholecalciferol) is recommended by clinical practice guidelines [5–7] despite its effectiveness for SHPT remaining unproven in randomized clinical trials (RCTs) [7–9]. Consensus, however, is lacking on the definition of vitamin D sufficiency in CKD. In 2003, the National Kidney Foundation defined vitamin D sufficiency as serum total 25-hydroxyvitamin D concentrations of ≥30 ng/mL [5], and in 2011, the Endocrine Society defined it as concentrations between 30 and 100 ng/mL [10]. The United States (US) Institute of Medicine (IOM) disagreed stating in 2011 that "practically all persons are sufficient at serum 25-hydroxyvitamin D levels of at least 20 ng/mL" [11].

Recent studies have demonstrated that serum total 25-hydroxyvitamin D levels well above 20 ng/mL are required to effectively treat SHPT in patients with CKD. In 2014, one RCT demonstrated that suppression of plasma iPTH with extended-release calcifediol (ERC) was directly proportional to increases in serum total 25-hydroxyvitamin D above 20 ng/mL in patients with estimated glomerular filtration rates (eGFR) of ≥25 to <70 mL/min/1.73 m^2. Levels as high as 85 ng/mL were insufficient to bring mean iPTH into the normal range [12]. A large cross-sectional study concluded that 25-hydroxyvitamin D levels well above 42–48 ng/mL were required for normalization of elevated PTH levels in more advanced CKD [13]. Data from 2 RCTs with ERC in patients with stage 3 or 4 CKD demonstrated that serum total 25-hydroxyvitamin D levels averaging approximately 68 ng/mL were required for effective iPTH suppression [14]. No evidence of increased rates of hypercalcemia or hyperphosphatemia with higher levels of 25-hydroxyvitamin D were observed in these studies. According to the IOM [11], "there may be reason for concern at serum 25-hydroxyvitamin D levels above 50 ng/mL," based on observational studies of all-cause mortality in elderly subjects [15, 16] and the general population [17], and on the incidence of cancer [18–20].

Herein, we report a secondary analysis of pooled data from the 2 most recent RCTs conducted with ERC in patients with stage 3 or 4 CKD [14]. This analysis focuses primarily on the observed changes in plasma iPTH, serum bone turnover markers, and widely accepted safety parameters as a function of gradual, progressive increases in mean serum total 25-hydroxyvitamin D well above 20 or 30 ng/mL and seeks to identify the minimum level of 25-hydroxyvitamin D required to halt SHPT progression.

Materials and Methods

Study Design

Two identical 26-week multicenter studies with randomized, double-blind, placebo-controlled designs enrolled a total of 429 subjects from 89 US sites with SHPT (plasma iPTH ≥85 and <500 pg/mL), stage 3 or 4 CKD (eGFR of ≥15 and <60 mL/min/1.73 m^2), and VDI (serum total 25-hydroxyvitamin D ≥10 and <30 ng/mL). Other eligibility criteria included serum calcium ≥8.4 and <9.8 mg/dL and serum phosphorus ≥2.0 and <5.0 mg/dL. Exclusion criteria included a spot urine calcium:creatinine (Ca:Cr) ratio of >0.2, nephrotic range proteinuria (>3 mg/mg Cr), and a history of parathyroidectomy for SHPT or renal transplantation. Subjects were enrolled progressively from December 2012 to January 2014 at sites of many different latitudes in order to minimize seasonal variation in mean baseline serum total 25-hydroxyvitamin D. Further details regarding these studies have been previously published [14, 21].

Subjects were stratified by CKD stage and were randomized in a 2:1 ratio to receive a once daily 30 μg oral dose of ERC (or matching placebo) for 12 weeks at bedtime followed by an additional 14 weeks of treatment with once daily bedtime doses of either 30 or 60 μg of ERC (or placebo). The daily dose was increased to 60 μg at the start of week 13 if plasma iPTH remained >70 pg/mL (the upper limit of the laboratory reference range), serum total 25-hydroxyvitamin D was <65 ng/mL (to reduce the risk of driving values above 100 ng/mL), and serum calcium was <9.8 mg/dL. The sole primary efficacy end point was the proportion of subjects in the intent-to-treat (ITT) population that attained a mean decrease of ≥30% in plasma iPTH from pretreatment baseline in the efficacy assessment period (EAP), defined as treatment weeks 20 through 26.

Study Populations

A total of 213 subjects participated in the first of these 2 RCTs (141 ERC and 72 placebo) and 216 subjects in the other (144 ERC and 72 placebo), and 354 subjects (83%) completed the studies. Data from both RCTs were pooled because: (a) the studies were governed by a common protocol; (b) they were conducted contemporaneously using multiple sites within the continental US; (c) the subject populations were similar according to selection

Table 1. Demographic and baseline data for PP subjects grouped by CKD stage

	CKD 3, mean (SE)	CKD 4, mean (SE)	Total
Number of subjects	185	171	356
Male	98	91	189
Female	87	80	167
Baseline eGFR	38.5 (0.6)	23.7 (0.4)[3]	31.4 (0.5)
Weight, kg	98.7 (1.8)	96.8 (1.9)	97.8 (1.3)
BMI, kg/m^2	35.1 (0.6)	34.2 (0.6)	34.7 (0.4)
Age, years	65.4 (0.8)	65.3 (0.9)	65.4 (0.6)
Serum total 25(OH)D, ng/mL	19.9 (0.4)	19.2 (0.4)	19.6 (0.3)
Serum total 1,25$(OH)_2$D, pg/mL	39.7 (1.0)	29.9 (1.0)[3]	34.5 (0.7)
Serum 24,25$(OH)_2D_3$, ng/mL	1.09 (0.04)	1.02 (0.03)	1.05 (0.02)
Plasma iPTH, pg/mL	129.5 (3.0)	160.1 (4.9)[3]	144.2 (2.9)
Serum total alkaline phosphatase, U/L	92.4 (2.1)	94.2 (2.5)	93.2 (1.6)
Serum BSAP, U/L	36.8 (1.3)	39.3 (1.5)	38.0 (1.0)
Serum CTx-1, pg/mL	602 (24)	841 (32)[3]	717.6 (20.8)
Serum P1NP, ng/mL	86.1 (3.5)	110.5 (5.5)[2]	98.0 (3.3)
Serum calcium, mg/dL	9.3 (0.03)	9.2 (0.03)[1]	9.3 (0.02)
Serum phosphorus, mg/dL	3.6 (0.04)	3.9 (0.04)[3]	3.7 (0.03)
Serum FGF-23, pg/mL	38.2 (3.2)	42.0 (4.0)	40.3 (2.6)
Urine Ca:Cr ratio, g/g creatine	0.046 (0.005)	0.031 (0.003)[1]	0.039 (0.003)

[1] Significantly different from CKD 3 subjects, $p < 0.05$.
[2] Significantly different from CKD 3 subjects, $p < 0.001$.
[3] Significantly different from CKD 3 subjects, $p < 0.0001$.

