A novel murine model of diet-induced pre-diabetic chronic kidney disease shows clear skeletal defects

IUPUI
DEPARTMENT OF BIOMEDICAL ENGINEERING

Rachel Kohler, Amy Creecy, and Joseph M. Wallace

BBML

INTRODUCTION

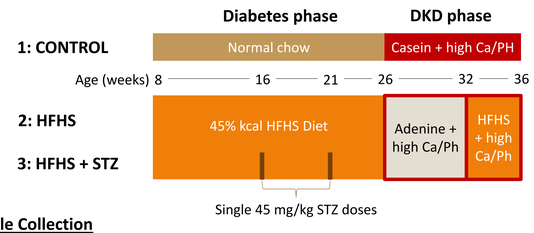
- Diabetes is the leading cause of chronic kidney disease and failure
- The US is facing an impending diabetes epidemic due to a large and growing population of pre-diabetic adults
- Diabetes-driven kidney disease (DKD) causes compounding skeletal deficits affecting fracture risk and fracture-related mortality rates.
- There is a **CRITICAL NEED** for interventions that treat DKD, but research has been inhibited by a lack of animal models

AIM: To create and characterize a mouse model combining obesity-associated type 2 diabetes with chronic kidney disease.

METHODS

Diet-induced Model

- Male C57BL/6J mice, 8-36 weeks of age
- 3 groups: control, high-fat high-sugar (HFHS), and HFHS + streptozotocin (STZ) groups, induced in 2 phases (below).



Sample Collection

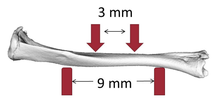
- Blood glucose and body weight measures were collected weekly.
- Glucose tolerance and insulin tolerance tests were performed at 25 weeks.
- Blood serum collected at study end to measure levels of blood urea nitrogen (BUN) and glycated hemoglobin (HbA1c)
- Right hindlimbs were harvested at sacrifice at 36 weeks.

Micro-computed Tomography (µCT)

- Femora, tibiae (RT) and hydroxyapatite phantoms were scanned via µCT (at 8 or 10 µm resolution) with a Bruker Skyscan 1172
- Scans were analyzed for cortical and trabecular properties using CTAn and MATLAB

Mechanical Testing

- RT were tested to failure in 4-point bending (right), at a displacement rate of 0.025 mm/s.
- µCT scans of failure sites were used to calculate mechanical properties.



Statistical Analysis

- One-way ANOVAs were performed with Tukey post-hocs for interaction.

DIABETIC MEASURES: HFHS+STZ developed glucose intolerance

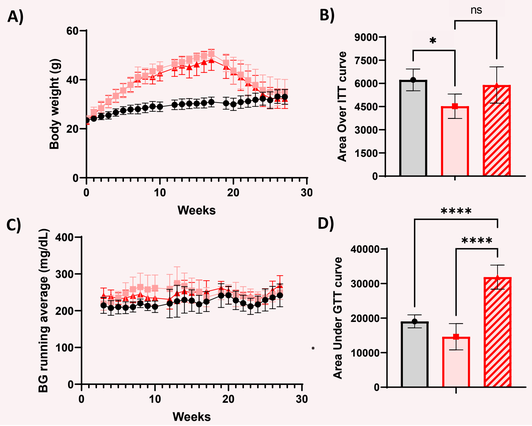
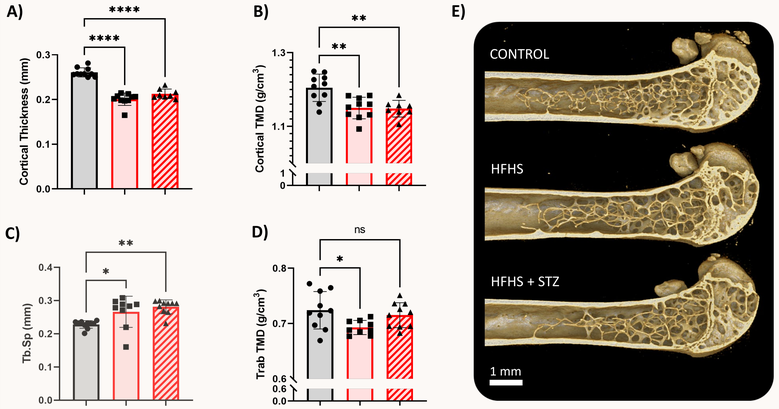


FIGURE 1. A) HFHS diet caused rapid weight gain while adenine diet caused weight loss, but B) insulin tolerance test showed little change in insulin tolerance and C) blood glucose levels did not rise consistently higher than controls. D) glucose tolerance tests showed HFHS+STZ group developed reduced glucose tolerance and further E) had the highest serum levels of HbA1c.

