Establishing Temporal Bone Quality Changes in Progressive Chronic Kidney Disease: A Translational Approach

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INTRODUCTION: In chronic kidney disease (CKD), the 2-14 fold increase in fracture rates compared to the age-matched population is a significant concern, and this increased fragility in CKD cannot be fully explained by mineral density and mass changes. Standard imaging for fracture risk assessment relies mainly on mineral density (BMD) measured through DEXA, overlooking crucial aspects of bone quality. Recent insights emphasize the importance of the organic matrix and bone water content in bone toughness, both of which are impacted in CKD. Conventional clinical assessments overwhelmingly prioritize mineral content and bone turnover, potentially overlooking crucial early bone changes relevant to fragility, where timely treatment would be most effective. We hypothesize that changes in bone water content and matrix organization precede mineral alterations during CKD progression, negatively affecting mechanical properties, and that these changes can be detected using standard clinical imaging methods.

METHODS: Male Cy/+ rats (n=40) and their normal littermates (NL, n=40) were utilized in this study. Cy/+ rats exhibit gradual disturbances in mineral metabolism and serve as an exceptional model for studying the progressive abnormalities associated with CKD. Notably, they develop evident skeletal changes by 28 weeks (wk), which intensify to severe phenotypic alterations by 34 wks. The rats were bred in-house and assigned to CKD or normal groups based on serum blood urea nitrogen (BUN) levels by 10 wks of age. A subgroup of n=8 rats per group was sacrificed at specific time points: 22, 25, 28, 31, and 34 wks (**Fig. 1A**). Tibiae resected from these rats underwent a series of assessments, including dual-energy X-ray absorptiometry (DXA) for BMD, micro computed tomography (microCT) for geometry and microarchitecture (12µm resolution), and ultrashort echo time magnetic resonance imaging (UTE MRI, 9.4T) for calculating bound water, porosity index, and matrix organization. Following imaging, tibia underwent whole bone dynamic mechanical analysis (DMA) to determine the elastic modulus (storage modulus, E'), viscous modulus (loss modulus, E", and damping coefficient (Tan δ) as a function of frequency (0.5-20 Hz) followed by thermogravimetric analysis (TgA, 3°C increase per min. until 650°C) to quantify free and bound water mass and organic mass in the tibial shaft. Where appropriate, data were analyzed by t-test or RM 2-way ANOVA. All animal procedures received Institutional Animal Care and Use Committee approval prior to initiating studies.

RESULTS: Rats from the 22-31 week (wk) time points have been sacrificed to date. At 10 wks, the Cy/+ rats exhibited significantly higher serum BUN levels compared to the NL rats (**Fig. 1B**). Both groups showed an increase in body mass up to 28 wks. However, by 31 wks, the Cy+ rats had significantly lower body mass compared to the 31 wk NL group, indicating a progression to a more severe disease state (**Fig. 1C**). Cy/+ rat BMD at the tibial midshaft was significantly lower than NL by week 28 by DXA (**Fig. 1D**). For microCT of the cortical bone, neither marrow area, total area, nor cortical porosity was significantly different between groups at each time point through 31wks, although Cy/+ had a higher average porosity vs. NL at each time point. Cortical thickness was significantly lower in Cy/+ rats at 22 wks (**Fig. 1E**), and bone area became significantly lower compared to NL by wk 28. **Figure 1F** depicts a representative UTE MRI image from the 28 wk NL and Cy/+ tibial shaft where the NL exhibited higher bound water content. Complete MRI analysis is ongoing. TgA revealed significantly higher free water (28 wks) and significantly lower bound water (22 wks), by mass, in Cy/+ rats vs. NL. DMA was performed on the 22-25 wk groups, showing a significant main effect of frequency for storage modulus (**Fig. 1H**), loss modulus, and Tan δ. Although a significant interaction term was not observed, there was a trend towards an increase in storage modulus, indicating a stiffer material, in the 25 wk Cy/+ rats.

