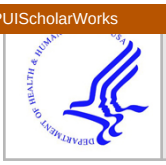

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Calcitriol suppression of parathyroid hormone fails to improve skeletal properties in an animal model of chronic kidney disease

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Abstract

Background—Chronic kidney disease leads to complex metabolic changes and an increased risk of fracture. Currently, calcitriol is the standard of care as it effectively suppresses parathyroid hormone levels in CKD patients. While calcitriol and its analogues 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 parathyroid hormone.

Methods—Male Cy/+ rats, a PKD-like CKD model, were treated with either vehicle or calcitriol for five 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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Keywords

animal models; bone disease; CKD; treatment; calcitriol

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 analogues, and calcimimetics all lower PTH, but data assessing 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]. Preclinical studies using calcium to suppresss 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 chronic kidney disease.

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 3x weekly; intraperitoneal) for 5 weeks. This treatment duration was chosen as it approximates an average bone remodeling cycle in skeletally mature rats (roughly six 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, Canton, MI, or Sigma kits). Intact PTH was determined by ELISA (Alpco, Salem, NH).

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 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 with tartrate-resistant acid phosphatase (TRAP) for osteoclast measurements (tibia only). For cancellous bone, a region of interest approximately 0.8 mm from the growth plate that was analyzed. Unstained sections were assessed for total bone surface, 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 bone surface, osteoclast number, and osteoclast surface to calculate the number of osteoclasts per unit bone surface (N.Oc/BS) and percent osteoclast surface (Oc.S/BS). All histomorphometric nomenclature follows standard usage [34].

Whole Bone Mechanics

Structural mechanical properties of femora were determined by four-point bending. The anterior surface was placed on two lower supports located +/−9 mm from the mid-diaphysis (18 mm span 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 (I_{ml}), and the distances from the centroid to the tensile surface using standard beam-bending equations for four-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 endplates were removed o 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 five 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, NJ). A 660 nm laser was focused on the bone surface using a 50X objective to a spot size of ~10 μm. Three locations were imaged ~3 mm apart with five 20 second acquisitions at each location as previously published [38]. A five-point linear baseline correction was applied in LabSpec 5 (HORIBA JobinYvon). Using OriginPro 8.6 (OriginLab, Northampton, MA), a single Gaussian peak was fit to the $PO_4^{3-}\nu1$ peak, and the areas under the $PO_4^{3-}\nu1$, $CO_3^{2-}\nu1$, and Amide I peaks were calculated at each location. Type B carbonate substitution was found by the band area ratio of $CO_3^{2-}\nu1/PO_4^{3-}\nu1$. The degree of matrix mineralization 3- was determined by the band area ratio of $PO_4^{3-}\nu1$/Amide I. Mineral maturity (crystallinity) was determined by the inverse of the full width at half maximum (FWHM) of the $PO_4^{3-}\nu1$ peak.

Nanoindentation

After Raman spectroscopy, nanoindentation was performed on the same samples using a Hysitron TI950 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 μm × 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 10s loading period, a 10s hold at 3000 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 40-95% 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 ANOVA 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 untreated CKD animals (−61%) but still higher than normal controls (+381%).

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 (Figure 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, cortical thickness, and bending moments of inertia compared to normal controls (Table 2). Animals treated with calcitriol displayed cortical values similar to CKD-vehicle animals. Calcitriol did not correct CKD-induced changes in cortical area, thickness, porosity, or polar moment of inertia of the femur or cortical thickness of the vertebra (Table 2).

Histology

Vehicle-treated CKD animals had higher trabecular bone formation rates in the tibia and vertebra as well as higher osteoclast number and percent osteoclast surface than their normal counterparts (Figure 2 and Table 3). Calcitriol animals displayed similar values to CKD-vehicle animals with regard to mineral apposition rate, osteoclast number, and percent osteoclast surface. While animals with calcitriol had a slightly higher proportion of mineralizing surfaces than CKD-vehicle animals (in the tibia only), bone formation rates 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 (Figure 3 and Table 4). Estimated material properties revealed a significantly lower modulus of toughness in CKD animals (Figure 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 (Figure 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 (Figure 3). Estimated material properties were similar among all three groups. As above, vertebrae from calcitriol animals were no different than their untreated CKD counterparts.

Bone Quality

Compared to normal controls, animals with CKD were found to have no differences in indentation properties. Calcitriol had no affect 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 due in part to the failure of calcitriol to suppress bone formation rates and osteoclast formation, both of which remained significantly higher than normal in long bones and vertebrae. These data raise important questions regarding how much PTH suppression 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 showed positive bone outcomes [39]. 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 the current study, and femoral neck BMD and mechanical properties were higher than CKD animals and equivalent to 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 differences, 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 calcitriol and its analogues 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, one 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/bone surface and osteoclast surface/bone surface in conjunction with a 73% decrease in PTH [51]. Conversely, other studies have shownno 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 osteoclast surface 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 analogues 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.