PP, per-protocol; BMI, body mass index; iPTH, intact parathyroid hormone; Ca:Cr, calcium:creatinine; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; FGF23, fibroblast growth factor 23; BSAP, bone-specific alkaline phosphatase; CTx-1, C-terminal collagen crosslinks; P1NP, procollagen I amino-terminal propeptide.

criteria and actual baseline demographic and biochemical characteristics; and (d) the changes observed in serum total 25-hydroxyvitamin D, serum total 1,25-dihydroxyvitamin D, and plasma iPTH were similar during ERC or placebo treatment. In aggregate, 222 subjects (51.7%) had stage 3 CKD (151 ERC and 71 placebo), and 207 subjects (48.3%) had stage 4 CKD (134 ERC and 73 placebo).

The ITT population included all subjects ($n = 429$) who were randomized to study drug. Subjects in the ITT population had a mean age of 66 years (range 25–85), 50% were male, 65% White, 32% African-American or Black, 21% Hispanic, and 3% other. The most common causes of CKD were diabetes and hypertension, and the mean eGFR was 31 mL/min/1.73 m^2. The per-protocol (PP) population included all subjects ($n = 356$) who did not have a major protocol deviation and for whom at least 2 serum total 25-hydroxyvitamin D and 2 plasma iPTH determinations were included in the calculated baseline value and in the EAP, defined as treatment weeks 20 through 26. Demographic and baseline data for the PP population are summarized in Table 1, grouped by CKD stage. Only analyses of the PP population are reported here as they yielded results that did not differ materially from those based on analyses of the ITT population, and because the number of subjects remained constant across the 26-week treatment period. Sixty-two ITT subjects were excluded because they discontinued treatment prior to the EAP, and 11 for major protocol violations: receipt of prohibited concomitant medication ($n = 4$); failure to meet all selection criteria ($n = 3$); dosing compliance <80% ($n = 3$); and premature unblinding ($n = 1$).

Laboratory and Clinical Procedures

Blood and spot urine samples were collected at weekly or biweekly intervals and analyzed during the applicable stability windows (documented in validation reports) at PPD Global Central Labs (Highland Heights, KY, USA). Plasma iPTH levels were determined by two-site sandwich electrochemiluminescence (Roche Elecsys; reference range 15–65 pg/mL; %CV 2.7). Serum total 25-hydroxyvitamin D was determined by chemiluminescence (DiaSorin), and serum total 1,25-dihydroxyvitamin D was determined by radioimmunoassay (IDS). Serum 25-hydroxyvitamin D_3 (lower limit of quantitation: 5.00 ng/mL; %CV of 0.82–1.84 within-run, 2.01–4.26% between-run) and 24,25-dihydroxyvitamin D_3 (lower limit of quantitation: 0.52 ng/mL; %CV 2.18–4.60 within-run, 3.79–9.29 between-run) were determined by LC–MS (Syneos) for the purpose of calculating the vitamin D metabolite ratio (VMR), calculated as serum 24,25-dihydroxyvitamin D_3/serum total 25-hydroxyvitamin D_3 * 100. Serum (rather than plasma) intact FGF23 levels were determined by enzyme-linked immunosorbent assay (Millipore; reference range 0–50 pg/mL; %CV 10.6) because of better recovery and long-term stability during validation. Serum collagen type 1 C-telopeptide (CTx-1) was measured

by electrochemiluminescence (Roche Cobas; reference range 0–856 pg/mL; %CV 1.4). Intact procollagen type 1 N-terminal propeptide (P1NP) was determined by chemiluminescence immunoassay (Roche Cobas; reference range 13.8–88 ng/mL; %CV 5.0), an assay which measures monomers that potentially accumulate in CKD patients, leading to falsely elevated results. Bone-specific alkaline phosphatase was determined by ELISA (Quidel; reference range 14.9–42.4 U/L, %CV 7.7), an assay which measures activity rather than mass. Total alkaline phosphatase was measured by enzymatic assay (Roche Cobas; reference range 43–115 U/L; %CV 2.0). Other parameters were determined by standard procedures. Serum calcium values were corrected for low albumin.

Results

Analyses by CKD Stage

Changes from pretreatment baseline in mean serum total 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and plasma iPTH in response to treatment with ERC or placebo were examined by CKD stage both midway through the study (at treatment weeks 8–12) and at the EAP (treatment weeks 20–26). ERC increased mean serum 25-hydroxyvitamin D similarly versus placebo ($p < 0.0001$) in both CKD stages at mid-study and at the EAP (Fig. 1a). ERC also increased mean serum 1,25-dihydroxyvitamin D and reduced mean plasma iPTH similarly versus placebo ($p < 0.05$ to 0.0001) in both CKD stages at mid-study and at the EAP (Fig. 1b, c). Values for serum 1,25-dihydroxyvitamin D and plasma iPTH were expressed as percentages of baseline since subjects with stage 3 CKD had different mean baseline values than subjects with stage 4 CKD (Table 1). Given the lack of stage-specific responses for these 3 key parameters, all further analyses were completed without regard to CKD stage.