GEOMETRY: Kidney damage associated with reduced bone mass

FIGURE 3. In right tibiae A) cortical thickness and B) cortical tissue mineral density were reduced in adenine-fed mice. Similar results were seen in trabecular bone with C) increased trabecular spacing and E) reduced trabecular tissue mineral density in HFHS mice. E) Cortical and trabecular thinning can be seen in qualitative comparison of representative femoral cross sections.



RESULTS & DISCUSSION

Control HFHS HFHS + STZ

Diabetic Measures

- HFHS mice grew to be almost twice as heavy as controls with little-to-no impact on insulin sensitivity or weekly blood glucose (Fig 1.A-C).
- Glucose tolerance tests and serum HbA1c were highest in HFHS + STZ group. (Fig 1.D-E)
- Addition of STZ may inhibit mice from efficiently processing glucose, leading to a buildup of HbA1c, modeling a pre-diabetic state.

Kidney Damage

- The HFHS+STZ group had the highest average serum BUN levels at study end, indicating reduced kidney function. (Fig 2.A)
- For all mice fed adenine, cortical bone became more porous than controls, indicative of KD-driven mineral and bone disorder. (Fig 2.B-C)

Geometry and Mechanics

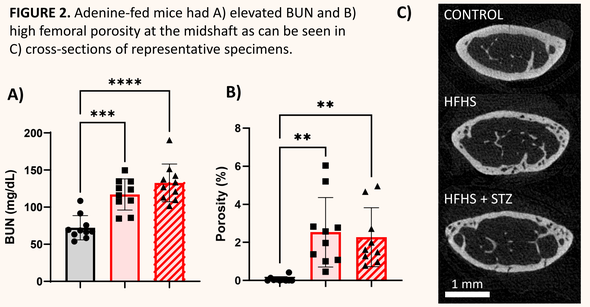
- Adenine-fed mice had reduced bone mass and tissue mineral density.
- This led to weakened bone at both the structural and tissue level
- There were few differences between HFHS and HFHS + STZ for all geometry and mechanics measures, indicating these deficiencies were primarily driven by the shared adenine diet.

Summary

- HFHS+STZ had strongest pre-diabetic state
- Kidney damage (seen by BUN), was strongly correlated with porous, weakened bone
- Addition of STZ dose had little-to-no affect on bone geometry and strength compared to HFHS alone

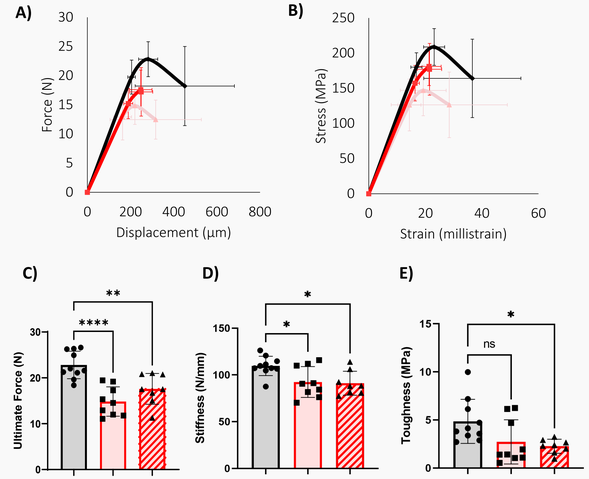
KIDNEY DAMAGE: High BUN and bone porosity

FIGURE 2. Adenine-fed mice had A) elevated BUN and B) high femoral porosity at the midshaft as can be seen in C) cross-sections of representative specimens.



MECHANICS: Kidney damage associated with weak bone

FIGURE 4. Average curves with SD bars from A) force displacement and B) stress-strain plots show that C) ultimate force, D) stiffness, and E) toughness were reduced in adenine-fed mice.



CONCLUSION: HFHS and adenine diets lead to kidney damage and skeletal defects but not overt diabetes, making this HFHS + STZ model useful for studying chronic kidney disease in the context of prediabetes.

FUTURE WORK

Our lab is continuing to investigate DKD by developing and studying T2D models using KK/Ay mice and T1D models induced with STZ doses.

ACKNOWLEDEGEMENTS

This research was funded by the NIH under training grant T32AR065971 and R01AR072609.