Calcitriol Suppression of Parathyroid Hormone Fails to Improve Skeletal Properties in an Animal Model of Chronic Kidney Disease

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Key Words

Animal models · Bone disease · Chronic kidney disease · Treatment · Calcitriol

Abstract

Background: Chronic kidney disease (CKD) leads to complex metabolic changes and an increased risk of fracture. Currently, calcitriol is the standard of care as it effectively suppresses parathyroid hormone (PTH) levels in CKD patients. While calcitriol and its analogs improve BMD and reduce fractures in the general population, the extension of these benefits to patients with advanced kidney disease is unclear. Here, the impact of calcitriol on the skeleton was examined in the setting of reduction in PTH. **Methods:** Male Cy/+ rats, a PKD-like CKD model, were treated with either vehicle or calcitriol for 5 weeks. Their normal littermates served as controls. Animals were assessed for changes in mineral metabolism and skeletal parameters (microCT, histology, whole bone mechanics and bone quality). **Results:** PTH levels were significantly higher (12-fold) in animals with CKD compared to normal controls. CKD animals also exhibited negative changes in bone structural and mechanical properties. Calcitriol treatment resulted in a 60% suppression of PTH levels in animals with CKD. Despite these changes, it had no impact on bone

volume (cortical or cancellous), bone turnover, osteoclast number or whole bone mechanical properties. **Conclusions:** These data indicate that while calcitriol effectively lowered PTH in rats with CKD, it did little to prevent the negative effects of secondary hyperparathyroidism on the skeleton.

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Introduction

Chronic kidney disease-mineral and bone disorder (CKD-MBD) represents a conglomeration of various metabolic and skeletal changes, such as hyperphosphatemia, secondary hyperparathyroidism and an increased fracture risk [1–3]. Currently, the primary goal of therapy is the suppression of elevated levels of parathyroid hormone (PTH) [4]. For FDA approval, the standard end point for the treatment of secondary hyperparathyroidism has been a reduction of PTH by 30% or more [5, 6]. Due to a paucity of data (detailed in the kidney disease improving global outcomes guidelines), the impact of these levels of suppression on vascular and skeletal outcomes is unclear [3, 7, 8].

Though imperfect, serum PTH is considered a surrogate biomarker for underlying renal osteodystrophy [9–11].

Calcitriol, vitamin D analogs and calcimimetics all lower PTH, but data that assess their efficacy on bone are rather limited. While most osteoporosis clinical studies in non-CKD patients demonstrate that calcitriol reduces fracture risk through improvements in BMD [12, 13], the extension of these benefits to patients with advanced kidney disease where cortical bone is adversely affected is unclear [4]. Pre-clinical studies using calcium to suppress PTH to normal (or below) levels largely preserves skeletal properties [14–17]. Unfortunately, this effectiveness comes at the cost of increased vascular calcification, at least when hypercalcemia ensues. These data are consistent with concerns raised in CKD patients about the use of calcium supplementation and calcium-based phosphate binders [18–23].

In light of these data, a more thorough examination of the effects of calcitriol therapy on specific skeletal outcomes seems warranted. Despite widespread use clinically, there are limited calcitriol intervention studies in humans and animal models that examine tissue properties (histomorphometry, trabecular architecture, cortical geometry, etc.). Fracture prevention studies have not been conducted for calcitriol in patients with CKD, and limited preclinical studies have evaluated mechanical properties of bone with intervention. The goal of the current study was to fill these voids in a preclinical model of CKD. Specifically, we sought to investigate how suppression of PTH levels with calcitriol would impact skeletal properties using an animal model of progressive CKD.

Methods

Animal Model

Cy/+ rats are characterized by autosomal dominant cystic disease [24]. These animals have a mutation (R823W) in *Anks6*, a gene that codes for the protein SamCystin. The exact function of this protein is unknown [25], but unlike many other cyst-related proteins, SamCystin does not localize to the primary cilia of kidney cells [26]. Recently, this gene was shown to be responsible for nephronophthisis in humans [27]. In this rat model, the mutation leads to a slow and gradual onset of CKD [24]. It parallels the human condition through the progressive development of hyperphosphatemia, secondary hyperparathyroidism and vascular calcification. Blood urea nitrogen (BUN) and creatinine are elevated by 20 weeks of age. Skeletal abnormalities consistent with osteitis fibrosa cystica are present by 30 weeks.

Experimental Design

At 24 weeks, male Cy/+ rats were placed on a casein diet (Purina AIN-76A; 0.7% Pi, 0.6% Ca), which we have shown to produce a more consistent kidney disease phenotype [24]. At this point, these animals have reached their full adult weights. Starting at 25 weeks of age, Cy/+ rats were treated with vehicle ($n = 9$) or calcitriol ($n = 11$; 10 ng/kg 3× weekly; intraperitoneal) for 5 weeks.

This treatment duration was chosen as it approximates an average bone remodeling cycle in skeletally mature rats (roughly 6 months in humans) and has been shown to be sufficient to detect treatment-induced skeletal changes in this model [28, 29]. Based on preliminary studies in these animals, calcitriol doses were chosen to achieve approximately 50% suppression of PTH levels. Non-affected male littermates served as controls ($n = 8$). All rats were injected with calcein (10 mg/kg; subcutaneous) 14 and 4 days prior to sacrifice to label surfaces with active bone formation.

