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The Impact of *in vitro* soaking with Calcitonin or Raloxifene on Mechanical Properties in Healthy and Diseased Mouse Bone

R.K. Surowiec* [1,2], R. Saldivar* [2], R.K. Rai [3], J.M. Wallace [1], and M.R. Allen [2]

[1]Dept. of Biomedical Engineering, Indiana University – Purdue University Indianapolis (IUPUI), Indianapolis, Indiana, USA

[2]Dept. of Anatomy, Cell Biology, and Physiology, Indiana University School of Medicine (IUSM), Indianapolis, Indiana, USA

[3]Dept. of Biochemistry and Molecular Biology, IUSM, Indianapolis, Indiana, USA

* Indicates co-first authors

IUPUI DEPARTMENT OF BIOMEDICAL ENGINEERING
INDIANA UNIVERSITY SCHOOL OF MEDICINE

INTRODUCTION

Raloxifene (RAL) reduces clinical fracture risk despite modest effects on bone mass/density[1]. This may be due to increased hydration, which we have shown improves material-level mechanical properties and works through cell- independent mechanisms[2-3]. Synthetic salmon calcitonin (CAL) has also demonstrated efficacy in fracture risk reduction and only modest improvements in mass/density[4] warranting further evaluation.

AIM

The goals of this study were to assess the ability of RAL and CAL to improve properties in bone from a chronic kidney disease (CKD) model and whether treatment could modify healthy/diseased bone through cell-independent mechanism that modify hydration. In doing so, we will evaluate any differential impacts between CAL vs. RAL treatment in both healthy and CKD bone tissue.

METHODS

Study Design:

- Male C57BL/6 mice were induced with chronic kidney disease (CKD) beginning at 16 wks of age via 0.2% adenine-laced casein-based (0.9% P, 0.6% C) chow or remained on casein-based control chow (control littermates (Con)). Adenine was discontinued after 6 wks, mice lived for an additional 4 wks until sacrifice at 26-wks of age.
- Right femora from CKD and Con mice were randomly assigned to the following treatment groups:
  - **Calcitonin (CAL),** 100 nM concentration, n=10 femora per group (Con, CKD)
  - **Raloxifiene (RAL)**, 2 µM concentration, n=10 femora/group
  - **Vehicle (VEH)**, equimolar dimethyl sulfoxide (DMSO), 0.04% vol/vol, n=9 femora/group
- Bones were incubated in PBS+drug solution at 37°C for 14 days using an established *ex vivo* soaking methodology.

Outcomes:

- A subset of femora underwent micro-computed tomography (µCT, 9 µm) to assess **cortical geometry**.
- Bones that underwent µCT were subject to a 3-point bending test to failure to assess **mechanical and material properties**.
- Remaining bones that had not undergone 3-point bending were subject to solid state nuclear magnetic resonance (ssNMR) spectroscopy to assess **hydration (total and bound water)**.
- Data were analyzed by two-tailed t tests (µCT) or 2-way ANOVA for main effects of disease, treatment, and their interaction.

RESULTS

Cortical Geometry and Microstructure

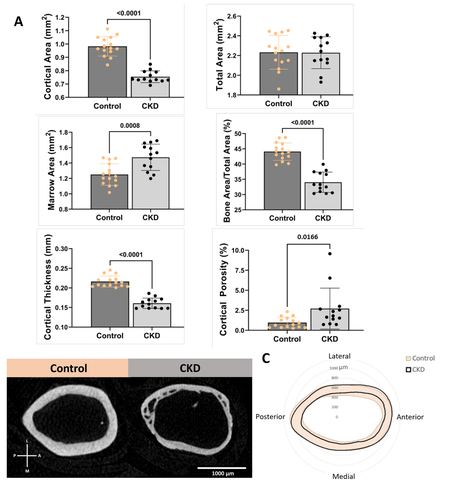

Figure 1. A) µCT results from Control (Con, n=15) and CKD (n=13) femora. B) Representative µCT images of cortical sections from C57BL/6 Con (left) and CKD (right) femora used for cortical geometry and microstructural analysis. Images show the elevated cortical porosity B) Average profiles of the femoral cortical ROIs from all bones in the control (orange) and CKD (black) group, demonstrating that cortical bone area and thickness is smaller in C57BL/6 mice induced with CKD. Only femora undergoing 3-pt bending were imaged. Data are mean ± SD, p-values are from t-tests.

