Skeletal Manifestations in a Streptozotocin-Induced Mouse Model of Diabetes

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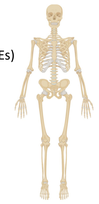
Background

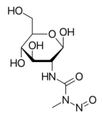
Diabetes and Bone

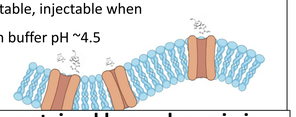
- ⇧ Advanced Glycation End Products (AGEs)
- ⇩ Ca^{2+}, Vitamin D
- ⇧ PTH, inflammatory cytokines
- ⇩ BMD
- ⇧ Risk of fracture



Streptozotocin (STZ)

- Competitively inhibits glucose
- High toxicity with specificity to pancreatic β-cells
- Highly unstable, injectable when dissolved in buffer pH ~4.5





AIM: Characterize the bone phenotype resulting from sustained hyperglycemia in male and female mice.

Methods

Study Design

- C57Bl/6J mice
- 4 groups, n = 15/group

Fem. Ctrl Vehicle Only | *Fem. STZ 90 mg/kg, citrate buffer* | *Male Ctrl Vehicle Only* | *Male STZ 65 mg/kg, citrate buffer*

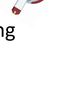
- IP injection, 5 consecutive days @ 8 weeks old

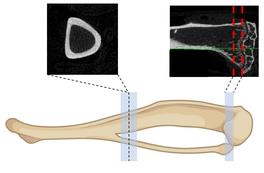
Statistical Analysis

- 2 – way ANOVA ($p < 0.05$) for main effects of treatment and sex with post-hoc Tukey's HSD evaluation when significant interaction found
- All data mean +/- SD

Glucose Measurements

- Fasted Glucose Tolerance Testing
  - N = 7 / group
  - 12+ hr. fast
  - 2g/kg glucose bolus
- Fasted Insulin Tolerance Testing
  - N = 8 / group
  - 2 hr. fast
- Weekly Non-fasted Blood Glucose
  - 0.75 U/kg insulin bolus





Hemoglobin A1C Analysis

- Blood collected via cardiac exsanguination
- Latex Agglutination Inhibition Assay
- Reported as $\frac{glycated\ hemoglobin}{total\ hemoglobin}$

Pancreatic Analysis

- Fixed in formalin
- Embedded in paraffin
- Insulin positive area stained

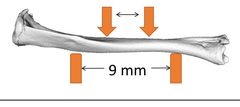
fAGE Analysis

- N = 5/group, random sampling
- Demineralized, dried, digested (6 M/mg HCL, 110°, 20 hrs.)
- Fluoresced @ 360 nm excitation and 460 nm emission
- Tested against quinine standard, normalized to collagen content (via hydroxyproline)

Microcomputed Tomography (µCT)

- 0.5 m Al filter (V = 60 kV, I = 167 µA)
- 9.8 µm/voxel resolution
- 0.7 degree interval
- 1-mm cortical region selected

Mechanical Testing



Results

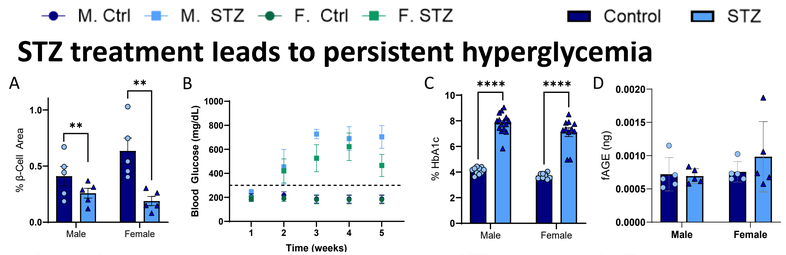


Figure 1. β-islet is successfully compromised by STZ treatment (A). Treated animals exceed hyperglycemic threshold (300 mg/kg) by two weeks after injection (B). By the end of the study, Glycated hemoglobin was increased by more than 50% in treated animals (C), although no increase in fluorescent AGEs was measurable.

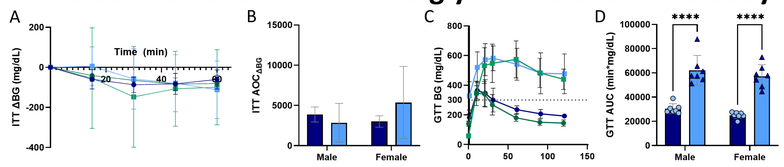


Figure 2. ITT results indicate the ability to process insulin is not compromised by STZ treatment (A, B), while GTT results show that STZ treated mice were unable to produce insulin endogenously in response to glucose injection (C,D). These results are in keeping with disease presentation of Type 1 Diabetic Humans.