Analyses by Posttreatment 25-Hydroxyvitamin D Quintile

All subjects, whether treated with ERC or placebo, were subsequently ranked by mean posttreatment (EAP) serum total 25-hydroxyvitamin D levels and divided into quintiles, with Quintile 1 being defined as subjects with the lowest levels and Quintiles 2–5 as those with progressively higher levels. The mean (SE) posttreatment 25-hydroxyvitamin D values in each quintile are noted at the top of Table 2 where the demographic and baseline characteristics of the PP subjects are summarized (by quintile) and at the left in Table 3. Means for Quintiles 2–5 were all significantly greater than the corresponding mean for Quintile 1 ($p < 0.0001$). The proportions of subjects treated with placebo in Quintiles 1 and 2 were 96 and 76%, respectively. There were no placebo subjects in Quintiles 3–5. Data from Quintiles 2–5 were compared to those from Quintile 1 by one-way ANOVA with subsequent Bonferroni's correction. Mean (SE) serum total 25-hydroxyvitamin D at baseline ranged from 16.1 (0.6) to 21.7 (0.6) ng/mL ($p < 0.05$) within the individual quintiles. Sig-

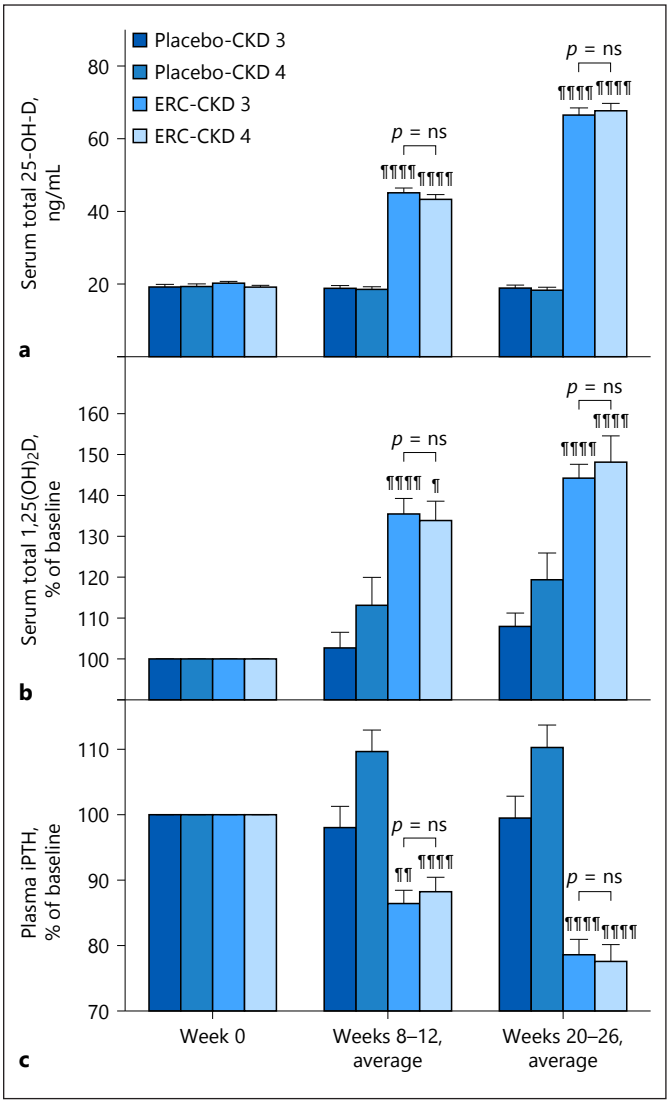


Fig. 1. Changes in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and plasma iPTH by treatment group and CKD stage. Mean (SE) data from PP subjects at pretreatment baseline (week 0), weeks 8–12 and weeks 20–26 were analyzed by treatment group and CKD stage. Differences between active and corresponding placebo groups or between CKD stages were calculated by *t* test. **a** Serum total 25-hydroxyvitamin D (25-OH-D). **b** Serum total 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). **c** Plasma iPTH. ¶ Significantly different from corresponding placebo group, $p < 0.05$; ¶¶ Significantly different from corresponding placebo group, $p < 0.01$; ¶¶¶¶ Significantly different from corresponding placebo group, $p < 0.0001$.

Table 2. Demographic and baseline data for PP subjects grouped by posttreatment 25-hydroxyvitamin D Quintile

	Quintile 1 25(OH)D 13.9 (0.4), ng/mL, mean (SE)	Quintile 2 25(OH)D 26.2 (0.6), ng/mL, mean (SE)	Quintile 3 25(OH)D 50.8 (0.8), ng/mL, mean (SE)	Quintile 4 25(OH)D 68.9 (0.6), ng/mL, mean (SE)	Quintile 5 25(OH)D 92.5 (1.4), ng/mL, mean (SE)
Number of subjects	71	71	72	71	71
Male	34	41	49	27	27
Female	37	30	23	44	44
Baseline eGFR	31.5 (1.0)	33.0 (1.4)	32.0 (1.3)	30.5 (1.1)	29.9 (1.2)
Weight, kg	96.5 (2.7)	101.2 (3.0)	105.9 (3.1)	96.2 (2.7)	89.0 (2.5)[3, 6]
BMI, kg/m^2	34.6 (0.9)	35.3 (1.0)	36.5 (1.0)	34.4 (0.8)	32.5 (0.9)[5]
Age, years	63.4 (1.3)	63.0 (1.5)	64.9 (1.2)	67.0 (1.3)	68.5 (1.0)[3]
Serum total 25(OH)D, ng/mL	16.1 (0.6)	21.7 (0.6)[2]	18.5 (0.6)[1, 4]	20.8 (0.6)[2]	20.7 (0.6)[2]
Serum total 1,25$(OH)_2$D, pg/mL	33.4 (1.6)	38.3 (1.8)	33.9 (1.6)	34.0 (1.6)	35.4 (1.5)
Serum 24,25$(OH)_2D_3$, ng/mL	0.8 (0.07)	1.1 (0.05)	0.9 (0.05)	1.0 (0.06)	1.2 (0.07)[2]
Plasma iPTH, pg/mL	157.0 (8.1)	140.6 (5.7)	142.3 (6.8)	134.5 (4.4)	146.7 (6.9)
Serum total alkaline phosphatase, U/L	97.8 (3.4)	85.9 (2.7)	93.7 (3.8)	94.4 (3.9)	94.4 (4.3)
Serum BSAP, U/L	40.8 (2.2)	35.0 (1.8)	35.5 (1.7)	39.3 (2.5)	39.4 (2.6)
Serum CTX-1, pg/mL	724 (49)	731 (53)	684 (41)	721 (44)	728 (46)
Serum P1NP, ng/mL	96.7 (6.3)	103.0 (10.7)	100.5 (6.6)	92.2 (5.9)	97.4 (6.4)
Serum calcium, mg/dL	9.3 (0.03)	9.2 (0.03)	9.3 (0.04)	9.2 (0.03)	9.2 (0.03)
Serum phosphorus, mg/dL	3.7 (0.06)	3.7 (0.07)	3.8 (0.06)	3.7 (0.07)	3.8 (0.07)
Serum FGF-23, pg/mL	34.7 (3.8)	44.1 (5.2)	45.4 (10.1)	36.1 (3.2)	43.1 (5.3)
Urine Ca:Cr ratio, g/g creatinine	0.038 (0.006)	0.045 (0.009)	0.036 (0.006)	0.039 (0.006)	0.037 (0.006)

