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# Compromised vertebral structural and mechanical properties associated with progressive kidney disease and the effects of traditional pharmacological interventions

Christopher L. Newman [a], Neal X. Chen [b], Eric Smith [a], Mark Smith [a], Drew Brown [a], Sharon M. Moe [b,c], Matthew R. Allen [a,b,*]

[a] Department of Anatomy and Cell Biology, Indianapolis, IN, United States
[b] Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States
[c] Roudebush VA Medical Center, Indianapolis, IN, United States

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ABSTRACT

*Background/Aims:* Patients with chronic kidney disease mineral and bone disorder (CKD-MBD) have a significantly higher vertebral and non-vertebral fracture risk than the general population. Several preclinical models have documented altered skeletal properties in long bones, but few data exist for vertebral bone. The goal of this study was to examine the effects of progressive CKD on vertebral bone structure and mechanics and to determine the effects of treatment with either bisphosphonates or anti-sclerostin antibody in groups of animals with high or low PTH.

*Methods:* Animals with progressive kidney disease were left untreated, treated with calcium to lower PTH, zoledronic acid to lower remodeling without affecting PTH, anti-sclerostin antibody, or anti-sclerostin antibody plus calcium. Non-diseased, untreated littermates served as controls. Vertebral bone morphology (trabecular and cortical) and mechanical properties (structural and material-level) were assessed at 35 weeks of age by microCT and mechanical testing, respectively.

*Results:* CKD with high PTH resulted in 6-fold higher bone formation rate, significant reductions in the amount of trabecular and cortical bone, and compromised whole bone mechanical properties in the vertebra compared to normal animals. Treatments that reduced bone remodeling were effective in normalizing vertebral structure and mechanical properties only if the treatment reduced serum PTH. Similarly, treatment with anti-sclerostin antibody was effective in enhancing bone mass and mechanical properties but only if combined with PTH-suppressive treatment.

*Conclusions:* CKD significantly altered both cortical and trabecular bone properties in the vertebra resulting in compromised mechanical properties and these changes can be normalized by interventions that involve reductions in PTH levels.



## Introduction

Patients with chronic kidney disease—mineral and bone disorder (CKD-MBD) have a significantly higher fracture risk than the general population [1–3]. This population also displays differences in fracture rates between long bones and vertebrae [3], suggesting that these two skeletal sites may be differentially affected by the disease. A study of Japanese men on dialysis who underwent screening lumbar spine imaging studies demonstrated that 20.9% of prevalent dialysis patients had evidence of spine fractures [4]. High resolution CT data have revealed significant increases in cortical porosity in the distal limbs with variable responses in trabecular bone [5]. Because vertebral elements are primarily composed of trabecular bone, the influence of secondary hyperparathyroidism on these sites could be potentially different than in long bone cortices [6]. Furthermore, the thin cortical shell of the vertebrae bears nearly 50% of the load [7]; thus, cortical bone changes at this site would also have dramatic effects on mechanical properties and fracture risk.

Several animal models of CKD have revealed significant detriments in the biomechanical properties of long bones [8–10]. For example, rodent models indicate that animals with CKD exhibit lower strength and stiffness compared to their normal counterparts [11–13]. While much of the decline in mechanical integrity can be attributed to structural changes resulting from high parathyroid hormone (PTH) and high turnover rates, recent studies indicate that bone quality also

* Corresponding author at: Dept. of Anatomy and Cell Biology, MS 5035, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202, United States. Fax: +1 317 278 2040.
*E-mail address:* matallen@iupui.edu (M.R. Allen).

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plays a role [9]. Unfortunately, vertebral bone in CKD models has yet to be examined at any of these levels. The goal of this study was to assess the effects of progressive CKD on vertebral bone structure and mechanics and to determine the effects of treatment with either anti-remodeling medications (bisphosphonates) or anabolic treatments (anti-sclerostin antibody). We hypothesized that a significant phenotype would exist in the vertebrae of CKD animals and that treatment would restore structural mechanical properties but not the material-level mechanical properties.

## Methods

### *Animal model*

Cy/+ rats exhibit the progressive onset of polycystic kidney disease due to transmission of an autosomal dominant missense mutation in the geneAnks6, which codes for the protein SamCystin [14–16]. Anks6 has been shown to be important in childhood recessive cystic kidney disease although heterozygote parents have no manifestations [17–19]. The course of kidney disease progression in the Cy/+ rats parallels the course of human CKD-Mineral Bone Disorder (CKD-MBD) [16]. A colony of these animals is maintained at the Indiana University School of Medicine. All procedures were reviewed and approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

### *Experimental design*

The animals described in this work were part of a large experiment that included numerous treatment and control groups. Male Cy/+ rats began the study at 25 weeks of age at which time animals were fed a casein-based diet (Purina AIN-76A, Purina Animal Nutrition, Shreevport, LA, USA; 0.53% Ca and 0.56% P) in order to accentuate the disease. Subsets of Cy/+ animals were divided into the following groups (Fig. 1):

Controls (CKD): These animals were left untreated. Based on the previously described phenotype these animals have high PTH and high bone turnover [11,20].

