Skeletal Effects of Raloxifene and Targeted Tibial Loading in the KK/Ay Murine Model of Type II Diabetes

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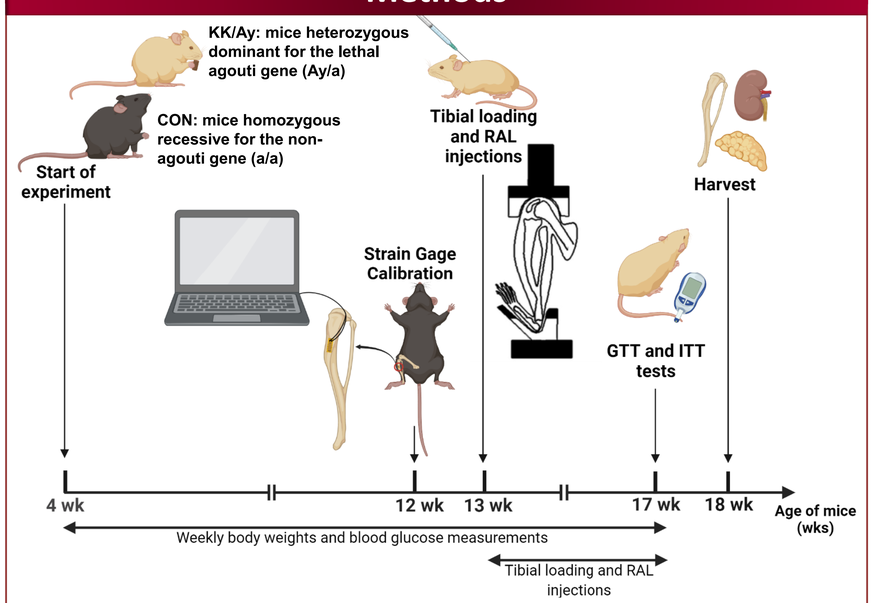
Introduction

- **Type 2 Diabetes (T2D)** affects an estimated 9.1% of the US population.
- Patients with T2D have a higher fracture risk compared to non-diabetes individuals.
- **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.

Goals

1) Are the biochemistries and bone phenotype of the KK/Ay genetic mutation mouse model reflective of the human condition of Type 2 diabetes?
2) What are the therapeutic effects of RAL, targeted mechanical loading, and the combined effects of RAL + mechanical loading on the T2D phenotype.

Methods



- **4 to 17 wks:** Weekly **body weights** and **blood glucose** measurements were obtained.
- **12 wks:** Mice were euthanized (n=4/grp) and used for **strain gage calibration** to determine the loaded required to achieve 2050με of tension during cyclic loading tibiae.
- **13 wks to 17 wks:** 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.
  - **Males:** 80 total – 40 KK/Ay (n=20 RAL, n=20 UN) and 40 CON (n=20 RAL, n=20 UN).
  - **Females:** 80 total – 40KK/Ay (n=20 RAL, n=20 UN) and 40 CON (n=20 RAL, n=20 UN).
- **17 wks:** Glucose Tolerance Tests (GTT) and Insulin Tolerance Tests (ITT) (n=6/grp).
- **18 wks:** Mice were **euthanized** via cardiac puncture. Whole blood, kidney, pancreas, bi-lateral tibiae were harvested.

Pending:

- Serum: Blood Urea Nitrogen (BUN), Parathyroid Hormone (PTH), Calcium, Phosphorous, Creatinine.
- Pancreas: beta-cell mass.
- Mechanical tests: 4-point bending tests (n=12/grp), fracture toughness test (n=6/grp).

Acknowledgments

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Figure 1 – Weekly Blood Glucose and Body Weights