[1] Significantly different from Quintile 1, $p < 0.05$.
[2] Significantly different from Quintile 1, $p < 0.001$.
[3] Significantly different from Quintile 2, $p < 0.05$.
[4] Significantly different from Quintile 2, $p < 0.01$.
[5] Significantly different from Quintile 3, $p < 0.05$.
[6] Significantly different from Quintile 3, $p < 0.001$.
PP, per-protocol; BMI, body mass index; iPTH, intact parathyroid hormone; Ca:Cr, calcium:creatinine; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; BSAP, bone-specific alkaline phosphatase; CTx-1, C-terminal collagen crosslinks; P1NP, procollagen I amino-terminal propeptide.

nificant variations between quintiles at baseline were also apparent for mean body weight, body mass index, and age, as noted, but no differences were detected for mean eGFR or for mean serum and urine parameters associated with mineral and bone metabolism.

Serum Total 1,25-Dihydroxyvitamin D

Mean (SE) posttreatment serum total 1,25-dihydroxyvitamin D increased progressively across the quintiles from 34.3 (1.3) pg/mL in Quintile 1 to 48.5 (2.1) pg/mL in Quintile 5. Means for Quintiles 2–5 were all significantly greater than the mean for Quintile 1 ($p < 0.01$).

Serum 24,25-Dihydroxyvitamin D_3 and VMR

Mean (SE) posttreatment serum 24,25-dihydroxyvitamin D_3 increased progressively from 0.7 (0.04) in Quintile 1 to 5.6 (0.27) ng/mL in Quintile 5. Values differed from Quintile 1 for only for the 2 highest quintiles ($p < 0.05$). Mean (SE) posttreatment VMR rose progressively from 3.6 (0.22) for Quintile 1 to 4.8 (0.22) in Quintile 4 but remained stable thereafter at 4.7 (0.19) in Quintile 5.

Plasma iPTH

Mean (SE) plasma iPTH trended upward during treatment in Quintiles 1 and 2, which included mostly placebo subjects, but decreased ($p < 0.05$) progressively in the 3 higher quintiles (Table 3; Fig. 2a). Mean posttreatment iPTH was 166 (10) pg/mL in Quintile 1 and was significantly lower ($p < 0.001$) in Quintiles 3–5, reaching 115 (6), 101 (5), and 97 (5) pg/mL, respectively (Fig. 2b). The observed reductions in iPTH appeared to attenuate as mean serum total 25-hydroxyvitamin D approached the highest level. The proportion of subjects who attained a mean decrease of ≥30% in plasma iPTH from pretreatment baseline in the EAP was 8.5% in Quintiles 1 and 2 and then increased in a linear fashion to 27.8% in Quintile 3, 42.3% in Quintile 4, and 57.7% in Quintile 5 (Fig. 3).

Serum Bone Turnover Markers

Changes in mean (SE) serum CTx-1, P1NP, bone-specific alkaline phosphatase, and total alkaline phosphatase within a given quintile with treatment duration and across quintiles at the end of treatment were similar to those observed for plasma iPTH (Fig. 2a, b). Mean post-

Table 3. Analysis of plasma iPTH and serum bone turnover markers by duration of treatment and posttreatment 25-hydroxyvitamin D Quintile

Quintile (mean serum total 25[OH]D at EAP)	PTH, pg/mL			CTX-1, pg/mL			P1NP, ng/mL			BSAP, U/L			Alkaline phosphatase, U/L		
	BL	Wk12	EAP	BL	Wk12	EAP	BL	Wk12	EAP	BL	Wk12	EAP	BL	Wk12	EAP
Quintile 1 (13.9 ng/mL)	157 (8)	165 (11)	166 (10)	724 (49)	722 (44)	763 (45)	97 (6.3)	99 (5.4)	111 (7.4)	41 (2.2)	38 (2.1)	35 (1.8)	98 (3.4)	105 (4.6)	101 (3.4)
	141 [84]	134 [85]	148 [73]	691 [420]	637 [533]	700 [374]	88 [76]	95 [73]	102 [71]	37 [21]	34 [23]	32 [22]	93 [39]	100 [39]	99 [33]
Quintile 2 (26.2 ng/mL)	141 (6)	146 (8)	149 (10)	731 (53)	739 (53)	747 (47)	103 (11)	104 (11)	107 (10)	35 (1.8)	34 (2.0)	33 (2.4)	86 (2.7)	89 (3.2)	91 (3.3)
	134 [51]	132 [77]	**130 [69]**	637 [424]	653 [547]	644 [487]	77 [78]	83 [41]	75 [64]	32 [18]	30 [20]	**27 [20]**	84 [27]	87 [36]	87 [29]
Quintile 3 (50.8 ng/mL)	142 (7)	123 (7)	**115 (6)**	684 (41)	675 (41)	616 (38)	101 (6.6)	93 (5.9)	94 (7.8)	35 (1.7)	32 (1.6)	**26 (1.3)**	94 (3.8)	91 (4.3)	88 (4.0)
	123 [50]	**112 [50]**	**106 [49]**	631 [529]	563 [594]	**632 [484]**	83 [68]	80 [60]	75 [68]	34 [17]	28 [19]	**24 [14]**	86 [37]	86 [33]	80 [36]
Quintile 4 (68.9 ng/mL)	134 (4)	**118 (5)**	**101 (5)**	721 (44)	664 (42)	**585 (39)**	92 (5.9)	92 (6.2)	82 (5.5)	39 (2.5)	35 (1.9)	**28 (1.7)**	94 (3.9)	90 (2.9)	89 (3.3)
	127 [49]	**117 [58]**	**99 [52]**	665 [587]	589 [406]	**517 [441]**	85 [47]	75 [50]	69 [57]	34 [25]	30 [19]	**25 [12]**	87 [36]	85 [35]	87 [36]
Quintile 5 (92.5 ng/mL)	147 (7)	**118 (6)**	**97 (5)**	728 (46)	686 (43)	**583 (40)**	97 (6.4)	95 (6.3)	81 (7.0)	39 (2.6)	35 (2.2)	**26 (1.4)**	94 (4.3)	89 (3.6)	83 (3.1)
	136 [70]	**106 [63]**	**85 [47]**	632 [433]	589 [567]	**476 [432]**	80 [63]	82 [68]	70 [44]	32 [26]	30 [23]	**23 [15]**	89 [46]	85 [35]	79 [32]

All entries are mean (SE) or median [IQR].
Bold: significantly different from baseline, $p < 0.05$.
: Significantly different from Quintile 1, $p < 0.05$.
: Significantly different from Quintile 1, $p < 0.001$.
iPTH, intact parathyroid hormone; EAP, efficacy assessment period; BSAP, bone-specific alkaline phosphatase; CTx-1, C-terminal collagen crosslinks; P1NP, procollagen I aminoterminal propeptide.