Calcium (CKD-Ca): These animals were treated with calcium-supplemented water (3%) starting at 30 weeks of age. Based on the previously described phenotype these animals have low PTH and low bone turnover [11,20].

Zoledronic acid (CKD-Z): These animals were treated with zoledronic acid (20 μg/kg/BW as a single subcutaneous injection at 30 weeks of age). Based on the previously described phenotype these animals have high PTH and low bone turnover [11,20]. Zoledronic acid was chosen as a representative bisphosphonate to study in CKD as it has an infrequent dosing schedule (1× per year in post-menopausal osteoporosis treatment) and has high affinity for the bone matrix making it likely to impart persistent treatment effects.

Anti-sclerostin antibody (CKD-SCL): These animals were treated with anti-sclerostin antibody (Scl-Ab; 100 mg/kg/week starting at 30 weeks of age). Scl-AB was provided by Novartis Institutes for BioMedical Research, Novartis Pharma AG. Based on the previously described phenotype these animals have high PTH and high bone turnover [11,20]. Anti-sclerostin antibody was chosen as it has emerged as a promising potential anabolic drug treatment.

Anti-sclerostin antibody plus calcium (CKD-SCL-Ca): These animals were treated with anti-sclerostin antibody and calcium (3% in water). Based on the previously described phenotype these animals have low PTH and high bone turnover [11,20].

Normal (NL): A group of non-diseased male littermates were untreated.

All animals were sacrificed at 35 weeks of age. For all experiments, serum biochemistries and long bone phenotypes have been previously reported in various publications, yet are reported in the results when deemed necessary [11,13,20].

### *Dynamic histomorphometry*

In order to describe the basal remodeling phenotype of the vertebra in NL and CKD animals, the third lumbar vertebra of animals from a separate experiment [13] was processed for undecalcified histomorphometry. The vertebral arch was removed, and the body was embedded in PMMA for sectioning. Thin sections (~4 μm) were

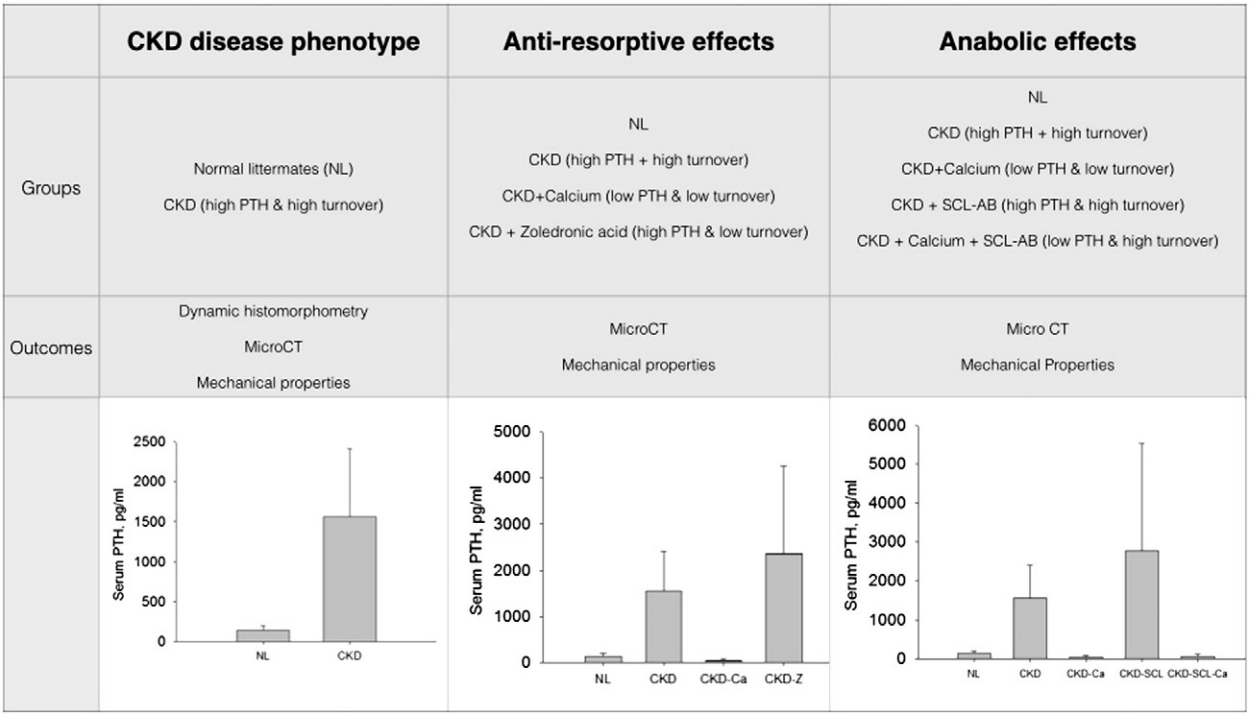


**Fig. 1.** Overview of experimental groups, outcome measures, and serum PTH levels for the planned comparisons. PTH values from previously published manuscript [20].