At 30 weeks of age, animals were anesthetized with isoflurane and underwent cardiac puncture for blood draw followed by exsanguination and bilateral pneumothorax to ensure death. The lumbar spine, tibiae and femora were removed and stored at -20°C for analysis. All procedures were approved by and carried out according to the rules and regulations of the Indiana University School of Medicine's Institutional Animal Care and Use Committee. Some of the data from normal and CKD-VEH animals have been published previously as these control groups were part of a larger study of which calcitriol treatment was one sub-group [54].

Biochemistry

Blood plasma at 30 weeks was analyzed for BUN, calcium and phosphorus using colorimetric assays (Point Scientific, Sigma kits, Canton, M.I., USA). Intact PTH was determined by ELISA (Alpco, Salem, N.H., USA).

MicroCT

Using microCT (Skyscan 1172), trabecular architecture was determined from the metaphysis of the proximal tibia and the full length of the L4 vertebra. Cortical bone geometry was determined from the femoral midshaft. Cortical thickness (Ct.Th) was assessed at 75% of the height of the vertebra (from cranial to caudal) because this represents a region free of zygapophyseal attachment. All bones were wrapped in parafilm to prevent drying and scanned at a resolution of 12 μm in accordance with standard guidelines [30].

Histomorphometry

Static and dynamic histomorphometric measures were obtained from the proximal metaphysis of the tibia as well as the caudal metaphysis of the L3 vertebra. Histological processing followed previously used protocols from this lab [31–33]. Tissues were embedded in methyl methacrylate for undecalcified sections. Transverse sections from the proximal tibia and frontal sections from the caudal portion of the L3 vertebra were cut and left unstained for dynamic histomorphometry or stained with tartrate-resistant acid phosphatase (TRAP) for osteoclast measurements (tibia only). For cancellous bone, a region of interest that was approximately 0.8 mm from the growth plate was analyzed. Unstained sections were assessed for total bone surface (BS), single-labeled surface and double-labeled surface to calculate mineral apposition rate (MAR), percent mineralizing surface (MS)/BS and bone formation rate (BFR)/BS. TRAP-stained sections were assessed for BS, osteoclast number and osteoclast surface (Oc.S) to calculate the number of osteoclasts per unit BS and percent Oc.S/BS. All histomorphometric nomenclature follows standard usage [34].

Whole Bone Mechanics

Structural mechanical properties of femora were determined by 4-point bending. The anterior surface was placed on 2 lower supports located ± 9 mm from the mid-diaphysis (18 mm span

Table 1. Biochemical profiles of animals at 30 weeks of age

	Normal	CKD (vehicle)	CKD (calcitriol)
BUN, mg/dl	14.62±1.95	48.32±8.20*	43.01±7.07*
Calcium, mg/dl	9.979±0.987	11.610±2.323	10.240±1.872
Phosphorus, mg/dl	4.527±0.579	6.682±2.408*	7.776±1.170*
PTH, pg/ml	181.97±105.05	2,194.39±1,811.01*	875.12±432.51*,#

* vs. normal; # vs. CKD (vehicle). BUN = Blood urea nitrogen; PTH = parathyroid hormone.

length) with an upper span length of 6 mm. Specimens were loaded to failure at a rate of 2 mm/min. Structural mechanical properties were obtained directly from the force–displacement curves, while apparent material properties were derived from the force–displacement curves, cross-sectional moments of inertia and the distances from the centroid to the tensile surface using standard beam bending equations for 4-point bending [35].

Structural mechanical properties of L4 vertebrae were determined by uniaxial compression. Vertebra height was assessed from microCT images. Prior to mechanical testing, the vertebral arch and end plates were removed to create parallel surfaces for compression testing. Specimens were loaded at a rate of 0.5 mm/min. Structural mechanical properties were obtained directly from the force–displacement curves, while apparent material properties were derived from the force–displacement curves, pre-test sample heights and the average bone area of 5 microCT slices (10, 30, 50, 70 and 90% slices of the pre-test sample height) using equations for uniaxial compression [36, 37].

Tissue Composition

The anteromedial portion of the tibial mid-diaphysis was polished with a 0.05 µm alumina suspension in order to create a flat region for spectroscopy and indentation testing. Composition was assessed using a LabRAM HR 800 Raman Spectrometer (HORIBA JobinYvon, Edison, N.J., USA). A 660 nm laser was focused on the BS using a 50X objective to a spot size of ~10 µm. Three locations were imaged ~3 mm apart with 520 s acquisitions at each location as previously published [38]. A 5-point linear baseline correction was applied in LabSpec 5 (HORIBA JobinYvon). Using OriginPro 8.6 (OriginLab, Northampton, Mass., USA), a single Gaussian peak was fit to the PO_4^{3-} ν1 peak, and the areas under the PO_4^{3-} ν1, CO_3^{2-} ν1 and Amide I peaks were calculated at each location. Type B carbonate substitution was found by the band area ratio of CO_3^{2-} ν1/ PO_4^{3-} ν1. The degree of matrix mineralization was determined by the band area ratio of PO_4^{3-} ν1/Amide I. Mineral maturity (crystallinity) was determined by the inverse of the full width at half maximum of the PO_4^{3-} ν1 peak.