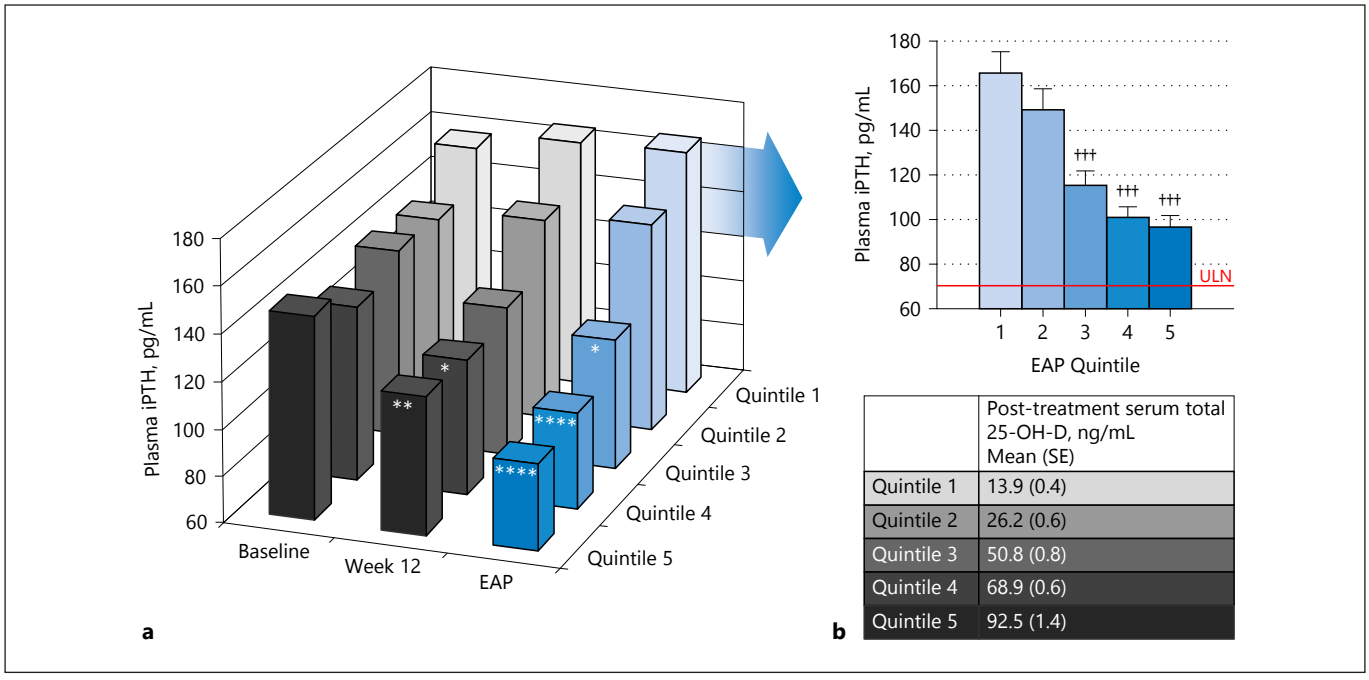
treatment values were within the laboratory normal ranges for all quintiles except for P1NP, which remained elevated in Quintiles 1–3.

Safety Parameters

Mean (SE) posttreatment levels of serum calcium and phosphorus were 9.3 (0.05) and 3.8 (0.06) mg/dL, respectively, in Quintile 1 and trended slightly upward across the other 4 quintiles, reaching 9.45 (0.03) and 4.0 (0.07) mg/dL, respectively, in Quintile 5. Mean (SE) posttreatment values for eGFR and the urine Ca:Cr ratio varied without apparent trends among the 5 quintiles between 27.8 (1.1) and 32.3 (1.6) mL/min/1.73 m^2, and 0.03 (0.004) to 0.04 (0.006), respectively. Mean (SE) posttreatment levels of serum intact FGF23 in Quintiles 1–5 were 51.7 (9.6), 63.3 (16.1), 50.6 (8.7), 44.9 (7.5), and 62.8 (7.9) pg/ mL, respectively. No significant differences were observed between Quintile 1 and any of the higher quintiles (p = NS) for these 5 parameters.

Discussion/Conclusion

This post hoc analysis of pooled data from 2 identical 26-week prospective, multicenter randomized, double-blind, placebo-controlled studies conducted with ERC in patients with stage 3 or 4 CKD showed that mean reductions in plasma iPTH and serum bone turnover markers were proportional to increases in mean serum total 25-hydroxyvitamin D and independent of CKD stage. These findings support the conclusion that ERC suppresses elevated iPTH and bone turnover markers by gradually raising the circulating level of 25-hydroxyvitamin D. They further show that reducing iPTH and bone turnover markers in CKD patients requires mean serum 25-hydroxyvitamin D levels of at least 50.8 ng/mL, well above the targets in clinical practice guidelines of 20 or 30 ng/mL [5, 10, 11], and suggest that normalization of iPTH, if desired, requires even higher levels than those evaluated here. Higher levels of serum 25-hydroxyvitamin D are readily achieved with ERC treatment and proportional to the administered dose [12]. iPTH normalization, however, may not be achievable in view of the apparent attenuation in mean iPTH reduction at the highest level of mean serum total 25-hydroxyvitamin D (92.5 ng/mL) examined herein. This attenuation may be overcome with longer treatment or it may offer both protection from iPTH oversuppression and an indication of the appropriate target for iPTH reduction in patients with stages 3–4 CKD.