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 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]. Further, 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 analogues.

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 osteoclast surfaces [55] without significant alterations in bone mass/geometry or mechanical properties. The current experiment 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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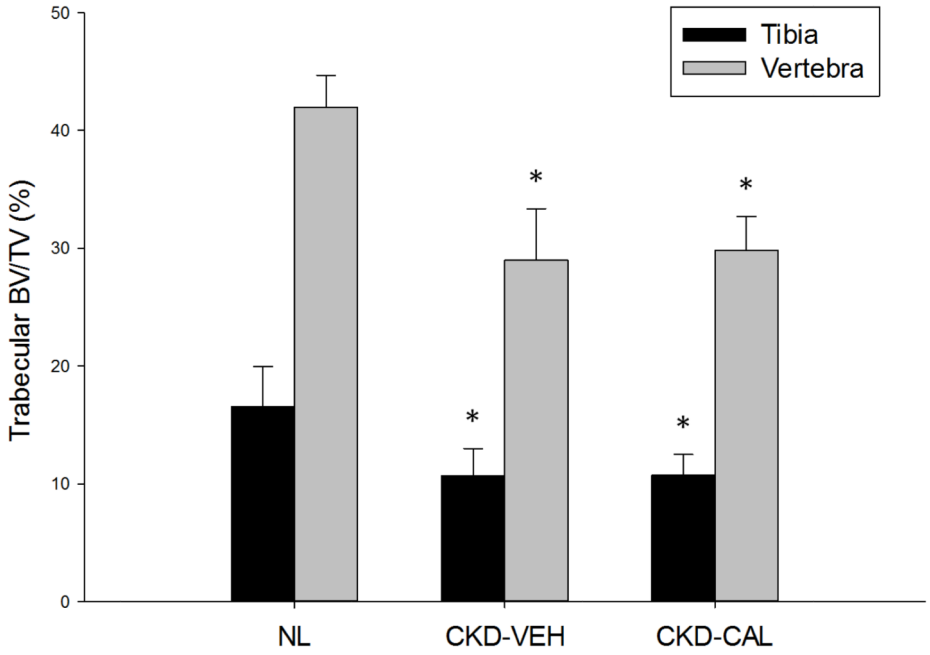


Figure 1.