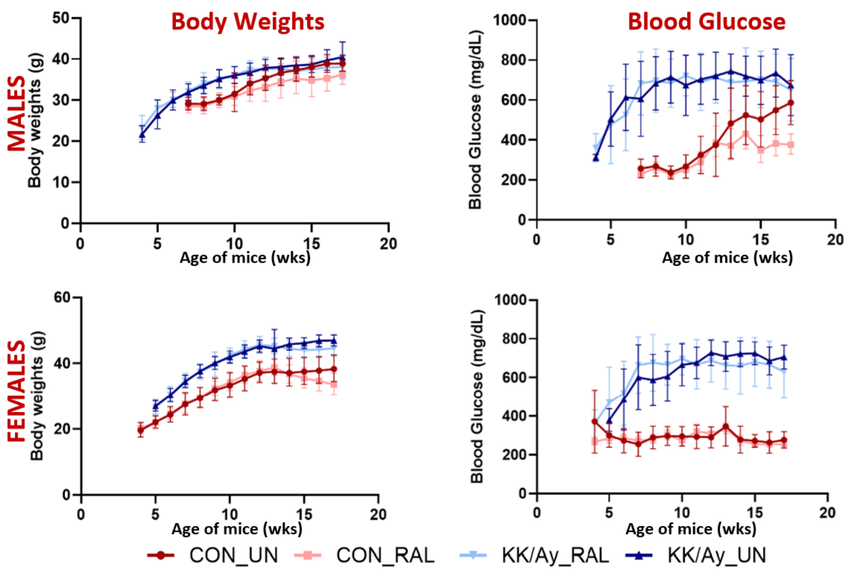

Figure 1: ***Body weights*** *– comparable between groups in both sexes, increases throughout the period of the study; lower average values are observed in mice administered with RAL, starting at 13wks of age, compared to UN.* ***Blood glucose*** *– KK/Ay have higher average values compared to CON. Both KK/Ay and CON males are severely hyperglycemic, but only KK/Ay females demonstrate severe hyperglycemia.*

Figure 2 – Cortical and Trabecular Data of Loaded and Non-loaded Tibiae

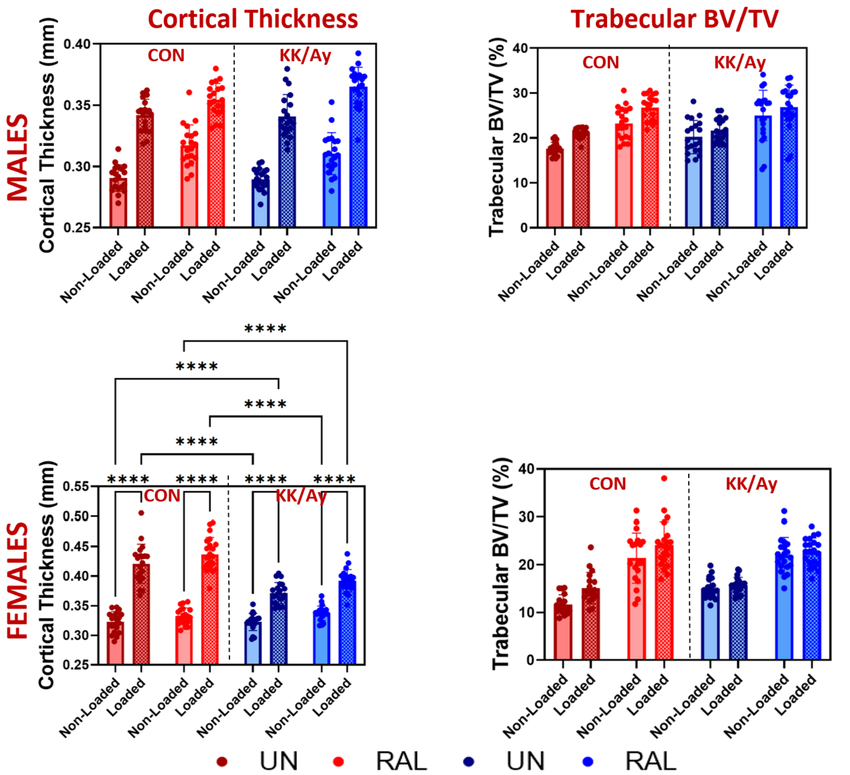

Figure 2: *Micro-computed tomography (μCT) – 8μm voxel size.* ***Cortical Thickness –*** *significantly higher for loaded compared to non-loaded in both males and females; significantly higher with RAL compared to UN; significant differences in cortical thickness between KK/Ay and CON in females only.* ***Trabecular BV/TV*** *– significantly higher for non-loaded compared to loaded; significantly higher with RAL compared to UN; significant differences in BV/TV between KK/Ay and CON in males only. 3-way ANOVA used for statistical analysis. Additional cortical and trabecular data pending.*

Variable	Genotype	Treatment	Loading	Interactions
		MALES		
Cortical Thickness	ns	ns	Loaded ↑ (p<0.0001)	none
Trabecular BV/TV	KK/Ay ↑ (p=0.0294)	RAL ↑ (p<0.0001)	Loaded ↑ (p<0.0001)	none
		FEMALES		
Cortical Thickness	KK/Ay ↓ (p<0.0001)	RAL ↑ (p<0.0001)	Loaded ↑ (p<0.0001)	Loading x Genotype (p<0.0001)
Trabecular BV/TV	ns	RAL ↑ (p<0.0001)	Loaded ↑ (p=0.0211)	none

Table 1: *Summary of statistical Analysis for Cortical Thickness and Trabecular BV/TV using 3-way ANOVA*