Nanoindentation

After Raman spectroscopy, nanoindentation was performed on the same samples using a Hysitron TI 950 TriboIndenter. Samples were partially submerged in ultrapure water with the surface remaining uncovered for optical imaging of the surface to determine indentation locations but then fully submerged during testing. Locations were imaged using in situ scanning probe imaging. Then, 6 indentations were performed on a 10 × 20 µm grid, avoiding interactions

from neighboring indentations. A previously calibrated fluid cell Berkovich diamond probe was used for the indentations. Machine calibrations were performed at the beginning of each day of testing. Tests were conducted in load control with a 10 s loading period, a 10 s hold at 3,000 µN and a 10 s unloading period. From the resulting load–displacement profiles, the indentation elastic modulus and hardness were calculated according to the following equations:

$$E_r = \frac{\sqrt{\pi}}{2\sqrt{A(h_c)}} \cdot S$$

$$H = \frac{P_{\max}}{A(h_c)}$$

where E_r is the reduced indentation elastic modulus of the sample, A is contact area, h_c is the contact displacement, S is the stiffness of the sample determined from 40 to 95% of the unloading slope, H is the hardness of the sample and $P_{\max}$ is the peak force. All of the individual indentations ($n = 6$ per location) were averaged to produce a single value for each location, and each of these locations was averaged to produce a single value for each sample.

Statistics

Comparisons among groups were assessed by one-way analysis of variance with Fisher's LSD post-hoc tests. A priori α -levels were set at 0.05 to determine significance.

Results

Mineral Metabolism

Animals with CKD had higher serum levels of BUN compared to normal littermates. Serum calcium was normal, while phosphorus and PTH levels were significantly higher than their normal counterparts (table 1). Animals treated with calcitriol had BUN, calcium and phosphorus values similar to their untreated CKD counterparts. PTH levels in calcitriol animals were significantly lower than in untreated CKD animals (–61%) but still higher than in normal controls (+381%).

Fig. 1. Cancellous bone structure in the proximal tibia and lumbar vertebra as determined by microCT. * $p < 0.05$ compared to NL.

Table 2. Bone architecture and geometry of long bones and vertebra

Proximal tibia	Normal	CKD (vehicle)	CKD (calcitriol)
BV/TV, %	17.04±3.34	11.26±1.51*	10.70±1.77*
Tb.Th, mm	0.106±0.010	0.108±0.004	0.101±0.005*
Tb.N, 1/mm	1.611±0.253	1.052±0.150*	1.057±0.163*
Tb.Sp, mm	0.369±0.044	0.604±0.114*	0.523±0.084*
<i>Femoral diaphysis</i>			
Ct.Th, mm	0.876±0.037	0.748±0.056*	0.769±0.045*
Ct.Ar, mm ²	8.767±0.631	7.324±0.358*	7.647±0.378*
I _{ap} , mm ⁴	15.00±2.59	12.40±0.58*	13.19±0.89*
I _{ml} , mm ⁴	10.23±1.56	7.50±0.59*	8.30±0.99*
Ct.Po, %	0.690±0.324	0.948±0.401	0.769±0.377
<i>Lumbar vertebra</i>			
BV/TV, %	41.88±2.92	30.01±3.98*	29.83±2.88*
Tb.Th, mm	0.119±0.004	0.110±0.007*	0.105±0.008*
Tb.N, 1/mm	3.581±0.258	2.726±0.324*	2.823±0.220*
Tb.Sp, mm	0.213±0.021	0.280±0.031*	0.275±0.018*
Ct.Th, mm	0.236±0.033	0.170±0.012*	0.191±0.035*

* vs. normal; # vs. CKD (vehicle).

BV/TV = Bone volume/tissue volume; Tb.Th = trabecular thickness; Tb.N = trabecular number; Tb.Sp = trabecular spacing; I_{ap} = moment of inertia in the anterior-posterior direction; I_{ml} = moment of inertia in the medial lateral direction; Ct.Po = cortical porosity.

MicroCT

Vehicle-treated CKD animals had lower trabecular bone volume than normal animals at the proximal tibia. A similar pattern was observed in the vertebra. In both cases, animals treated with calcitriol displayed no differences than their CKD vehicle counterparts (fig. 1 and table 2). All other trabecular parameters were similar between calcitriol animals and the untreated CKD animals.

Cortical bone of the femoral midshaft and lumbar vertebra was also negatively affected by CKD. CKD animals had lower cortical area (Ct.Ar), Ct.Th and bending moments of inertia compared to those of normal controls (table 2). Animals treated with calcitriol displayed cortical values similar to CKD vehicle animals. Calcitriol did not correct CKD-induced changes in Ct.Ar, thickness, porosity or polar moment of inertia of the femur or Ct.Th of the vertebra (table 2).

Fig. 2. BFRs in the proximal tibia and caudal lumbar vertebra as determined by dynamic histomorphometry. * $p < 0.05$ compared to NL.

Table 3. Trabecular bone histomorphometry

Tibia	Normal	CKD (vehicle)	CKD (calcitriol)
MAR, $\mu\text{m}/\text{day}$	1.305 ± 0.185	$2.470 \pm 0.569^*$	$2.285 \pm 0.343^*$
MS/BS, %	26.56 ± 5.14	33.32 ± 4.11	$35.86 \pm 3.26^{*,\#}$
BFR/BS, $\mu\text{m}^3/\mu\text{m}^2/\text{year}$	126.65 ± 31.02	$299.62 \pm 74.35^*$	$298.51 \pm 47.41^*$
Oc.S/BS, %	7.157 ± 1.250	$15.739 \pm 3.332^*$	$13.538 \pm 2.790^*$
N.Oc/BS, $1/\text{mm}$	1.966 ± 0.412	$4.125 \pm 0.785^*$	$3.604 \pm 0.590^*$
<i>Vertebra</i>			
MAR, $\mu\text{m}/\text{day}$	1.057 ± 0.339	$1.983 \pm 0.876^*$	$1.87 \pm 0.52^*$
MS/BS, %	13.62 ± 3.98	$27.81 \pm 5.73^*$	$31.39 \pm 4.58^*$
BFR/BS, $\mu\text{m}^3/\mu\text{m}^2/\text{year}$	52.14 ± 21.76	$211.94 \pm 128.49^*$	$220.61 \pm 94.32^*$

* vs. normal; # vs. CKD (vehicle).

