# Molecular Scale Structural and Compositional Alterations in Chronic Kidney Disease (CKD) Bone Treated with Synthetic Salmon Calcitonin

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INTRODUCTION: Chronic kidney disease (CKD) affects an estimated 15% of adults in the US, leading to a staggering 2-14-fold increase in fracture susceptibility compared to the general population. Changes in bone quantity (bone mass) among CKD patients alone fail to fully account for the heightened fracture risk observed, emphasizing the contribution of bone material alterations (bone quality) as a key factor governing fracture susceptibility. As a result, therapeutic strategies focusing solely on increasing bone mass may prove insufficient in addressing CKD-associated fractures. Recent investigations in our laboratory have demonstrated that synthetic salmon calcitonin can enhance matrix-bound water content and improve critical post-yield mechanical properties in CKD bone, as evidenced by ex vivo experiments where non-viable bone was treated with calcitonin for 14 days [1]. However, whether calcitonin can produce similar improvements in bone quality in an in vivo setting of CKD remains unexplored. Therefore, our study aims to assess the effects of calcitonin treatment on micro-, nano-, and molecular-scale structural and bone composition changes in a CKD model after five weeks of treatment with calcitonin in skeletally mature mice.

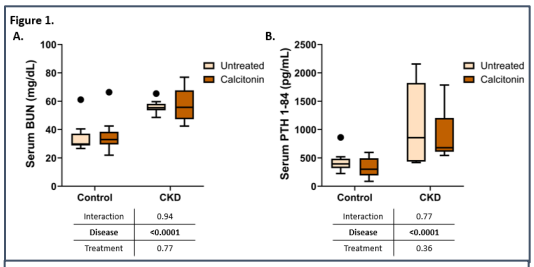


Fig 1. Serum biochemistry results for A) BUN and B) PTH 1-84. 2-Way ANOVA results are bolded when significant.

METHODS: Sixteen-week-old male C57BL/6 mice (Jackson Laboratories) underwent a 10-week CKD induction period via a 0.2% adenine-laced casein- based (0.9% P, 0.6% C) diet (n=20) or remained as non-CKD littermate controls (Con, n=20). Half of the mice (equal number CKD and Con) received subcutaneous injections of 50 IU/kg/day of calcitonin 5x a week for five weeks, and the other half remained untreated (UN). Mice were sacrificed at 31 weeks of age. Serum biochemistries were performed to assess the presence of altered kidney function (blood urea nitrogen (BUN)) and for alterations in parathyroid hormone (PTH 1-84). Right femora were assessed for cortical geometry and microarchitecture at the midshaft (microCT, 7.9 µm resolution). At the same location of microCT, marrow was flushed, and the cortical midshaft was prepared to evaluate molecular and nanoscale changes from CAL treatment. Specifically, wide-angle X-ray scattering (WAXS) was used to characterize tropocollagen and carbonated apatite dimensions and phases, while small-angle X-ray scattering (SAXS) was used to analyze mineralized collagen fibril D-spacing. Biochemistry and microCT data were analyzed using a 2-way Mixed Effects Model (treatment x disease). Initial WAXS and SAXS outcomes were analyzed with unpaired t-tests to determine changes within groups (Con or CKD) due to CAL treatment. All animal procedures received Institutional Animal Care and Use Committee approval before initiating.

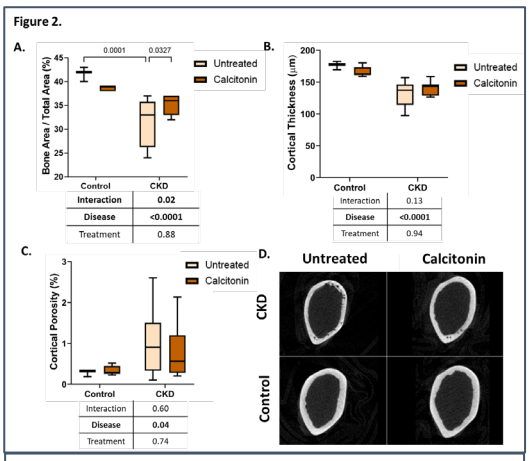


Fig 2. MicroCT results (A-C) and representative microCT crosssections of the femoral midshaft.