Figure 3 – GTT and ITT at 17 wks of age

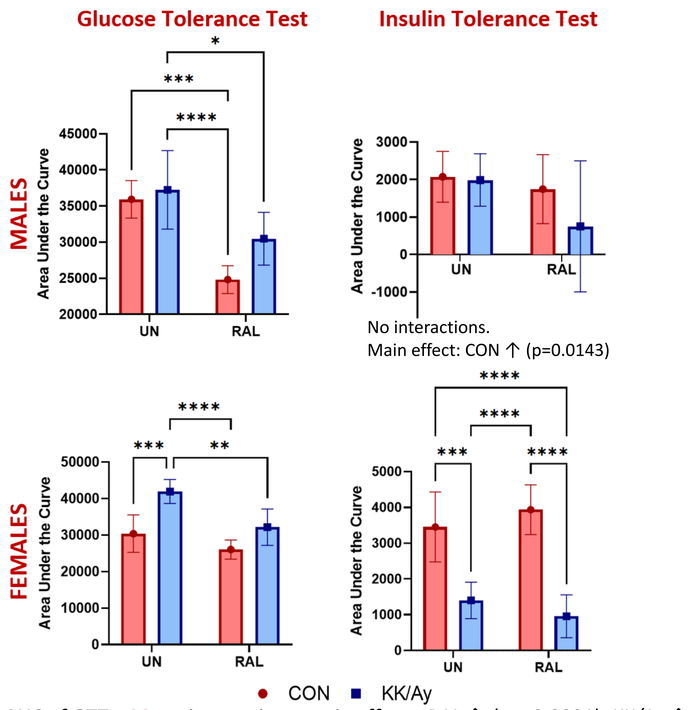

Figure 3: ***AUC of GTT*** *–* ***M:*** *no interactions; main effects: RAL ↑ (p=<0.0001), KK/Ay ↑ (p=0.0297); *p=0.0223, ***p=0.0070, p<0.0001.* ***F:*** *No interaction; main effects: RAL ↑ (p=0.0005), KK/Ay ↑ (p<0.0001); post-hoc comparison: **p=0.0031, ***p=0.0006, ****p<0.0001.* ***AUC of ITT*** *–* ***M:*** *no interactions, no main effects.* ***F:*** *no interactions; main effects: KK/Ay ↓ (p<0.0001); post-hoc comparison: ***p=0.0004, ****p<0.0001. 2-way ANOVA used for statistical analysis.*

Figure 4 – GTT and ITT at 17 wks of age

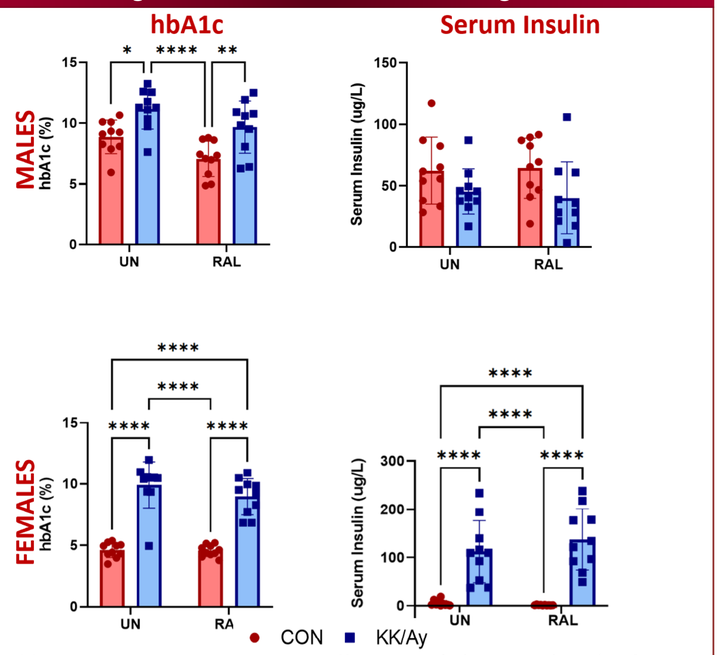

Figure 4: ***hbA1c*** *–* ***M:*** *no interactions; main effects: RAL ↓ (p=0.0038), KK/Ay ↑ (p<0.0001); post-hoc comparison: *p=0.0223, **p=0.0070, ****p<0.0001.* ***F:*** *no interaction; main effects: KK/Ay ↑ (p<0.0001); post-hoc comparison: ****p<0.0001.* ***Serum insulin*** *–* ***M:*** *no interaction; main effects: KK/Ay ↓ (p=0.0143).* ***F:*** *no interactions; main effects: KK/Ay ↑ (p<0.0001); post-hoc comparison: ****p<0.0001. 2-way ANOVA used for statistical analysis.*

CONCLUSION

- The data supports the use of the KK/Ay model as a suitable model for the study of Type-II diabetes.
- The blood glucose values are higher in KK/Ay compared to CON in both males and females.
- Serum insulin confirms that the mice are still producing insulin, which is characteristic of early stages of Type-II Diabetes. This is in contrast with Type-I, which is characterized by completed destruction of beta-cells and inability to produce insulin.
- Cortical thickness and trabecular BV/TV increased with loading indicating that T2D does not diminish mechanosensitivity.
- RAL increased cortical thickness and BV/TV in both CON and KK/Ay.