MAR = Mineral apposition rate; MS/BS = mineralizing surface/bone surface; BFR/BS = bone formation rate per bone surface; Oc.S/BS = osteoclast surface per bone surface; N.Oc/BS = number of osteoclasts per bone surface.

Histology

Vehicle-treated CKD animals had higher trabecular BFRs in the tibia and vertebra as well as higher osteoclast number and percent Oc.S than their normal counterparts (fig. 2 and table 3). Calcitriol animals displayed similar values to CKD vehicle animals with regard to MAR, osteoclast number and percent Oc.S. While animals with calcitriol had a slightly higher proportion of MS than CKD vehicle animals (in the tibia only), BFRs at both sites were similar between these groups.

Whole Bone Mechanics

Animals with CKD had lower femoral ultimate force, stiffness and energy to fracture compared to normal animals (fig. 3 and table 4). Estimated material properties re-

vealed a significantly lower modulus of toughness in CKD animals (fig. 3) with no differences in the ultimate stress or elastic modulus (table 4). Animals treated with calcitriol displayed no treatment effects compared to vehicle-treated CKD. They were similar with regard to ultimate force, stiffness and energy to fracture (fig. 3). Calcitriol treatment also had no effect on estimated material properties (table 4).

Vertebral compression tests, which assess both trabecular and cortical bone, revealed that CKD animals had lower ultimate force and energy to ultimate force but no differences in stiffness compared to normal animals (fig. 3). Estimated material properties were similar among all 3 groups. As above, vertebrae from calcitriol animals were no different from their untreated CKD counterparts.

Fig. 3. Whole bone mechanical properties of the femur as determined by 4-point bending: (a) ultimate load, (b) stiffness and (c) energy to fracture. * $p < 0.05$ compared to NL.

Table 4. Structural and material mechanical properties

	Normal	CKD (vehicle)	CKD (calcitriol)
<i>Femur</i>			
Ultimate force, n	272.08±17.75	204.81±23.20*	214.00±20.04*
Stiffness, n/mm	531.67±47.41	412.96±56.28*	446.99±58.57*
Energy to fracture, mJ	119.16±15.33	91.65±14.13*	101.305±17.98*
Ultimate stress, MPa	153.81±15.68	143.94±17.93	136.49±9.60
Elastic modulus, MPa	4.649±0.633	5.144±0.971	4.830±0.696
Toughness, MPa	4.336±0.382	3.652±0.545*	3.815±0.518*
<i>Vertebra</i>			
Ultimate force, n	256.89±60.01	187.06±48.03*	171.31±38.19*
Stiffness, n/mm	952.09±314.20	866.74±260.33	845.67±144.32
Energy, mJ	46.27±10.60	28.05±8.93*	25.38±10.88*
Ultimate stress, MPa	42.49±10.00	38.74±9.16	35.33±10.88
Elastic modulus, MPa	954.37±334.35	1,174.00±447.08	1,131.84±170.17
Toughness, MPa	1.310±0.470	0.924±0.347	0.819±0.386

* vs. normal.

Table 5. Bone quality

	Normal	CKD (vehicle)	CKD (calcitriol)
Raman spectroscopy			
Crystallinity (1/FWHM PO_4^{3-} ν 1)	0.0529 $\pm$ 0.0003	0.0534 $\pm$ 0.0008	0.0534 $\pm$ 0.0004
Carbonate substitution (CO_3^{2-} ν 1/ PO_4^{3-} ν 1)	0.247 $\pm$ 0.008	0.242 $\pm$ 0.013	0.238 $\pm$ 0.013
Relative mineralization (PO_4^{3-} ν 1/Amide I)	2.598 $\pm$ 0.341	2.403 $\pm$ 0.442	2.759 $\pm$ 0.299
Nanoindentation			
Elastic modulus, GPa	11.066 $\pm$ 2.766	9.666 $\pm$ 1.021	9.987 $\pm$ 2.610
Hardness, MPa	202.62 $\pm$ 22.86	192.68 $\pm$ 47.35	209.426 $\pm$ 43.65

Bone Quality

Compared to normal controls, animals with CKD were found to have no differences in indentation properties. Calcitriol had no effect on these properties either (table 5). Tissue composition assessed by Raman spectroscopy revealed no differences in any of the compositional parameters among the groups either.

Discussion

The goal of this study was to assess the impact of PTH suppression on CKD-induced bone disease. Using calcitriol, the current standard of care in patients with secondary hyperparathyroidism, PTH levels were suppressed by 60% in animals with CKD. Despite this reduction, no skeletal benefits were observed. This is likely in part due to the failure of calcitriol to suppress BFRs and osteoclast formation, both of which remained significantly higher than normal in long bones and vertebrae. These data arise important questions regarding the PTH suppression that is needed to produce skeletal benefits in the setting of CKD and whether PTH is an accurate biomarker of bone remodeling and mechanical properties.