Mechanical and Material-Level Properties

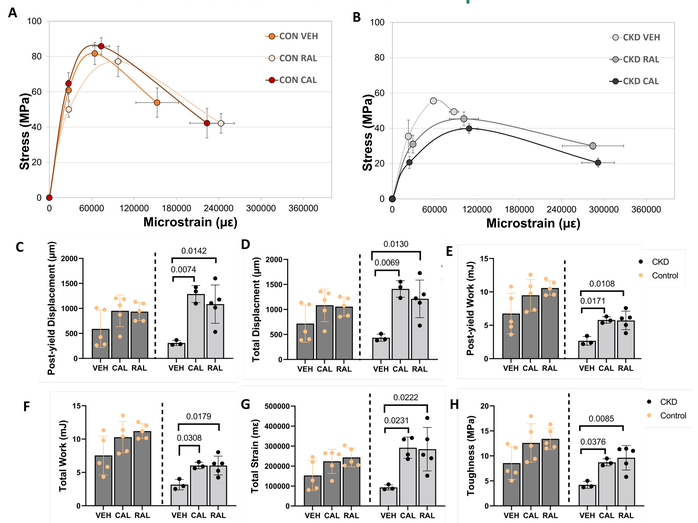

Figure 2. Average stress-strain plots from Con (A) and CKD (B) show CKD bones treated with RAL or CAL had increased post-yield displacement, toughness, and total strain with effects more pronounced in CKD vs. Con bone. Data points are mean values ± standard error of the mean. C-H) a sig. main effect of Treatment (via 2-way ANOVA) was followed by a Tukey post-hoc test to determine the source of differences. Treatment with CAL and RAL significantly improved post-yield displacement (C), total displacement (D), post-yield work (E), total work (F), total strain (G), and toughness (H) compared to VEH in the CKD bones only. No significant impact of either treatment was observed in Con. CAL vs. RAL was not sig. different for any measure. Data are mean ± SD, p-values are from Tukey post-hoc analysis.

Bone Hydration

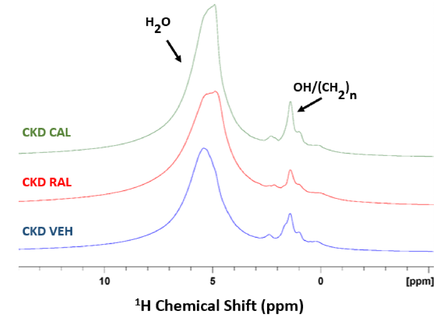

Figure 3. Representative ^1H chemical shift spectra (ppm) from CKD femora treated with CAL (green), RAL (red), or VEH (blue). Femora treated with CAL and RAL demonstrate higher total water content with broader peaks following treatment via soaking. Peaks associated with water (H_2O), inorganic OH- are denoted.

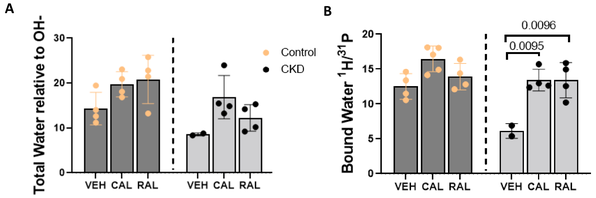

Figure 4. A) Average total water content relative to OH- measured via ^1H ssNMR demonstrated sig. main effects of 'Treatment' and 'Disease', but no sig. differences between groups in post-hoc analyses. B) ANOVA results for bound water, measured via ^1H-^{31}P HetCOR, revealed sig. main effects of 'Treatment' (p=0.0002), 'Disease' (p=0.0009), and a sig. interaction term (p=0.04). Post-hoc analysis demonstrate that both CAL and RAL treatment significantly increased bound water vs. VEH. There were no sig. differences due to treatment in the Con group. Data are mean ± SD and p-values are from Tukey post-hoc analysis.

CONCLUSIONS

- RAL and CAL improved important post-yield properties and toughness in CKD bone in a non-cell mediated manner (via *ex-vivo* soaking treatment).
- **CKD bones treated with RAL or CAL had significantly higher bound water content compared to VEH control.**
- There were no significant differences in mechanical properties or hydration for Con bones although there was a trend towards higher total and bound water with both treatments.
- Ongoing work in our labs is evaluating the impact of CAL on material properties and hydration using *in vivo* disease models.

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CONTACT INFORMATION

For any additional questions, please contact Dr. Rachel Surowiec: rksurowi@iu.edu.