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

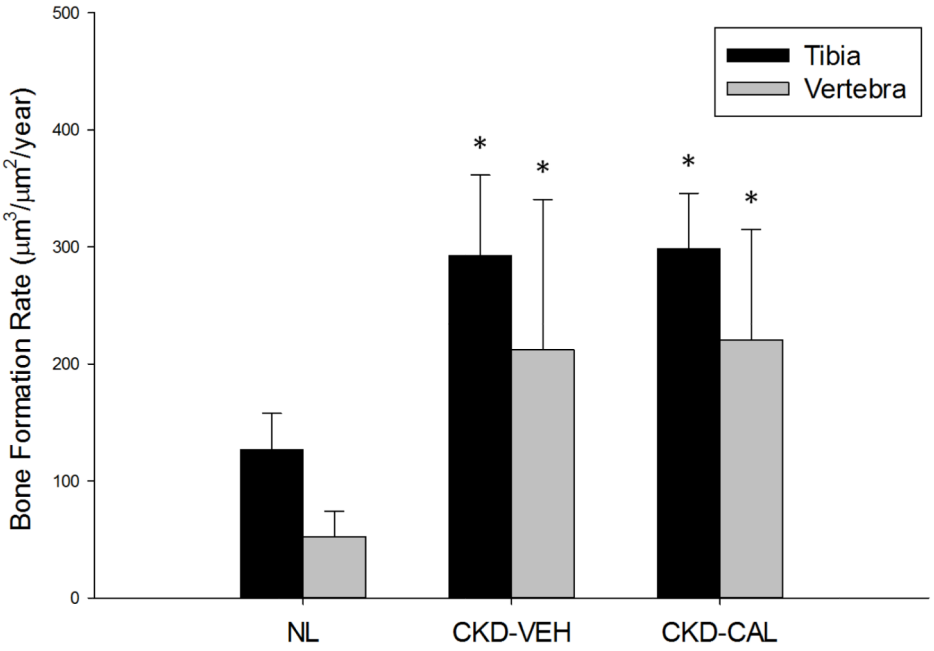
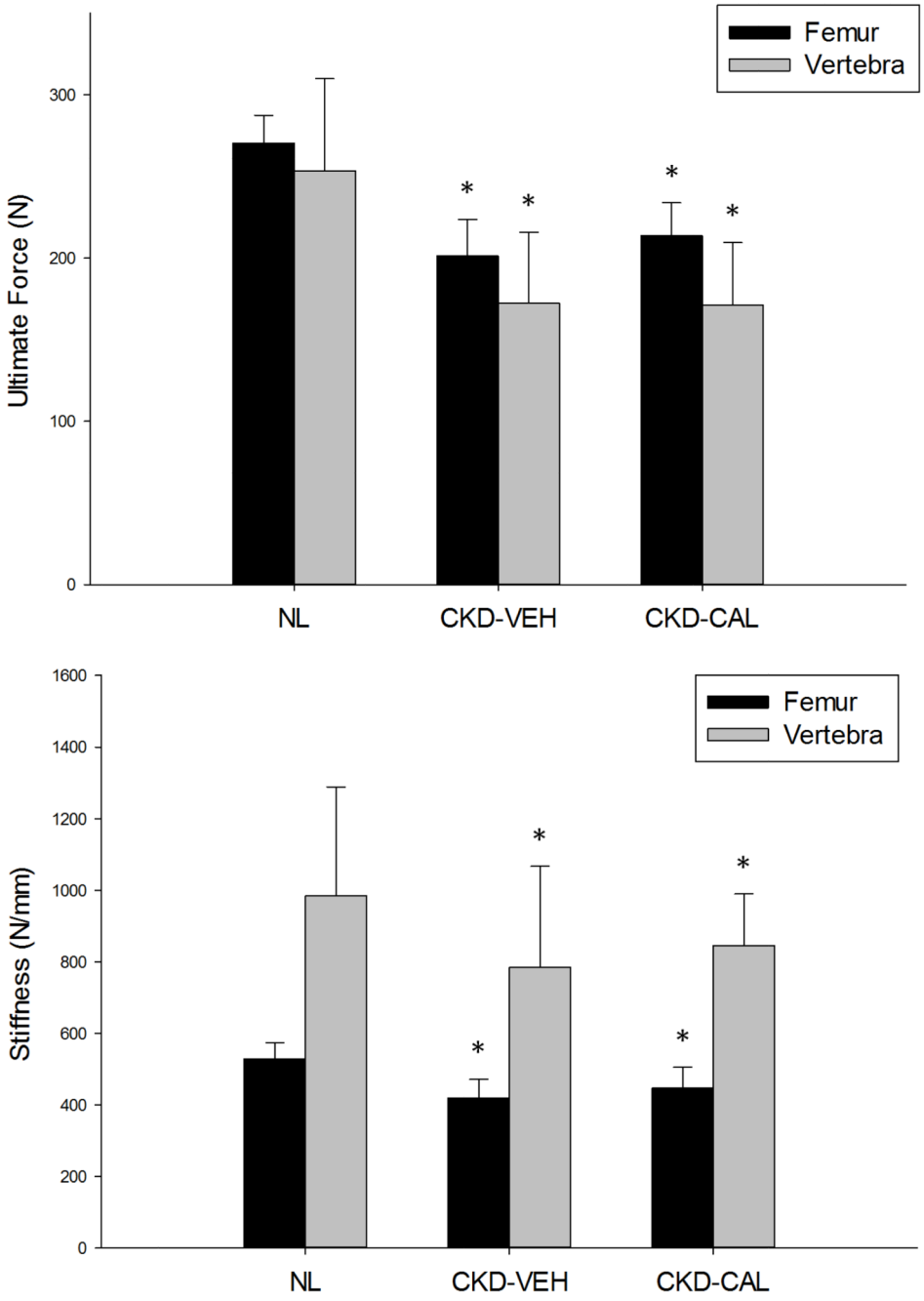


Figure 2.

Bone formation rates in the proximal tibia and caudal lumbar vertebra as determined by dynamic histomorphometry. *, $p<0.05$ compared to NL