In animal studies using calcitriol and paricalcitol in the 5/6th or 7/8th nephrectomy model, the results on bone have been mixed (table 6) [39–42]. Even in the presence of PTH suppression, only Jokihaara et al. [39] showed positive bone outcomes. In that study, 8-week-old animals underwent 5/6 nephrectomy and were treated with paricalcitol for 15 weeks. Levels of PTH suppression were similar to those in the current study, and femoral neck BMD and mechanical properties were higher than those in CKD animals and equivalent to those in normal animals. Yet, no beneficial effects were observed in BMD or mechanical properties at the femoral diaphysis (a site assessed in the current study). While variations in these models may explain some of the skeletal differ-

ences, one possible explanation is that calcitriol has a preferential effect at certain skeletal sites. Although the femoral neck is a clinically relevant site, why it would be positively affected when the proximal tibia, femoral diaphysis and lumbar vertebra in the current study were not is unclear.

Despite the plethora of data showing the effectiveness of calcitriol and its analogs to suppress PTH in patients with advanced CKD [4, 43–46], far fewer clinical studies have examined the impact of these treatments on bone outcomes (table 7). As reviewed in the KDIGO CKD-MBD guidelines, the evidence supporting a skeletal benefit of calcitriol or its analogs is weak [3]. In pediatric populations, calcitriol therapy has consistently shown effective suppression of bone turnover, though the associated suppression of PTH has been much more variable [47–49]. Skeletal results in adult populations have been far less congruous. For example, 1 study in dialysis patients revealed increased BMD at the spine and the hip, even though PTH levels were not impacted [50]. Studies have described significant reductions in osteoblast surface/BS and Oc.S/BS in conjunction with a 73% decrease in PTH [51]. Conversely, other studies have shown no difference in these osteoblast/osteoclast parameters associated decrease in PTH [52, 53], although these studies were not designed to specifically study suppression of PTH to a given level. Despite some studies documenting changes in osteoblast and Oc.S measures, no study has documented differences/changes in the dynamic measures provided by fluorescent labeling, further adding to the obscurity. In summary, pediatric populations appear to be responsive to calcitriol and its analogs as they exhibit PTH suppression with associated changes in dynamic histology parameters. In adults, however, PTH changes are much less consistent, and the only histological changes observed are limited static parameters. These studies demonstrate the need for a fracture prevention clinical trial with calcitriol or its analogs.

Table 6. Summary of pre-clinical assessment of calcitriol (and analogs) on bone properties

Reference	Animal model	Baseline fold increase in PTH in uremic vs. normal	Treatment	Percent change in PTH with treatment	Bone histomorphometry response to treatment		CT (bone volume or diameter) response to treatment	Mechanics
					turnover ^a	mineralization ^b	volume ^c	
Jablonski et al. [41], 1995	5/6th Nx rats out 4 months from surgery	1.2X	Calcitriol 0.17 µg/100 g BW daily for 12 weeks	Not changed	Not done, but static eroded surface improved	↑	↑	↑ Stiffness ↑ Time to fracture NS toughness
Jokihaara et al. [39], 2006	5/6th Nx rats out 16 weeks from surgery	13.0X	Paricalcitol 100 ng/rat 3 times per week for 12 weeks	↓ 61%	NA	NA	↑	↑ Ultimate load at femoral neck NS ultimate load at femoral diaphysis
Naves Diaz et al. [42], 2007	7/8 Nx, started treatment 1 week later	No normal animals	Calcitriol 10 ng/kg BW 5 times per week for 8 weeks	↓ 38%	Labels too weak	NS	↑	NA
Finch et al. [40], 2010	5/6th Nx started treatment when 'uremic'	9.27X	Paricalcitol 0.16 µg/kg BW 3 times per week for 6 weeks	↓ 49%	NS	NS	↓	NA
Newman (current study)	Cy/+ spontaneous CKD rat	12X	Calcitriol 10 ng/kg BW 3 times per week for 5 weeks	↓ 60%	NS	NS	NS	NS (ultimate force, stiffness, energy to fracture, ultimate stress, elastic modules, toughness in both femur diaphysis and vertebra)

Nx = Nephrectomy; FN = femoral neck, CT = microCT (animals) or DXA (dual X-ray absorptiometry); ↑ = improves with treatment; ↓ = worsens with treatment; NS = not statistically different from uremic controls); NA = not available.

^a Bone formation rate, mineral apposition rate; ^b mineralizing surface/bone surface (MS/BS) or osteoid volume; ^c bone volume/total volume.

Table 7. Summary of clinical assessment of calcitriol (and analogs) on bone properties

Reference	Patient characteristics	Treatment	Bone histomorphometry response to treatment		DXA Fracture
			turnover ^a	mineralization ^b	
Baker et al. [53], 1989	54 patients at baseline, 20 follow-up bone biopsies in patients with CKD 3–5 not ESRD	Oral calcitriol vs. placebo 12–57 months of treatment	No dynamic labeling; worsened in 50% of placebo; 40% of calcitriol (10% higher; 30% very low)	Worsened in 40% placebo and 30% of calcitriol	
Hamdy et al. [52], 1995	134 patients with CKD 3–5 not ESRD	Oral alfacalcidol vs. placebo for 24 months	Improved in 32%, worsened in 11% of alfacalcidol; placebo group improved in 3% and worsened in 13%	Improved in alfacalcidol treatment	NS
Nordal [56], 1988	30 patients with CKD 3–5 not ESRD	28 patients with biopsy after placebo or calcitriol for 8 months	Mean BFR decreased in calcitriol, increased in placebo. 25% of the calcitriol patients had too low BFR		
Salusky et al. [48], 1998	46 children on peritoneal dialysis	IP vs. PO calcitriol for 12 months	IP calcitriol: 44% lower; 37% too low PO calcitriol increased in 12%, lowered in 23%; too low in 29%		
Wesseling-Perry et al. [47], 2011	60 children on peritoneal dialysis	PO calcitriol vs. doxercalciferol; in each group sevelamer or calcium as phosphate binder	BFR ↓ in all 4 groups; 72% achieved NL BFR; only 1 patient had very low BFR	↓	NS
Przedlacki [57], 1995	25 patients with CKD stages 4–5	PO calcitriol vs. placebo for 12 months			↑
Rix [58], 1999	113 patients with CKD stages 4–5	Alfacalcidol vs. placebo for 12 months			↑

↑ = Improves with treatment; ↓ = worsens with treatment; NS = not statistically different from controls; blank cell = not done.

