Skeletal Benefits of Combination Mechanical Loading and Raloxifene Treatment in the KK/Ay Mouse Model of Type II Diabetes

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Introduction

- **Type 2 Diabetes (T2D)** affects an estimated 1 in every 10 individuals in the US.
- Chronic kidney disease (CKD) affects half of the individuals with T2D.
- Both CKD and T2D independently increase fracture risk.
- **Raloxifene (RAL)** is an FDA-approved drug that can reduce fracture risk by improving bone structure and increasing tissue hydration which in turn enhances bone quality and ductility.
- In-vivo **mechanical loading** is a potent anabolic stimulus that can induce bone formation and increase bone mass.
- Combining an anabolic stimulus with a quality-based therapy could have an interactive effect.

KK/Ay Mouse Model:

- In KK/Ay mice, the agouti gene is ectopically expressed in other tissues.
  - **Agouti gene (Ay):** controls the production of melanin and is typically only expressed in hair follicles.
- Mice homozygous for the agouti mutation are not viable embryos.
- Heterozygous mice (Ay/a) develop obesity and diabetes.
- Wild-type mice (a/a) without the agouti gene develop obesity but not diabetes.

Goals

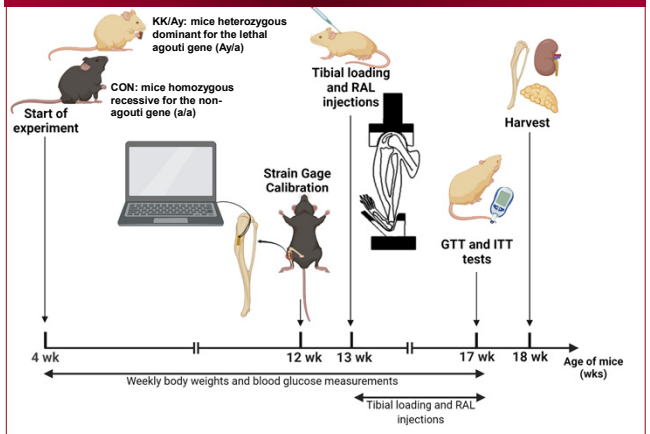
Hypothesis 1: The KK/Ay mice model is suitable for the study of T2D.

Hypothesis 2: The KK/Ay mice model will have signs of kidney dysfunction.

Hypothesis 3: The KK/Ay mice will have skeletal deterioration due to the disease state.

Hypothesis 4: Combination RAL and tibial loading will improve bone strength and bone quality.

Methods



Experimental Groups:

- **Females:** 40KK/Ay (n=20 RAL, n=20 UN) and 40 CON (n=20 RAL, n=20 UN).
- At 12wks, strain gages were applied to standardize strain between groups (n=4/group).
- From 13 to 17wks of age, all mice underwent in-vivo tibial loading 3x/wk. Half of the mice from each group were also subcutaneously injected with 0.5mg/kg of RAL 5x/wk.
- Mice were euthanized at 18wks. Bi-lateral tibiae were µCT scanned (8µm res) and used for architectural analysis prior to 4-point bending tests to failure.

Acknowledgments

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Figure 1 – Weekly Blood Glucose (BG) and Body Weights

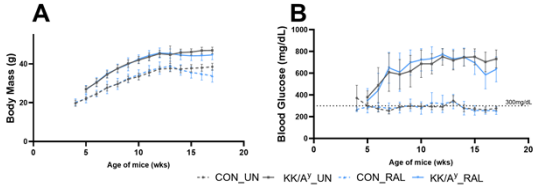

Figure 1: Average body mass for KK/Ay mice trended higher than CON throughout the study duration. At the study endpoint, KK/Ay mice were severely diabetic (BG>300mg/dL) but CON animals were in a non-diabetic range.

Figure 2 – Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

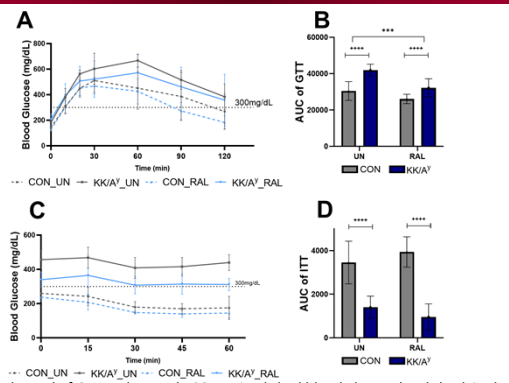

Figure 2: By the end of GTT and ITT, only CON animals had blood glucose levels back in the normal range and KK/Ay animals maintained their diabetic state. Area Under the Curve (AUC) of GTT for KK/Ay animals was greater than CON, and RAL lowered the AUC of GTT. AUC of ITT for CON animals was higher than KK/Ay animals.