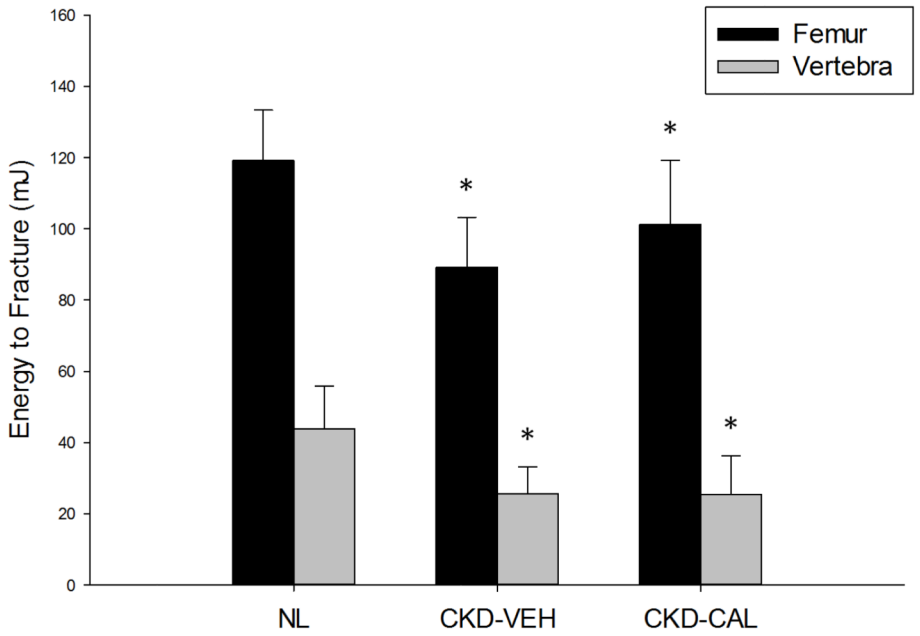


Figure 3.

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

Table 1

Biochemistry 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	2194.39 ± 1811.01 *	875.12 ± 432.51 *#

BUN, blood urea nitrogen; PTH, parathyroid hormone

* vs. Normal;

vs. CKD (Vehicle);

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 *
Femoral Diaphysis			
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
Lumbar Vertebra			
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. BV/TV, bone volume/tissue volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Ct.Th, cortical thickness; Ct.Ar, cortical area; I_{ap}, moment of inertia in the anterior-posterior direction; I_{ml}, moment of inertia in the medial-lateral direction; Ct.Po, cortical porosity.

Table 3

Trabecular bone histomorphometry

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

* vs. Normal. MAR, mineral apposition rate; MS/BS, mineralizing surface/bone surface; BFR/BS, bone formation rate per bone surface; OcS/BS, osteoclast surface per bone surface; N.Oc/BS, number of osteoclasts per bone surface.

Table 4

Structural and material mechanical properties

Femur	**Normal**	**CKD (Vehicle)**	**CKD (Calcitriol)**
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 *
Vertebra			
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	1174.00 ± 447.08	1131.84 ± 170.17
Toughness (MPa)	1.310 ± 0.470	0.924 ± 0.347	0.819 ± 0.386

* vs. Normal

Table 5

Bone Quality

Raman Spectroscopy	**Normal**	**CKD (Vehicle)**	**CKD (Calcitriol)**
Crystallinity (1/FWHM PO_4^{3-}-ν1)	0.0529 ± 0.0003	0.0534 ± 0.0008	0.0534 ± 0.0004
Carbonate Substitution (CO_3^{2-}-ν1/ PO_4^{3-}-ν1)	0.247 ± 0.008	0.242 ± 0.013	0.238 ± 0.013
Relative Mineralization (PO_4^{3-}-ν1/Amide I)	2.598 ± 0.341	2.403 ± 0.442	2.759 ± 0.299
Nanoindentation			
Elastic Modulus (GPa)	11.066 ± 2.766	9.666 ± 1.021	9.987 ± 2.610
Hardness (MPa)	202.62 ± 22.86	192.68 ± 47.35	209.426 ± 43.65

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]	Minerali-zation[b]	Volume[c]		
Jablonski 1995	5/6th NX rats out 4 months from surgery	1.2X	Calcitriol 0.17μg/100 g BW daily for 12 weeks	Not changed	Not done, but static eroded surface improved	↑	↑	↑	↑ Stiffness ↑ Time to fracture NS Toughness
Jokihaara 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	NA	↑	↑ Ultimate load at femoral neck NS Ultimate load at femoral diaphysis
Naves-Diaz 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	NA
Finch 2010	5/6th Nx started treatment when “uremic”	9.27X	Paricalcitol 0.16 ug/kg BW three times per week for 6 weeks	↓49%	NS	NS	↓	NA	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	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 Xray 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*	Volume[c]		
Baker 1986	54 patients at baseline, 20 follow up bone biopsies in patients with CKD 3-5 not ESRD	Oral calcitriol vs. placebo 12 to 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 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 1988	30 patients with CKD 3-5 not ESRD	28 pts 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 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 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 pt had very low BFR	↓	NS		
Przedlacki 1995	25 patients with CKD stages 4-5	PO calcitriol vs. placebo for 12 months				↑	
Rix 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] = bone formation rate (BFR), mineral apposition rate;

b = Mineralizing surface/bone surface (MS/BS) or osteoid volume;

[c] = bone volume/ total volume