^a BFR, mineral apposition rate; ^b MS/BS or osteoid volume; ^c bone volume/total volume; IP = intraperitoneal; PO = per os (by mouth); ESRD = end-stage renal disease; DXA = dual energy x-ray absorptiometry.

In the current study, dynamic measures were consistent with the human studies, though osteoclast number and percent surface remained at the level of untreated CKD animals. Most importantly, this study showed that calcitriol had no impact on any whole bone mechanical parameters or on those assessing bone quality. Unfortunately, none of these previous studies revealed clear relationships between bone remodeling and PTH suppression. In contrast, by using calcium in the same animal model, we did demonstrate efficacy in improving bone histology and CT [29]. However, it is unclear if this was a result of increased calcium or more aggressive suppression of PTH. Unfortunately, this approach also led to an exacerbation of arterial calcification. Taken together, these data lend support to previous suggestions that calcitriol may have a direct effect on bone independent of its effect on PTH [46]. Furthermore, because there have been no fracture trials using calcitriol in CKD, the extension of its positive effects in patients with osteoporosis [12, 13] is simply unknown. The future of calcitriol therapy in patients with CKD requires fracture assessments for further evaluation. This raises the question of the goal of treatment with calcitriol and its analogs.

Our study has some limitations. We do not have baseline biochemistries or bone assessments. Previous work in this model shows that at 25 weeks, when we initiated treatment in the current study, the biochemistries are roughly comparable with respect to calcium and phosphorus, while BUN and PTH are already higher in Cy/+ compared to normals [16, 55]. We have not extensively studied the bone phenotype at 25 weeks of age. At 20 weeks of age, Cy/+ animals already have higher osteoblast and Oc.S [55] without significant alterations in bone mass/geometry or mechanical properties. The current ex-

periment was also limited in that only a single dose of calcitriol and a single duration of treatment were studied; it is possible that the outcomes would have differed with modification of either of these variables. Although we achieved our target goal of suppressing PTH by ~50%, which matches the clinical goal of treatment with calcitriol, the levels of PTH in our model (12X normal) far exceed those of humans. Most clinical studies have excluded patients with very high PTH levels and thus it is possible that the degree of hyperparathyroidism or the severity of hyperphosphatemia could influence the efficacy of calcitriol on bone.

The current study demonstrated that calcitriol treatment, while efficacious in terms of PTH suppression in animals with CKD, did little to curb the detrimental effects of secondary hyperparathyroidism from CKD on the skeleton. This was demonstrated by its lack of effect on biochemical, histomorphometric and, most importantly, of all mechanical measures. Taken together, this indicates that a greater degree of PTH suppression may be required for efficacy in skeletal properties to occur in the setting of CKD. Alternatively, there may not be efficacy of calcitriol or its analogs on the skeleton. Fracture studies are greatly needed in this patient population.

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References

- 1 Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G: Definition and classification of chronic kidney disease: a position statement from kidney disease: improving global outcomes (KDIGO). *Kidney Int* 2005;67:2089–2100.
- 2 Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL: 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;71:31–38.
- 3 KDIGO: Clinical practice guidelines for the management of CKD–MBD. *Kidney Int* 2009;76:S1–S130.
- 4 Kandula P, Dobre M, Schold JD, Schreiber MJ Jr, Mehrotra R, Navaneethan SD: Vitamin D supplementation in chronic kidney disease: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Clin J Am Soc Nephrol* 2011;6:50–62.
- 5 Coyne D, Acharya M, Qiu P, Abboud H, Batlle D, Rosansky S, Fadem S, Levine B, Williams L, Andress DL, Sprague SM: Paricalcitol capsule for the treatment of secondary hyperparathyroidism in stages 3 and 4 CKD. *Am J Kidney Dis* 2006;47:263–276.
- 6 Coburn JW, Maung HM, Elangovan L, Germain MJ, Lindberg JS, Sprague SM, Williams ME, Bishop CW: Doxercalciferol safely suppresses PTH levels in patients with secondary hyperparathyroidism associated with chronic kidney disease stages 3 and 4. *Am J Kidney Dis* 2004;43:877–890.
- 7 Moorthi RN, Kandula P, Moe SM: Optimal vitamin D, calcitriol, and vitamin D analog replacement in chronic kidney disease: to D or not to D: that is the question. *Curr Opin Nephrol Hypertens* 2011;20:354–359.

