Pain during Murine Tibial Loading Impacts Quality-Based Improvements to the Bone Tissue

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Introduction

Murine Tibia Axial Loading

- Common modality used to assess bone mechanical adaption
- Lacks standardization across labs
- In our hands, mice have recently shown signs of discomfort (e.g. limping) following a loading bout, but recover within one hour.

Previous work in our lab has suggested that while bone mass increases in response to loading, this may be decoupled from quality-based mechanical improvements in the bone tissue, possibly due to observed limping.

Study Aims:

1) Assess alternate loading profiles to reduce pain (assessed by limping) while maintaining a robust bone formation response
2) Evaluate if quality-based changes are influenced by animal limping.

Methods

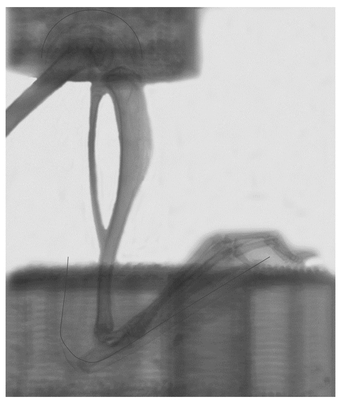
4 Groups: MoTuWe High, MoTuWe Low, MoWeFr Low, TuFr Low

In vivo Tibial Loading

3 Week with loading on days noted
220 loading cycles per bout

- Max Load: 10.6 N (2050 με)
- 4 cycles at 2 Hz
- 3 second dwell held at:
  - 10.6 N for "High" group
  - 2 N for "Low" groups
- Repeat 55 times

Limping assessed after each bout

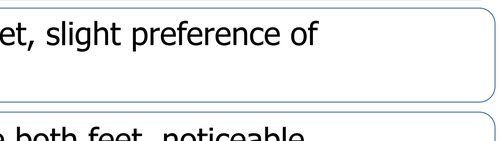


Semi-Quantitative Assessment of Limping

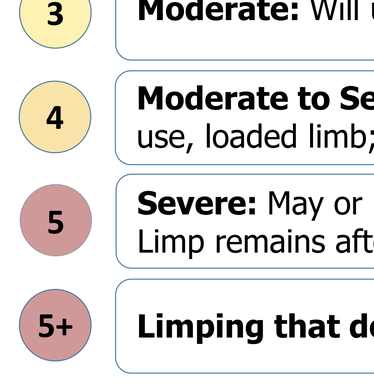
0	**No Limping**
1	**Mild Limp:** Will use both feet, slight preference of contralateral limb
2	**Mild to Moderate:** Will use both feet, noticeable preference of contralateral limb
3	**Moderate:** Will use both feet, hobbles on the loaded limb
4	**Moderate to Severe:** May or may not touch, but not use, loaded limb; Uses both limbs within 1 hr of loading
5	**Severe:** May or may not touch, but not use, loaded limb; Limp remains after 1 hr, but mouse is recovered by next day.
5+	**Limping that does not recover by the next day**

Microcomputed Tomography (μCT)

- 8.4 μm voxel size
- Shadow scan: damage assessment
- Cortical ROI: 50% of bone length



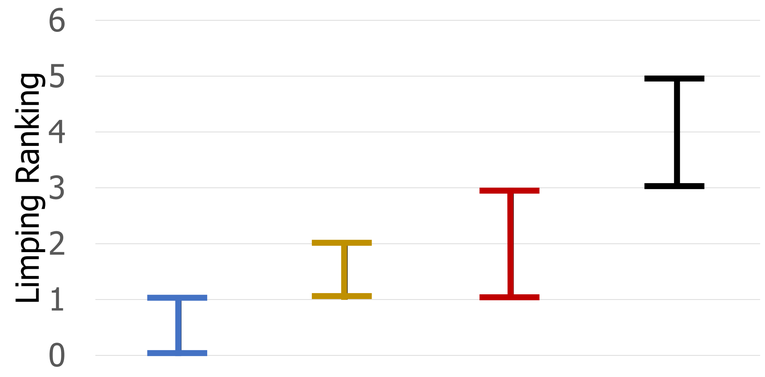
Fracture Toughness (tissue quality)



- Notched on anterior surface
- 3 point bending at 0.001 mm/sec
- Graded dehydration (70%-100%)
- SEM to determine angles of stable and unstable crack growth
- CT at fracture site to determine geometry
- Analysis of toughness at crack initiation, maximum load and crack instability

Results and Discussion

Limping Assessment



General Observation

TuFr Low: None to Mild

MoWeFr Low: Mild to Mild-Moderate

MoTuWe Low: Mild to Moderate

MoTuWe High: Moderate to Severe

Microcomputed Tomography (μCT)

