Structural and Mechanical Improvements to Bone are Strain Dependent in a Targeted Tibial Loading Model of Young Female C57BL/6 Mice

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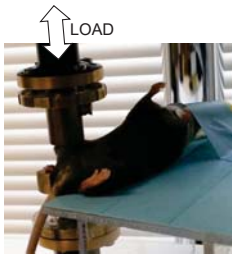
INTRODUCTION

- Rodent models often used to assess bone's adaptive response to loading (running, climbing, targeted loading, etc.)
- Targeted loading provides controlled way to assess bone's response to load
- Recently, the usage of murine tibial loading has exploded
- Many studies investigate morphological changes, but do not assess mechanical outcomes of the loading



HYPOTHESIS

Mechanical loading will increase bone architecture and improve cortical mechanical properties in a dose-dependent fashion

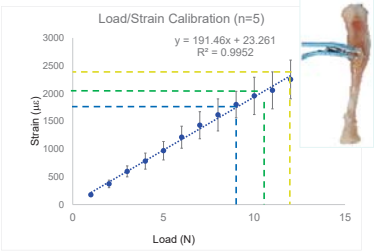
MATERIALS AND METHODS

Animals

- Female C57Bl6 mice at 8 weeks of age (n=35)
  - Calibration group (n=5)
  - Low Strain Group (1700 με; n=10)
  - Mid Strain Group (2050 με; n=10)
  - High Strain Group (2400 με; n=10)

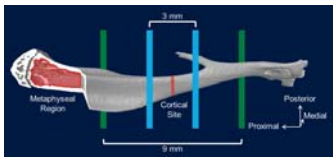
Strain Calibration

- Strain gauged anteromedial portion of the tibial mid-diaphysis of the tibiae
- Cyclically loaded (2 Hz) in compression
  - Load stepped from 2 to 12 N in 1 N increments



In Vivo Loading

- Right tibiae loaded
- Left tibiae served as non-loaded control
- Loading Bout
  - 4 cycles at 2 Hz; Hold 3 seconds
  - Repeat 55 times for 220 total cycles
- Loading Schedule
  - 3 days loaded, 1 day rest
  - Repeat 3 times for a total of 2 weeks



Microcomputed Tomography (CT)

- 10.2 μm resolution
- Cancellous Analysis (Proximal Metaphysis)
  - Region of interest: 12% of bone length
    - Start at the distal end of the proximal growth plate and extending distally
- Cortical Analysis
  - Standard site at 50% of bone length

Mechanical Testing

- 4-point bending (9 mm support span; 3 mm loading span)
- Tested in medial-lateral direction with medial surface in tension
- Displacement control at 0.025 mm/sec
- CT images at fracture site used to normalize Force-Displacement into Stress-Strain

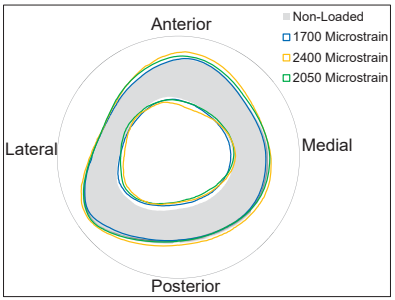
Statistical Analysis

- Repeated measures ANOVA (main effect of loading and strain level)
  - Tukey HSD Post-hoc to examine pairwise difference between strain level
- In the case of interactions:
  - Paired t-tests to evaluate the effect of loading at each of the three strain levels
  - One-way ANOVA to evaluate the effect of strain level in loaded and non-loaded limbs
  - Bonferroni correction (α<0.01)

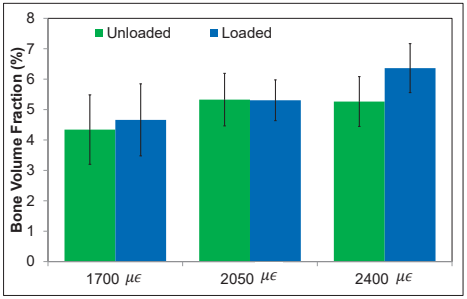
RESULTS AND DISCUSSION

Cortical Architecture (mid-diaphysis)

- Woven bone formation in half of the 2400 με animals
  - Removed from analysis
  - Sample size of 2400 με decreased to n=5
- In non-loaded limbs, no systemic effects
- Loading resulted in:
  - ↑ Cortical area and thickness
  - ↑ Maximum and minimum principal moments of inertia
- Loading caused periosteal expansion and endocortical contraction



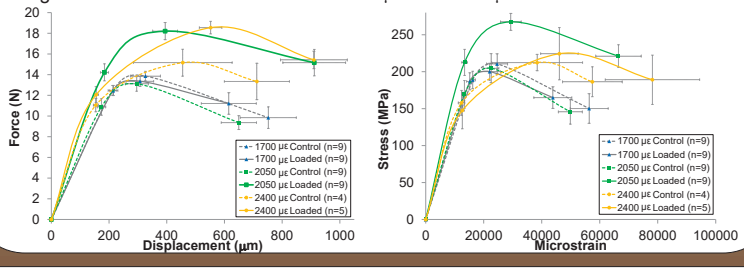
Cancellous Architecture (proximal methaphysis)



- Loading resulted in:
  - ↑ Percent bone volume
  - ↑ Trabecular thickness
  - ↓ Trabecular separation
  - ↓ Trabecular number
- Loading caused fewer, thicker trabeculae resulting in a greater bone volume fraction

Mechanical Properties

- At 2050 με, ↑ ultimate force, ↑ postyield work, ↑ work to failure and ↑ ultimate stress
- No significant effect of load detected at 1700 με and 2400 με



CONCLUSIONS

- **With loading, bone was positively impacted in a dose dependent manner**
  - As expected, loading had a positive impact on cortical and cancellous architecture
  - In addition to morphological improvements, loading caused increases in structural- and tissue-level mechanical behavior
- **Woven cortical response at the highest load level resulted in increased bone mass, but at the cost of animal discomfort**
  - Load at the highest strain level should be avoided

A moderate load level results was largely beneficial in young female mice with respect to both cortical and cancellous structure and cortical mechanical function