DISCUSSION: Although BMD differences between Cy/+ and NL became significant only at 28 weeks, notable changes in parameters like cortical thickness (measured by microCT) and crucially, bound water, were observed starting at week 22. These findings suggest the potential identification of new biomarkers using clinically relevant imaging techniques to detect bone changes earlier than conventional methods like DXA. Ongoing efforts involve completing UTE MRI assessments, DMA for all time points, and Fourier transform near-infrared spectral imaging to examine the spatial dynamics of cortical water, collagen, and glycosaminoglycans throughout disease progression.

SIGNIFICANCE: To enhance the evaluation of fracture risk in CKD, it's essential to gain a deeper understanding of the significant skeletal changes that take place as the disease progresses and support these efforts with clinically feasible techniques capable of detecting these factors beyond BMD.

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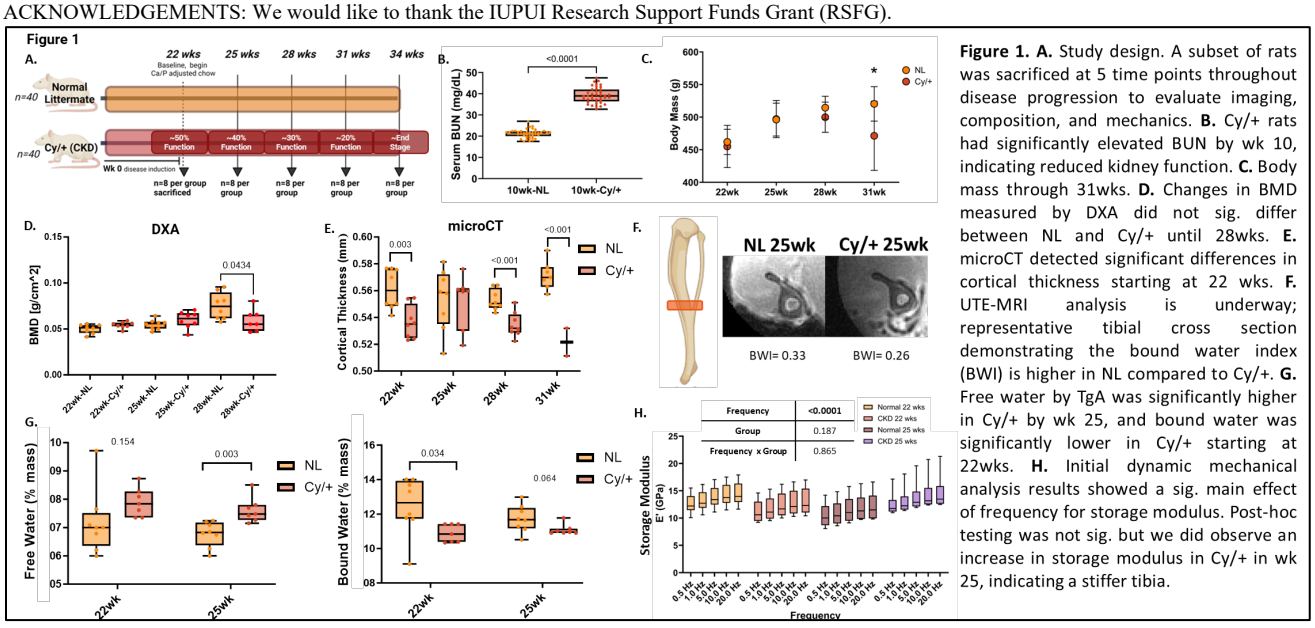

Figure 1. A. Study design. A subset of rats was sacrificed at 5 time points throughout disease progression to evaluate imaging, composition, and mechanics. **B.** Cy/+ rats had significantly elevated BUN by wk 10, indicating reduced kidney function. **C.** Body mass through 31wks. **D.** Changes in BMD measured by DXA did not sig. differ between NL and Cy/+ until 28wks. **E.** microCT detected significant differences in cortical thickness starting at 22 wks. **F.** UTE-MRI analysis is underway; representative tibial cross section demonstrating the bound water index (BWI) is higher in NL compared to Cy/+. **G.** Free water by TgA was significantly higher in Cy/+ by wk 25, and bound water was significantly lower in Cy/+ starting at 22wks. **H.** Initial dynamic mechanical analysis results showed a sig. main effect of frequency for storage modulus. Post-hoc testing was not sig. but we did observe an increase in storage modulus in Cy/+ in wk 25, indicating a stiffer tibia.