- 8 Moe SM, Thadhani R: What have we learned about chronic kidney disease-mineral bone disorder from the EVOLVE and PRIMO trials? *Curr Opin Nephrol Hypertens* 2013;22:651–655.
- 9 Moe S, Drueke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G: Definition, evaluation, and classification of renal osteodystrophy: a position statement from kidney disease: improving global outcomes (KDIGO). *Kidney Int* 2006;69:1945–1953.
- 10 Goodman WG: The consequences of uncontrolled secondary hyperparathyroidism and its treatment in chronic kidney disease. *Semin Dial* 2004;17:209–216.
- 11 Martin KJ, Olgaard K, Coburn JW, Coen GM, Fukagawa M, Langman C, Malluche HH, McCarthy JT, Massry SG, Mehls O, Salusky IB, Silver JM, Smogorzewski MT, Slatopolsky EM, McCann L: Diagnosis, assessment, and treatment of bone turnover abnormalities in renal osteodystrophy. *Am J Kidney Dis* 2004;43:558–565.
- 12 Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B: Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* 2005;293:2257–2264.
- 13 Peppone LJ, Hebl S, Purnell JQ, Reid ME, Rosier RN, Mustian KM, Palesh OG, Huston AJ, Ling MN, Morrow GR: The efficacy of calcitriol therapy in the management of bone loss and fractures: a qualitative review. *Osteoporos Int* 2010;21:1133–1149.
- 14 Allen MR, Chen NX, Gattone VH 2nd, Chen X, Carr AJ, LeBlanc P, Brown D, Moe SM: Skeletal effects of zoledronic acid in an animal model of chronic kidney disease. *Osteoporos Int* 2013;24:1471–1481.
- 15 Moe SM, Chen NX, Newman CL, Gattone VH 2nd, et al: A comparison of calcium to zoledronic acid for improvement of cortical bone in an animal model of CKD. *J Bone Miner Res* 2014;29:902–910.
- 16 Moe SM, Chen NX, Newman CL, Organ JM, Kneissel M, Kramer I, Gattone VH 2nd, Allen MR: Anti-sclerostin antibody treatment in a rat model of progressive renal osteodystrophy. *J Bone Miner Res* 2015;30:499–509.
- 17 Allen MR, Newman CL, Chen N, Granke M, Nyman JS, Moe SM: Changes in skeletal collagen cross-links and matrix hydration in high- and low-turnover chronic kidney disease. *Osteoporos Int* 2015;26:977–985.
- 18 Goodman WG, et al: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;342:1478–1483.
- 19 Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM: Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int* 2007;71:438–441.
- 20 Russo D, Miranda I, Ruocco C, Battaglia Y, Buonanno E, Manzi S, Russo L, Scafarto A, Andreucci VE: The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. *Kidney Int* 2007;72:1255–1261.
- 21 Elder GJ: Calcium supplementation: lessons from the general population for chronic kidney disease and back. *Curr Opin Nephrol Hypertens* 2011;20:369–375.
- 22 West SL, Swan VJ, Jamal SA: Effects of calcium on cardiovascular events in patients with kidney disease and in a healthy population. *Clin J Am Soc Nephrol* 2010;5(suppl 1):S41–S47.
- 23 Spence LA, Weaver CM: Calcium intake, vascular calcification, and vascular disease. *Nutr Rev* 2013;71:15–22.
- 24 Moe SM, Chen NX, Seifert MF, Sindors RM, Duan D, Chen X, Liang Y, Radcliff JS, White KE, Gattone VH 2nd: A rat model of chronic kidney disease-mineral bone disorder. *Kidney Int* 2009;75:176–184.
- 25 Nagao S, Morita M, Kugita M, Yoshihara D, et al: Polycystic kidney disease in Han:SPRD Cy rats is associated with elevated expression and mislocalization of SamCystin. *Am J Physiol Renal Physiol* 2010;299:F1078–F1086.
- 26 Stagner EE, Bouvrette DJ, Cheng J, Bryda EC: The polycystic kidney disease-related proteins Bicc1 and SamCystin interact. *Biochem Biophys Res Commun* 2009;383:16–21.
- 27 Hoff S, et al: ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3. *Nat Genet* 2013;45:951–956.
- 28 Allen MR, Chen NX, Gattone VH 2nd, Chen X, Carr AJ, LeBlanc P, Brown D, Moe SM: Skeletal effects of zoledronic acid in an animal model of chronic kidney disease. *Osteoporos Int* 2013;24:1471–1481.
- 29 Moe SM, Chen NX, Newman CL, Gattone VH 2nd, et al: A comparison of calcium to zoledronic acid for improvement of cortical bone in an animal model of CKD. *J Bone Miner Res* 2014;29:902–910.
- 30 Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R: Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res* 2010;25:1468–1486.
- 31 Allen MR, Follet H, Khurana M, Sato M, Burr DB: Antiremodeling agents influence osteoblast activity differently in modeling and remodeling sites of canine rib. *Calcif Tissue Int* 2006;79:255–261.
- 32 Allen MR, Erickson AM, Wang X, Burr DB, Martin RB, Hazelwood SJ: Morphological assessment of basic multicellular unit resorption parameters in dogs shows additional mechanisms of bisphosphonate effects on bone. *Calcif Tissue Int* 2010;86:67–71.
- 33 Allen MR, Kubek DJ, Burr DB: Cancer treatment dosing regimens of zoledronic acid result in near-complete suppression of mandible intracortical bone remodeling in beagle dogs. *J Bone Miner Res* 2010;25:98–105.
- 34 Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR, Parfitt AM: Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 2012;28:2–17.
- 35 Turner CH, Burr DB: Experimental techniques for bone mechanics; in Cowin SC (ed): *Bone Mechanics Handbook*. Boca Raton, CRC Press, 2002.
- 36 Hogan HA, Ruhmann SP, Sampson HW: The mechanical properties of cancellous bone in the proximal tibia of ovariectomized rats. *J Bone Miner Res* 2000;15:284–292.
- 37 Bloomfield SA, Allen MR, Hogan HA, Delp MD: Site- and compartment-specific changes in bone with hindlimb unloading in mature adult rats. *Bone* 2002;31:149–157.
- 38 Hammond MA, Gallant MA, Burr DB, Wallace JM: Nanoscale changes in collagen are reflected in physical and mechanical properties of bone at the microscale in diabetic rats. *Bone* 2014;60:26–32.
- 39 Jokihäärä J, Porsti I, Pajamäki I, Vuohelainen T, Jolma P, Koobi P, Kalliovalkama J, Niemela O, Kannus P, Sievanen H, Jarvinen TL: Paricalcitol [19-nor-1,25-(OH)₂D₂] in the treatment of experimental renal bone disease. *J Bone Miner Res* 2006;21:745–751.
- 40 Finch JL, Tokumoto M, Nakamura H, Yao W, Shahnazari M, Lane N, Slatopolsky E: Effect of paricalcitol and cinacalcet on serum phosphate, FGF-23, and bone in rats with chronic kidney disease. *Am J Physiol Renal Physiol* 2010;298:F1315–F1322.
- 41 Jablonski G, Mortensen BM, Klem KH, Mosekilde L, Danielsen CC, Gørdeladze JO: Vitamin D₃ analogs and salmon calcitonin partially reverse the development of renal osteodystrophy in rats. *Calcif Tissue Int* 1995;57:385–391.
- 42 Naves Diaz M, Rodriguez Rodriguez A, Fernandez Martin JL, Serrano Arias M, Menendez Rodriguez P, Cannata Andia JB: Effects of estradiol, calcitriol and both treatments combined on bone histomorphometry in rats with chronic kidney disease and ovariectomy. *Bone* 2007;41:614–619.
- 43 Ott SM: Therapy for patients with CKD and low bone mineral density. *Nat Rev Nephrol* 2013;9:681–692.
- 44 Dogan E, Erkok R, Sayarlioglu H, Soyoral Y, Dulger H: Effect of depot oral cholecalciferol treatment on secondary hyperparathyroidism in stage 3 and stage 4 chronic kidney diseases patients. *Ren Fail* 2008;30:407–410.
- 45 Chandra P, Binongo JN, Ziegler TR, Schlanger LE, Wang W, Someren JT, Tangpricha V: Cholecalciferol (vitamin D₃) therapy and vitamin D insufficiency in patients with chronic kidney disease: a randomized controlled pilot study. *Endocr Pract* 2008;14:10–17.
- 46 Coburn JW: Use of oral and parenteral calcitriol in the treatment of renal osteodystrophy. *Kidney Int Suppl* 1990;29:S54–S61.