RESULTS: BUN was higher in CKD mice regardless of intervention (Fig. 1A). CKD mice exhibited significantly elevated PTH levels. While treatment with CAL led to a reduction in PTH (1068 vs. 899.7 pg/mL for CKD UN vs. CKD CAL), this decrease did not reach statistical significance (Fig. 1B). Imaging confirmed a cortical phenotype reflective of CKD, presenting with a lower bone area ratio (BA/TA, $p < 0.0001$), lower cortical thickness ($p < 0.0001$), and higher cortical porosity ($p = 0.04$) compared to Con (Fig. 2A-D). A significant interaction term was observed for BA/TA, and subsequent post hoc tests revealed that CKD CAL-treated animals exhibited a significantly higher bone area fraction than CKD UN (Fig. 2A). At the molecular level, analysis of WAXS peaks from collagen helix (3.5nm-1 < q-1 < 5.0nm-1, Fig.3A) and carbonated apatite mineral (17.5nm-1 < q-1 < 190nm-1, Fig.3B) disclosed indications of enhanced structure in diseased samples treated with CAL, featuring subtle increases in mineralization and more swollen collagen helices. This stands in contrast to untreated bones from the CKD cohort. Initial SAXS analysis revealed no discernible changes in the arrangement or dimensions of the mineralized collagen fibril between treated and untreated CKD bones. Further assessment of remaining CKD and Con bone and evaluation of bone water content by thermogravimetric analysis (TGA) is underway.

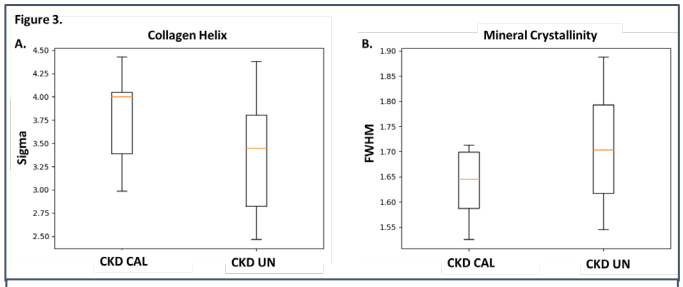


Fig 3. Normalized intensities from WAXS peaks of A.) the collagen triple helix peak at ~ 40 $nm^{-1}$ fit with a gaussian model and B.) the carbonated apatite crystal peak ~ 18 $nm^{-1}$ fit with a Lorentz peak model. This work benefited from the use of the SasView application.

DISCUSSION: In light of the prevalence of CKD and its association with heightened fracture susceptibility, the limitations of focusing on bone mass to address this risk have become evident. CAL treatment resulted in minor bone mass/quantity changes, aligning with previous clinical experience. Our investigation builds on recent laboratory insights that calcitonin has the potential to enhance matrix-bound water content and improve post-yield mechanical properties in CKD [1]. Notably, our in vivo study on mice with CKD demonstrates an intriguing increase in swelling of collagen helices following CAL administration and a subtle rise in carbonated apatite mineral. Whether collagen swelling is due to increased tightly bound water or increased mineral crystallinity is under investigation. Further analysis is necessary to include additional samples and to determine whether these structural changes result from interactions with collagen, mineral, or potential alterations in physicochemical environmental factors influenced by CAL introduction.

SIGNIFICANCE/CLINICAL RELEVANCE: Therapeutic modulation of aspects of bone quality across multiple length scales may be an attractive approach to improving mechanical properties and potentially reducing fracture risk in CKD. REFERENCES: [1]Surowiec RK, et al. Bone. 2023 Aug;173:116805.
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