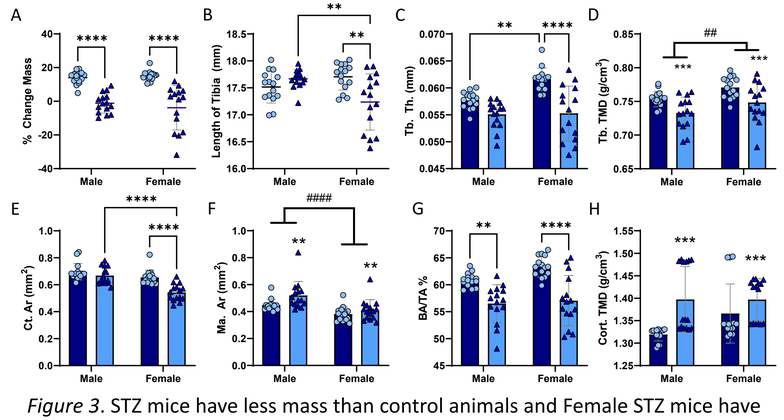


Figure 3. STZ mice have less mass than control animals and Female STZ mice have shorter tibiae than controls similar to patients with childhood onset Type 1 Diabetes (A,B). Both trabecular thickness and cortical area are is significantly less in STZ females suggesting dysregulation of the bone modeling system (C, E). Both lower cortical area and lower marrow area drive a lower percent bone area in treated animals (G). TMD in both types of bone vary significantly with sex and disease (D, H).

Results

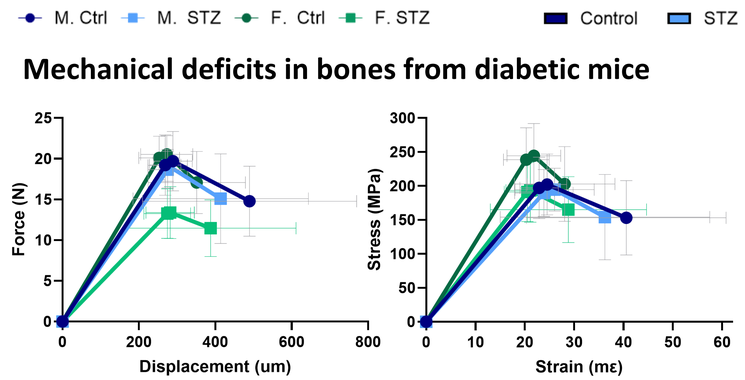


Figure 4. A. Force displacement data averaged by group. B. Normalized Stress/strain averages by group.

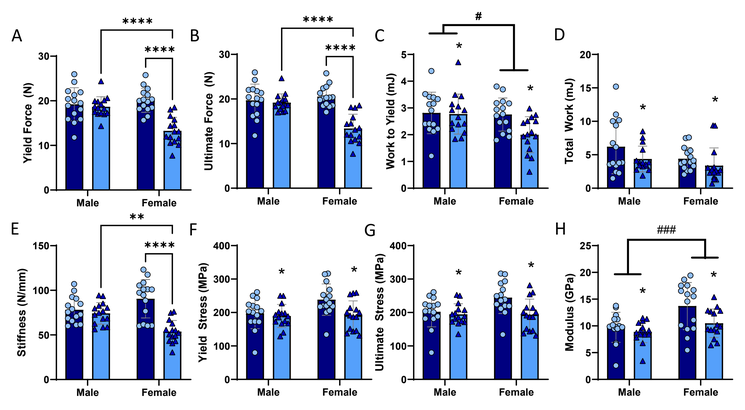


Figure 5. Bones from STZ-treated females require less force to yield and break (A, B). All STZ-treated animals underwent less work before yield and failure (C,D). The reduced ability to withstand force is represented as a significantly reduced stiffness in treated female bones (E). On a material level, yield and ultimate stresses were lower in STZ mice leading to a modulus which was reduced in STZ-treated bones of both sexes (F, G, H).

Conclusions

- STZ treatment successfully induced diabetic state
- Skeletal manifestations paralleled clinical diabetic outcomes
  - Reduced BMD, compromised bone quality, and reduced longitudinal growth.
- Useful model for study of diabetic bone
- Future Work:
  - Extend age of mice to explore effects in aging population,
  - Explore effect of loading on bone in STZ mice,
  - Combine with other clinically relevant disease models.