	Post-treatment serum total 25-OH-D, ng/mL Mean (SE)
Quintile 1	13.9 (0.4)
Quintile 2	26.2 (0.6)
Quintile 3	50.8 (0.8)
Quintile 4	68.9 (0.6)
Quintile 5	92.5 (1.4)

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Fig. 2. Analysis of plasma iPTH by duration of treatment and post-treatment 25-hydroxyvitamin D Quintile. Mean (SE) data from PP subjects were analyzed by duration of treatment (Baseline [week 0], week 12 [average of treatment weeks 8–12] and EAP [EAP, average of treatment weeks 20–26]) within a given quintile, and by posttreatment 25-hydroxyvitamin D quintile (n = 71–72 in each). Differences from baseline (**a**) and from Quintile 1 at EAP (**b**) were calculated by ANOVA with subsequent Bonferroni's correction. * Significantly different from baseline, $p < 0.05$; ** Significantly different from baseline, $p < 0.01$; **** Significantly different from baseline, $p < 0.0001$; ††† Significantly different from Quintile 1, $p < 0.0001$. ULN, upper limit of normal; EAP, efficacy assessment period; iPTH, intact parathyroid hormone.

It is currently unknown whether normalization of iPTH is an appropriate goal of vitamin D repletion therapy given that the optimal iPTH level in patients with stage 3 or 4 CKD remains undefined. The updated KDIGO CKD-mineral and bone disorder guideline (7) suggests that modest increases in PTH represent an appropriate adaptive response to declining kidney function and that overly aggressively use of vitamin D receptor (VDR) activators increases the risk of adynamic bone disease. Therefore, reducing iPTH toward or into the normal range needs to be carefully evaluated in future studies.

Optimal levels of serum total 25-hydroxyvitamin D for controlling SHPT in predialysis CKD patients also remain undefined. Previous studies have documented similar low levels of serum 25-hydroxyvitamin D levels in patients with stage 3 or 4 CKD, consistent with the data presented herein [22, 23]. Observational studies have shown that morbidity and mortality are increased in CKD patients with low serum 25-hydroxyvitamin D concentrations [24–28], and a recent prospective study suggested that increasing mean serum 25-hydroxyvitamin D in CKD patients to 94 ng/mL had a beneficial effect on cardiovascular parameters such as reducing pulse wave velocity [29]. Few other studies have examined the effects of 25-hydroxyvitamin D at levels substantially above 30 ng/mL in CKD patients. A cross-sectional study [13] concluded that PTH levels in patients with stages 1–5 CKD progressively declined as serum 25-hydroxyvitamin D increased above 30 ng/mL and that 25-hydroxyvitamin D levels well above 42–48 ng/mL would be required for iPTH normalization in the more advanced CKD. Data from 3 prospective controlled studies [12, 14], including the 2 presented herein, support higher serum total 25-hydroxyvitamin D targets in patients with stage 3 or 4 CKD.

Of concern is the purported safety risk which the IOM has associated with serum total 25-hydroxyvitamin D levels above 50 ng/mL [11] and speculations by others that the vitamin D toxicity can be encountered at levels of 100 or 150 ng/mL [30, 31]. Elevated serum calcium (hypercalcemia) is the first sign of vitamin D toxicity. Severe hypercalcemia involving serum calcium levels far above normal can cause cardiac arrest and death. More moder-

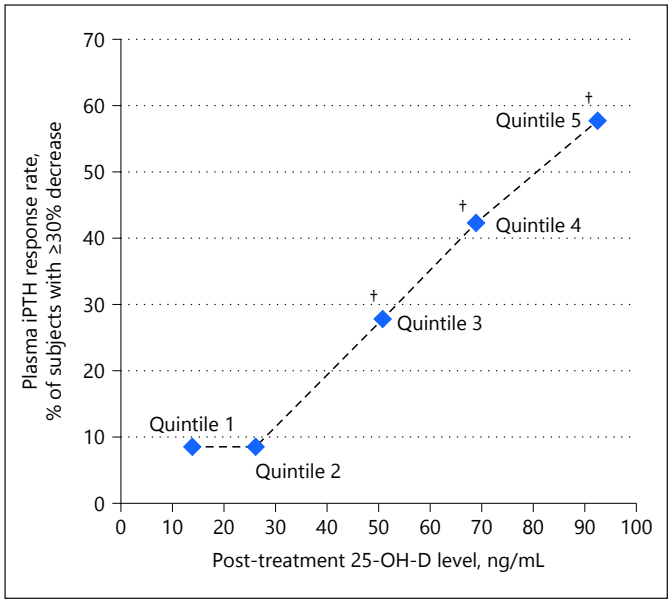

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Fig. 3. Analysis of plasma iPTH response rates by posttreatment 25-hydroxyvitamin D Quintile. The proportion of PP subjects achieving an iPTH response, defined as a mean decrease of ≥30% in plasma iPTH from pretreatment baseline, was analyzed as a function of mean posttreatment serum total 25-hydroxyvitimain D quintile. † Significantly different from Quintile 1, $p < 0.05$. iPTH, intact parathyroid hormone.

ate elevations in serum calcium levels, if allowed to persist for long periods, can lead to calcification of soft tissues, including kidneys and the vasculature [32], which can eventually be fatal. Clearly, massive doses of immediate-release cholecalciferol, ergocalciferol, or calcifediol can produce severe hypercalcemia, hyperphosphatemia, and related mortality in man and animals, but the clinical threshold of toxicity remains poorly characterized. High serum levels of calcifediol or total 25-hydroxyvitamin D have been demonstrated to exert mass action effects on the VDR, mimicking the actions of 1,25-hydroxyvitamin D [33]. However, calcifediol has a low affinity for the VDR, estimated to be 500–1,000-fold lower than that of calcitriol [34, 35]. As a consequence, physiological concentrations of calcifediol exert little, if any, biological actions. Early signs that high serum calcifediol is exerting a mass action effect are suppression of CYP27B1 expression in the kidney, and the concomitant observation that serum total 1,25-dihydroxyvitamin D levels have been suppressed [36, 37]. These signs are not observed in rodent models until serum total 25-hydroxyvitamin D is elevated to >400 ng/mL [33, 37]. Previous studies with ERC have shown that the mean terminal elimination half-life of serum calcifediol is shorter (11 days in healthy volunteers and 25–50 days in patients with stage 3 or 4 CKD [21]) than those of cholecalciferol or ergocalciferol [38], thereby reducing the risk of toxicity.