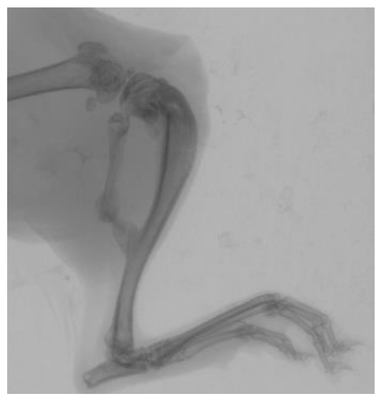
- No sign of injury in MoWeFr Low or TuFr Low

MoTuWe High

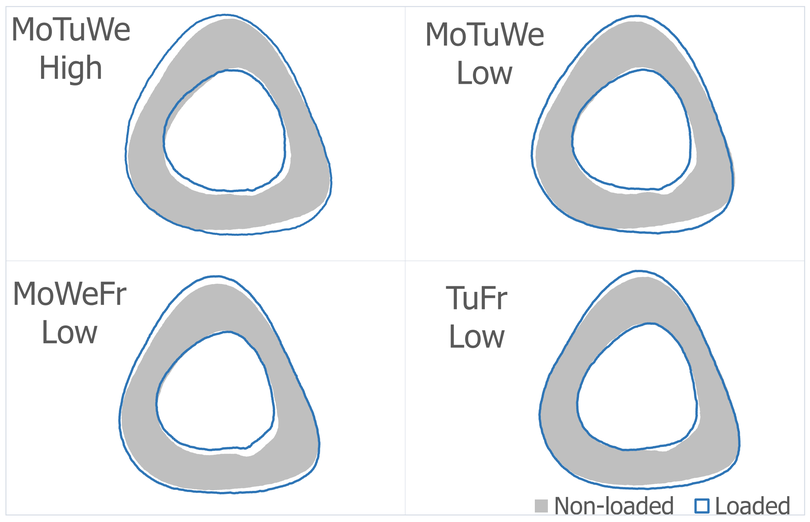
- Broken fibula and crushed metaphysis: 2 mice
- Damaged epiphysis: 2 mice

MoTuWe Low

- Broken fibula: 1 mouse
- Crushed proximal metaphysis: 2 mice
- Deformed epiphysis: 1 mouse



Example of damage, as seen in MoTuWe High and Low Groups



Cortical Analysis

Periosteal Expansion (all groups)

↑ Total Cross-Sectional Area
↑ Cortical Area
↑ Cortical Thickness
↑ Periosteal Bone Surface

No Endocortical Contraction

All four groups

MoWeFr Low and TuFr Low

↑ Tissue Mineral Density

Fracture Toughness

Crack Initiation

Trend toward ↑ in MoTuWe Low (p=0.07)

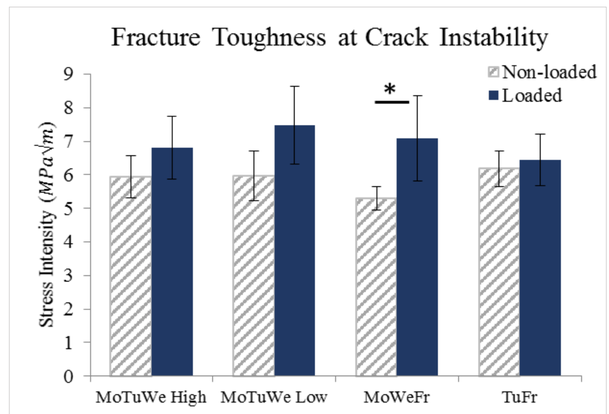
Maximum Load

↑ for MoTuWe Low (p<0.05)

Crack Instability

↑ for MoWeFr Low (p<0.05)

Trend toward ↑ in MoTuWe Low (p=0.06)



Conclusion

MoTuWe High	CT shadow scans clearly showed damage in the tibia and fibula; Little effect of loading on bone fracture toughness ⇒ ***Increased mass, but not quality***
MoTuWe Low	CT shadow scans clearly showed damage in the tibia and fibula; Increased fracture toughness ⇒ ***Both quantity and quality-based improvements***
MoWeFr Low	No damage; Increase fracture toughness ⇒ ***Both quantity and quality-based improvements***
TuFr Low	No damage; Increased cortical bone (mild effects); No effect on fracture toughness ⇒ ***Modest effect of loading***

1) Limping may prevent quality-based improvements even when bone mass is increased
2) The minimal pain and improved bone structure and fracture toughness observed in the MoWeFr Low group suggest that **loading on alternate days and holding at a low force level was best able to reduce pain while improving both bone quantity and quality**

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