In vivo mechanical loading in murine tibiae as a function of applied strain results in differential changes in microstructure and fatigue response

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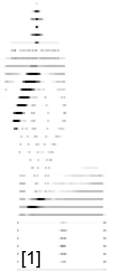
INTRODUCTION

- *In vivo* loading provides control over loading parameters such as magnitude, orientation, frequency, number of cycles, and rest time that are not controllable using exercise models.
- Few groups that use tibial loading report mechanical properties.
- Some studies have indicated that loading to moderate levels can induce joint damage [2]. Low strain levels may eliminate this issue while still inducing bone formation.

Exercise → Geometry / Bone Mass / Material Properties → Mechanics


[1]

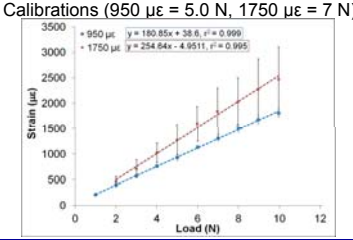
HYPOTHESIS

Loading to the higher of two strains, 1750 με, will engender a more robust cortical and trabecular response and better protect bone against fatigue than the lower strain, 950 με.

METHODS

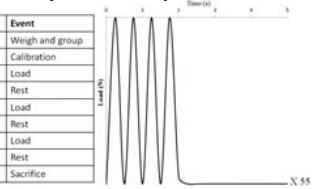
In Vivo Tibial Loading

- Calibrations (950 με = 5.0 N, 1750 με = 7 N)



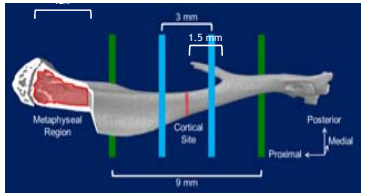
- 8 wk old, female, C57BL/6 mice
- Calcein injections on days 3 and 10

Day	Event
-1	Weigh and group
0	Calibration
1-3	Load
4	Rest
5-7	Load
8	Rest
9-11	Load
12-13	Rest
14	Sacrifice



Microcomputed Tomography (μCT)

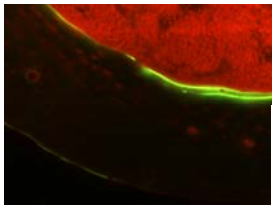
- 17 μm resolution, V = 60 kV, I = 167 μA
- HA calibration phantoms (0.25 and 0.75 g/cm^3 CaHA)
- Reconstructed, rotated, and batch processed with a grayscale value of 60
- Parameters evaluated at standard ROIs



Ex Vivo Fatigue

- 20,000 cycles, 2 Hz, 120 MPa tension (8 N)
- Load-unload curve recorded before and after fatigue (0.5 Hz, 8 N)
- Stained with basic fuchsin
- Embedded in PMMA
- Sectioned with a diamond-wire saw

Dynamic Histomorphometry



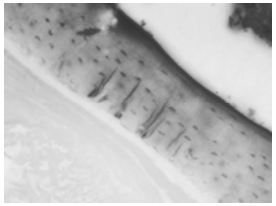
MS/BS = mineralizing surface

MAR = mineral apposition rate

BFR = bone formation rate

Parameters calculated following ASBMR standards [3].

Microdamage (1750 με)



Cr.N = number of cracks

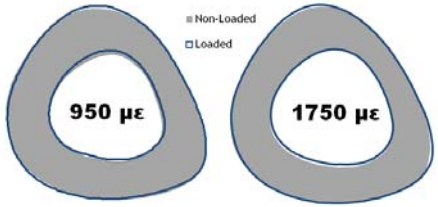
Cr.Le = length of cracks

Cr.D = Cr.N/Area

Cr.S.D = Cr.Le/Area

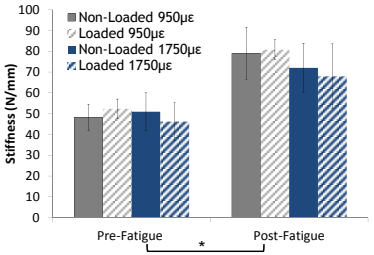
RESULTS

Microcomputed Tomography (μCT)



- Increase in cortical thickness ($p<0.05$) for both strain levels.
- Trabecular thickness increased ($p<0.05$) with loading in the 1750 με group.
- There was no change in trabecular number or BV/TV for either strain level.

Fatigue Analysis — Mean ± SD



Histomorphometry

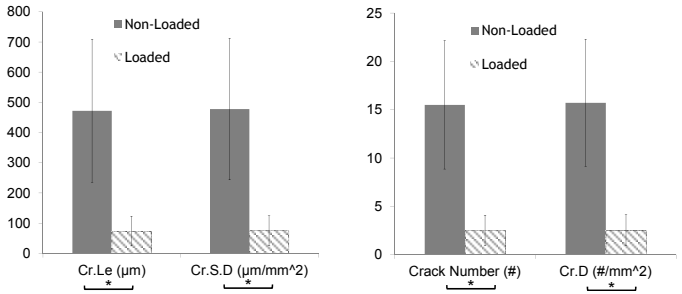
Periosteal Surface

- No significant effects of loading on histomorphometric parameters for either strain level.

Endocortical Surface

- No significant effects of loading on histomorphometric parameters for either strain level.

Microdamage — Mean ± SE — Mean ± SE



* indicates $p<0.05$

- Microdamage was not quantifiable in the 950 με groups.

CONCLUSIONS

- Both strain levels had an increase in cortical thickness. 1750 με increased trabecular number. Qualitatively, 1750 με engendered more bone growth than 950 με.
- Neither strain level was enough to protect bones against the mechanical property changes due to fatigue. Stiffness significantly increased with fatigue in all groups, indicating a possible fatigue-hardening effect.
- While there were few effects of loading on geometric properties, the reduced microdamage accrual indicates that some tissue-level change is occurring that is not translating to cortical or trabecular properties. *In vivo* loading protected bones from *ex vivo* damage accrual.

While loading to 1750 με mildly effects bone geometry and fatigue response, there was not a robust formation response. Future studies are being performed to investigate higher strain levels.

REFERENCES

[1] Main, R. P., et al. (2014). "Load-induced changes in bone stiffness and cancellous and cortical bone mass following tibial compression diminish with age in female mice." J Exp Biol **217**(Pt 10): 1775-1783.

[2] F. C. Ko, C. Dragomir, D. A. Plumb, S. R. Goldring, T. M. Wright, M. B. Goldring, *et al.*, "In vivo cyclic compression causes cartilage degeneration and subchondral bone changes in mouse tibiae," *Arthritis Rheum*, vol. 65, pp. 1569-78, Jun 2013.

[3] D. W. Dempster, J. E. Compston, M. K. Drezner, F. H. Glorieux, J. A. Kanis, H. Malluche, *et al.*, "Standardized nomenclature, symbols, and units for bone histomorphometry: A 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee," *Journal of Bone and Mineral Research*, vol. 28, pp. 2-17, 2013.