The present studies show that gradual elevation of mean serum total 25-hydroxyvitamin D with ERC to levels as high as 92.5 ng/mL over a 26-week period had no adverse effects on mean serum calcium, phosphorus, FGF23, eGFR, VMR, or the urine Ca:Cr ratio and did not increase mean serum 1,25-dihydroxyvitamin D above the ULN (62 pg/mL). Extension of these studies to 52 weeks of ERC treatment [14] demonstrated no increased risks related to these parameters. One observational study has suggested a J-shaped association between serum 25-hydroxyvitamin D levels and all-cause mortality [39], while another study has shown an increased hazard ratio only at low levels of serum 25-hydroyvitamin D [40]. In the present studies, a positive correlation was observed between serum total 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, but no correlation was observed between serum total 25-hydroxyvitamin D and serum calcium or phosphorus. The few episodes of hypercalcemia, observed in 2% of subjects treated with ERC, appeared unrelated to serum total 25-hydroxyvitamin D, as previously described [14]. The safety of serum total 25-hydroxyvitamin D levels above the range of 30–100 ng/mL, which has been approved for ERC by the US Food and Drug Administration, is unknown and is under investigation in further studies with ERC in CKD and other disease populations.

Data from the present studies also showed that increasing 25-hydroxyvitamin D exposures not only attenuated the progressive rise in serum bone turnover markers but also actually reduced the levels of these markers, suggesting improved control of high turnover bone disease and a reduction in the risk of related adverse sequelae. Bone degradation and resulting fractures are a significant source of morbidity and mortality in CKD patients with SHPT [41]. Even mildly elevated PTH has recently been demonstrated to produce significant changes in bone architecture and reduce BMD at the spine [42]. Poor bone health has been strongly associated with vascular calcification and the associated high rates of cardiovascular morbidity and mortality in CKD [43] fostering considerable interest in improving bone health and reducing health care costs by diagnosing and correcting the bone disease in patients with kidney disease.

Surprisingly, ERC treatment had similar effects in patients with either stage 3 or 4 CKD on serum total 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and on plasma iPTH. This finding goes against conventional wis-

dom that calcifediol is less likely to be converted to 1,25-dihydroxyvitamin D_3 as CKD advances, due to declining expression of CYP27B1 in the residual kidneys. However, calcifediol can be activated extrarenally by CYP27B1 in parathyroid and many other tissues [44, 45]. Extrarenal hormone production depends on sufficient circulating levels of 25-hydroxyvitamin D and may be enabled by levels well above 20–30 ng/mL. The present findings indicate that (a) there is adequate renal CYP27B1 activity in predialysis patients to activate 25-hydroxyvitamin D and/or that (b) 25-hydroxyvitamin D is activated by CYP27B1 expressed outside the kidneys and released into circulation. It has been previously demonstrated that serum levels of 1,25-dihydroxyvitamin D rise in response to administered calcifediol in anephric patients [44]. It is plausible, therefore, that ERC raises serum total 25-hydroxyvitamin D to high enough levels to enable sufficient extrarenal 1,25-dihydroxyvitamin D production for PTH control. Additional studies are underway with ERC to ascertain the extent and regulation of extrarenal activation, which is poorly understood in CKD patients. While serum 24,25-dihydroxyvitamin D_3 levels increased with ERC treatment, the VMR rose only moderately, suggesting that there was no substantial induction of CYP24A1.

In conclusion, pooled data from 2 large prospective RCTs demonstrated that ERC safely increased 25-hydroxyvitamin D exposures in patients with stage 3 or 4 CKD to levels well above those recommended in current clinical practice guidelines. Mean levels of serum total 25-hydroxyvitamin D of at least 50.8 ng/mL were associated with proportional increases in serum 1,25-hydroxyvitamin D and decreases in plasma iPTH and serum bone turnover markers, and not associated with adverse changes in mean serum calcium, phosphorus, FGF23, eGFR or the urine Ca:Cr ratio. Elevation of mean serum total 25-hydroxyvitamin D to 92.5 ng/mL was insufficient to normalize plasma iPTH, suggesting that higher exposures may be needed to optimally treat SHPT in stage 3 or 4 CKD.

Acknowledgments

None.

Ethics Statement

Subjects included in the clinical studies described in this article gave written informed consent. The study protocol was approved by an Institutional Review Board.

Disclosure Statement

Three of the authors of this study (S.A.S., A.A., and C.W.B.) are employees of the Renal Division of OPKO Health, Inc. Two of the authors of this study (S.M.S. and M.P.) are consultants for the Renal Division of OPKO Health, Inc.

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Author Contributions

S.M.S., M.P., and C.W.B.: designed the studies. S.A.S.: carried out data analyses and preparation of the figures and tables. S.A.S., A.A., and C.W.B.: drafted and revised the paper; all authors approved the final version of the manuscript.

References

1 Cunningham J, Locatelli F, Rodriguez M. Secondary hyperparathyroidism: pathogenesis, disease progression, and therapeutic options. Clin J Am Soc Nephrol. 2011 Apr;6(4):913–21.

2 Nigwekar SU, Tamez H, Thadhani RI. Vitamin D and chronic kidney disease-mineral bone disease (CKD-MBD). Bonekey Rep. 2014 Feb;3:498.

3 González EA, Sachdeva A, Oliver DA, Martin KJ. Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. Am J Nephrol. 2004 Sep-Oct;24(5):503–10.

4 Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, et al. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney Int. 2007 Jan;71(1):31–8.

5 National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 2003;42:S1-201.

6 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl. 2009 Aug;(113):S1-130.

7 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl (2011). 2017 Jul;7(1):1–59.

8 Agarwal R, Georgianos PI. Con: nutritional vitamin D replacement in chronic kidney disease and end-stage renal disease. Nephrol Dial Transplant. 2016 May;31(5):706–13.