- 47 Wesseling-Perry K, Pereira RC, Sahney S, Gales B, Wang HJ, Elashoff R, Juppner H, Salusky IB: Calcitriol and doxercalciferol are equivalent in controlling bone turnover, suppressing parathyroid hormone, and increasing fibroblast growth factor-23 in secondary hyperparathyroidism. *Kidney Int* 2011;79: 112–119.
- 48 Salusky IB, Kuizon BD, Belin TR, Ramirez JA, Gales B, Segre GV, Goodman WG: Intermittent calcitriol therapy in secondary hyperparathyroidism: a comparison between oral and intraperitoneal administration. *Kidney Int* 1998;54:907–914.
- 49 Goodman WG, Ramirez JA, Belin TR, Chon Y, Gales B, Segre GV, Salusky IB: Development of adynamic bone in patients with secondary hyperparathyroidism after intermittent calcitriol therapy. *Kidney Int* 1994;46: 1160–1166.
- 50 Ruedin P, Rizzoli R, Slosman D, Leski M, Bonjour JP: Effects of oral calcitriol on bone mineral density in patients with end-stage renal failure. *Kidney Int* 1994;45:245–252.
- 51 Costa AF, dos Reis LM, Ribeiro MC, Moyses RM, Jorgetti V: Effects of calcitriol on parathyroid function and on bone remodelling in secondary hyperparathyroidism. *Nephrol Dial Transplant* 2003;18:743–749.
- 52 Hamdy NA, Kanis JA, Beneton MN, Brown CB, Juttmann JR, Jordans JG, Josse S, Meyrier A, Lins RL, Fairey IT: Effect of alfacalcidol on natural course of renal bone disease in mild to moderate renal failure. *BMJ* 1995;310:358–363.
- 53 Baker LR, Abrams SM, Roe CJ, Faugere MC, Fanti P, Subayti Y, Malluche HH: Early therapy of renal bone disease with calcitriol: a prospective double-blind study. *Kidney Int Suppl* 1989;27:S140–S142.
- 54 Newman CL, Creecy A, Granke M, Nyman JS, Tian N, Hammond MA, et al: Raloxifene improves skeletal properties in an animal model of cystic chronic kidney disease. *Kidney Int* 2015, Epub ahead of print.
- 55 Moe SM, Radcliffe JS, White KE, Gattone VH 2nd, Seifert MF, Chen X, et al: The pathophysiology of early-stage chronic kidney disease-mineral bone disorder (CKD-MBD) and response to phosphate binders in the rat. *J Bone Miner Res* 2011;26:2672–2681.
- 56 Nordal KP, Dahl E: Low dose calcitriol versus placebo in patients with predialysis chronic renal failure. *J Clin Endocrinol Metab* 1988;67:929–936.
- 57 Przedlacki J, Manelius J, Huttunen K: Bone mineral density evaluated by dual-energy X-ray absorptiometry after one-year treatment with calcitriol started in the predialysis phase of chronic renal failure. *Nephron* 1995;69: 433–437.
- 58 Rix M, Andreassen H, Eskildsen P, Langdahl B, Olgaard K: Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. *Kidney Int* 1999;56:1084–1093.