Figure 3 – Serum Assays

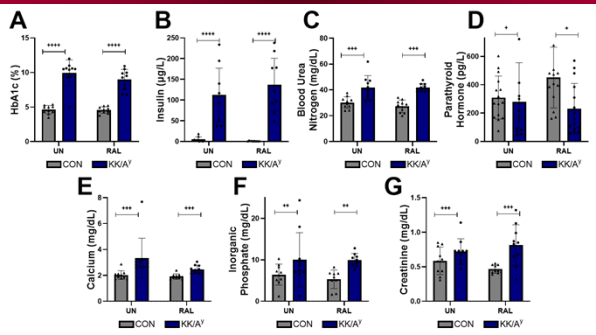

Figure 3: Glycated Hemoglobin (HbA1c) and serum concentrations of insulin, blood urea nitrogen (BUN), calcium, phosphorous, and creatinine were all higher in KK/Ay mice. Serum concentration of parathyroid hormone was higher in CON compared to KK/Ay mice.

Figure 4 – Trabecular and Cortical Properties

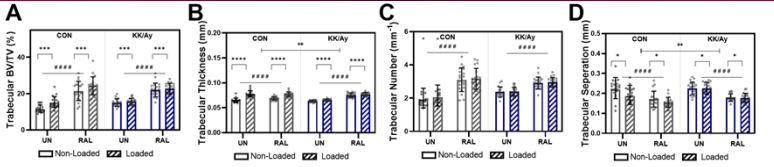

Figure 4: CON mice had thicker trabeculae and lesser trabecular separation compared to KK/Ay mice. Mice administered with RAL had greater trabecular bone volume fraction, thicker trabeculae, greater trabecular number, and less trabecular separation compared to CON mice. Tibial loading increased trabecular bone volume fraction, trabecular thickness, and decreased trabecular separation in mice.

Figure 5 – Cortical Properties

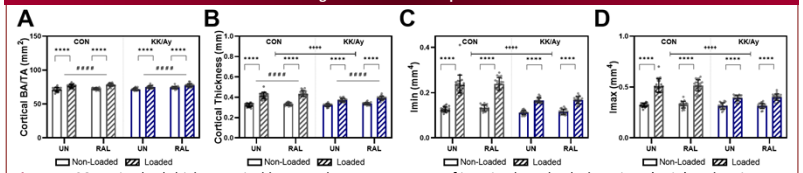

Figure 5: CON mice had thicker cortical bone and greater moment of inertia about both the minor (Imin) and major (Imax) axes. Mice administered with RAL had greater bone area fraction and a thicker cortex compared to untreated mice. Tibial loading increased bone area fraction, cortical thickness, Imin and Imax in mice.

Figure 6 – Mechanical Properties

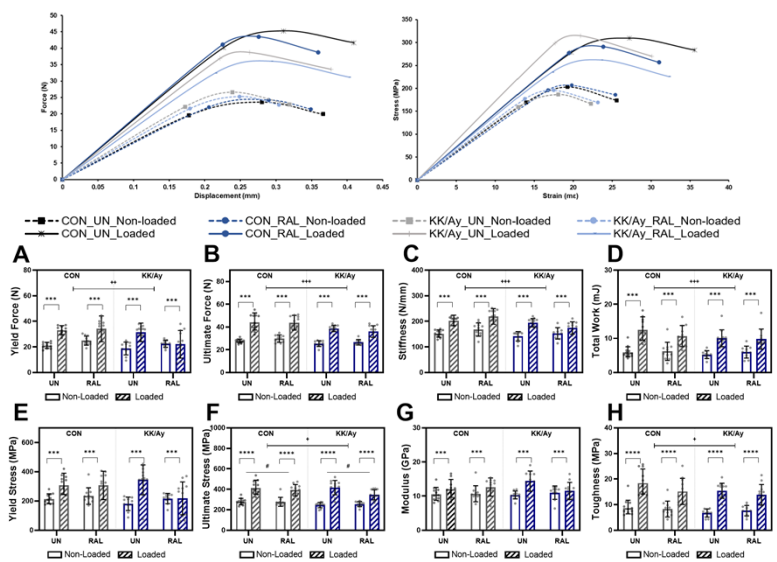

Figure 5: CON mice had greater yield force, ultimate force, stiffness, ultimate stress, and toughness. Mice administered with RAL had lower ultimate stress, but other mechanical properties were unaffected. Tibial loading increased all mechanical properties.

CONCLUSION

- **Hypothesis 1 Conclusion:** KK/Ay animals had elevated blood glucose levels, HbA1c, and serum insulin. KK/Ay mice also impaired glucose and insulin tolerance. Therefore, the data support the use of the KK/Ay model as a suitable model for the study of T2D.
- **Hypothesis 2 Conclusion:** The KK/Ay animals had elevated serum concentrations of BUN, PTH, Ca, and creatinine and lowered PTH, which are all indicative of kidney dysfunction.
- **Hypothesis 3 Conclusion:** Diabetic KK/Ay mice had deterioration in their trabecular, cortical and mechanical properties. Therefore, confirming that the disease states did impair skeletal properties.
- **Hypothesis 4 Conclusion:** The interactive effects of RAL and tibial loading were minimal. Even though RAL improved overall trabecular and cortical properties, the mechanical properties were not improved in mice administered with RAL. Tibial loading improved trabecular, cortical, and mechanical properties of mouse tibiae.