9 Kalantar-Zadeh K, Kovesdy CP. Clinical outcomes with active versus nutritional vitamin D compounds in chronic kidney disease. Clin J Am Soc Nephrol. 2009 Sep;4(9):1529–39.
10 Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011 Jul;96(7):1911–30.
11 Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011 Jan;96(1):53–8.
12 Sprague SM, Silva AL, Al-Saghir F, Damle R, Tabash SP, Petkovich M, et al. Modified-release calcifediol effectively controls secondary hyperparathyroidism associated with vitamin D insufficiency in chronic kidney disease. Am J Nephrol. 2014;40(6):535–45.
13 Ennis JL, Worcester EM, Coe FL, Sprague SM. Current recommended 25-hydroxyvitamin D targets for chronic kidney disease management may be too low. J Nephrol. 2016 Feb;29(1):63–70.
14 Sprague SM, Crawford PW, Melnick JZ, Strugnell SA, Ali S, Mangoo-Karim R, et al. Use of extended-release calcifediol to treat secondary hyperparathyroidism in stages 3 and 4 chronic kidney disease. Am J Nephrol. 2016;44(4):316–25.
15 Visser M, Deeg DJ, Puts MT, Seidell JC, Lips P. Low serum concentrations of 25-hydroxyvitamin D in older persons and the risk of nursing home admission. Am J Clin Nutr. 2006 Sep;84(3):616–22.
16 Jia X, Aucott LS, McNeill G. Nutritional status and subsequent all-cause mortality in men and women aged 75 years or over living in the community. Br J Nutr. 2007 Sep;98(3):593–9.
17 Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med. 2008 Aug;168(15):1629–37.
18 Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T, et al.; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of breast cancer. J Natl Cancer Inst. 2008 Nov;100(22):1581–91.
19 Stolzenberg-Solomon RZ, Jacobs EJ, Arslan AA, Qi D, Patel AV, Helzlsouer KJ, et al. Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: cohort consortium vitamin D pooling project of rarer cancers. Am J Epidemiol. 2010 Jul;172(1):81–93.
20 Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, et al. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer. 2004 Jan;108(1):104–8.
21 Sprague SM, Strugnell SA, Bishop CW. Extended-release calcifediol for secondary hyperparathyroidism in stage 3-4 chronic kidney disease. Expert Rev Endocrinol Metab. 2017 Sep;12(5):289–301.
22 Al-Aly Z, Qazi RA, González EA, Zeringue A, Martin KJ. Changes in serum 25-hydroxyvitamin D and plasma intact PTH levels following treatment with ergocalciferol in patients with CKD. Am J Kidney Dis. 2007 Jul;50(1):59–68.
23 Zisman AL, Hristova M, Ho LT, Sprague SM. Impact of ergocalciferol treatment of vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. Am J Nephrol. 2007;27(1):36–43.
24 Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, et al. Vitamin D levels and patient outcome in chronic kidney disease. Kidney Int. 2009 Jan;75(1):88–95.
25 Mehrotra R, Kermah DA, Salusky IB, Wolf MS, Thadhani RI, Chiu YW, et al. Chronic kidney disease, hypovitaminosis D, and mortality in the United States. Kidney Int. 2009 Nov;76(9):977–83.
26 Pilz S, Tomaschitz A, Friedl C, Amrein K, Drechsler C, Ritz E, et al. Vitamin D status and mortality in chronic kidney disease. Nephrol Dial Transplant. 2011 Nov;26(11):3603–9.
27 de Boer IH, Katz R, Chonchol M, Ix JH, Sarnak MJ, Shlipak MG, et al. Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. Clin J Am Soc Nephrol. 2011 Sep;6(9):2141–9.
28 Navaneethan SD, Schold JD, Arrigain S, Jolly SE, Jain A, Schreiber MJ Jr, et al. Low 25-hydroxyvitamin D levels and mortality in non-dialysis-dependent CKD. Am J Kidney Dis. 2011 Oct;58(4):536–43.
29 Levin A, Tang M, Perry T, Zalunardo N, Beaulieu M, Dubland JA, et al. Randomized controlled trial for the effect of vitamin D supplementation on vascular stiffness in CKD. Clin J Am Soc Nephrol. 2017 Sep;12(9):1447–60.
30 Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005 Feb;135(2):317–22.
31 Holick MF. Vitamin D for health and in chronic kidney disease. Semin Dial. 2005 Jul-Aug;18(4):266–75.
32 Towler DA, Demer LL. Thematic series on the pathobiology of vascular calcification: an introduction. Circ Res. 2011 May;108(11):1378–80.
33 Deluca HF, Prahl JM, Plum LA. 1,25-Dihydroxyvitamin D is not responsible for toxicity caused by vitamin D or 25-hydroxyvitamin D. Arch Biochem Biophys. 2011 Jan;505(2):226–30.
34 Bikle DD. Clinical counterpoint: vitamin D: new actions, new analogs, new therapeutic potential. Endocr Rev. 1992 Nov;13(4):765–84.
35 Hughes MR, Baylink DJ, Jones PG, Haussler MR. Radioligand receptor assay for 25-hydroxyvitamin D_2/D_3 and 1 alpha, 25-dihydroxyvitamin D_2/D_3. J Clin Invest. 1976 Jul;58(1):61–70.
36 Jones G. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr. 2008 Aug;88(2):582S–6S.
37 Shephard RM, Deluca HF. Plasma concentrations of vitamin D_3 and its metabolites in the rat as influenced by vitamin D_3 or 25-hydroxyvitamin D_3 intakes. Arch Biochem Biophys. 1980 Jun;202(1):43–53.
38 Torres U, Cozzolino PA, Vervloet M, editors. Vitamin D in chronic kidney disease. New York (NY): Springer Berlin Heidelberg; 2016.
39 Durup D, Jørgensen HL, Christensen J, Tjønneland A, Olsen A, Halkjær J, et al. A reverse J-shaped association between serum 25-hydroxyvitamin D and cardiovascular disease mortality: the COPD study. J Clin Endocrinol Metab. 2015 Jun;100(6):2339–46.
40 Shardlow A, McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Associations of fibroblast growth factor 23, vitamin D and parathyroid hormone with 5-year outcomes in a prospective primary care cohort of people with chronic kidney disease stage 3. BMJ Open. 2017 Aug;7(8):e016528.
41 West SL, Patel P, Jamal SA. How to predict and treat increased fracture risk in chronic kidney disease. J Intern Med. 2015 Jul;278(1):19–28.
42 Rødbro LL, Bislev LS, Sikjær T, Rejnmark L. Bone metabolism, density, and geometry in postmenopausal women with vitamin D insufficiency: a cross-sectional comparison of the effects of elevated parathyroid levels. Osteoporos Int. 2018 Oct;29(10):2211–8.
43 Tomiyama C, Carvalho AB, Higa A, Jorgetti V, Draibe SA, Canziani ME. Coronary calcification is associated with lower bone formation rate in CKD patients not yet in dialysis treatment. J Bone Miner Res. 2010 Mar;25(3):499–504.
44 Dusso A, Lopez-Hilker S, Rapp N, Slatopolsky E. Extra-renal production of calcitriol in chronic renal failure. Kidney Int. 1988 Sep;34(3):368–75.
45 Ritter CS, Haughey BH, Armbrecht HJ, Brown AJ. Distribution and regulation of the 25-hydroxyvitamin D_3 1α-hydroxylase in human parathyroid glands. J Steroid Biochem Mol Biol. 2012 May;130(1-2):73–80.