Combined Effects of Hind-Limb Suspension and Immobilization on Bone Fracture Mechanics

Rosalie Connell[1], Matthew Sanseverino[1], Jennifer Pierchia[1] Anthony G. Lau[1]

[1]The College of New Jersey, Department of Biomedical Engineering

Introduction

A mission to Mars is quickly becoming an obtainable goal by NASA. Scientists around the world are working to address not only how to get to Mars, but also how to make it possible for humans to safely make the journey and live on Mars. Microgravity environments, outside of Earth's gravitational field, however, raise concerns musculoskeletal health. This environment reduces the loads experienced by bone and muscle, which can lead to tissue degradation. Hind-limb suspension (HLS) and cast mediated immobilization (IMM) can be used to model disue effects of gravity on Earth [1]. These methods have proven to cause bone mineral density and muscle losses of up to 10% and 12% respectively [2][3]. Some studies have been able to find relationships between the tissue generation [4], but there is still a lack of understanding how these structural changes are related to the mechanical performance of the system, and therefore its overall health. This study related the combined effects of HLS and IMM on bone mechanical properties through an analysis of FEA and mechanical loading.

Methods

Study Design

All animal work was performed at Pennsylvania State University under their IACUC approvals. No live animal work is conducted at The College of New Jersey. Forty C57BL/6J male mice were randomly assigned to HLS (n=20) or control group (n=20) at age 20 week. Pre-treatment microCT scans were taken of hind-limbs. HLS mice were suspended for 14 days with their left leg casted throughout the period. At day 14, mice were euthanized and hind limbs were collected. Post-treatment microCT scans were then taken ex-vivo.

MicroCT, Image Processing, FEA Analysis

All microCT scans of the proximal tibia were taken at 10.5 µm resolution. Mimics 19 was used to process the scans to consistent sets of 75 slices. 3D models were generated to align corresponding pre and post treatment scans using anatomical landmarks (Figure 1). Only accurately matched pairs were further analyzed in Abaqus 6.9. A hexahedral 8-point voxel mesh was created for the 3D models; bones were models as isotropic and homogeneous with E = 10 GPa and v=0.3. Axial compressions were applied to the proximal face of the model to simulated 0.5% compression (Figure 1). The software collected the resultant force and calculated bone volume, structural efficiency and FEA stiffness for each bone.

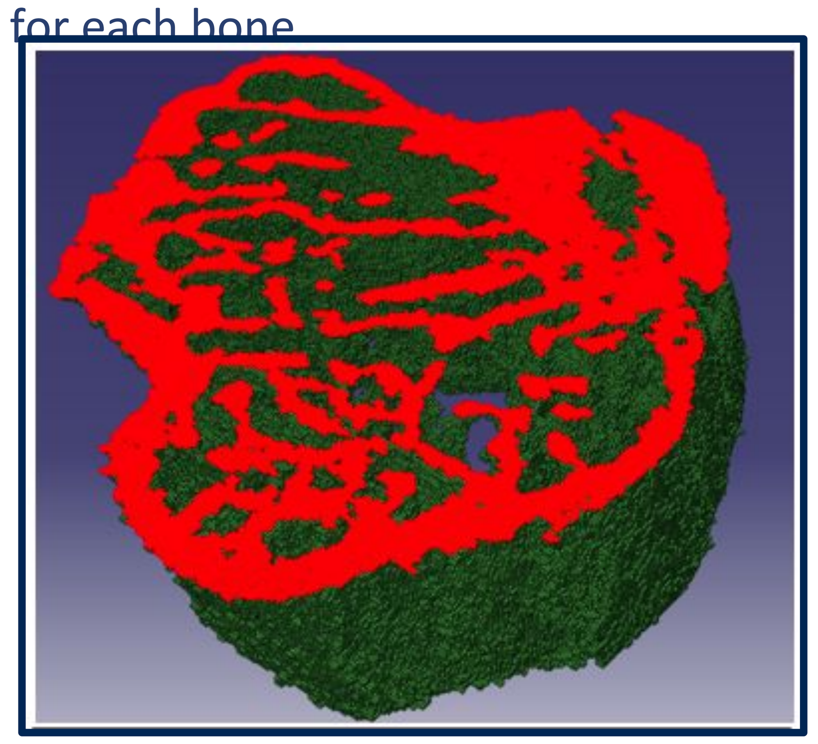


Figure 1: (Left):Pre-treatment (green) and post-treatment (yellow) alignment after 3D registration. (Right): FEA load simulation of 3D model of tibial. Area highlighted in red shows application of the force for finite element analysis.

Biomechanical Testing

Bones were brought to room temperature and rehydrated in saline solution 24 hours prior to mechanical testing. Femurs were loaded along the anterior shaft in three-point bending to fracture (Figure 2). The tests were conducted using a 10 mm span at a rate of 0.05 mm/s. An Instron mechanical testing frame was used to collected force and displacement data, which was then compiled to create force vs. deflection curves. A custom MATLAB script was used to analyze these curves to obtain mechanical strength values including: linear stiffness, max load, fracture load and energy to fracture.

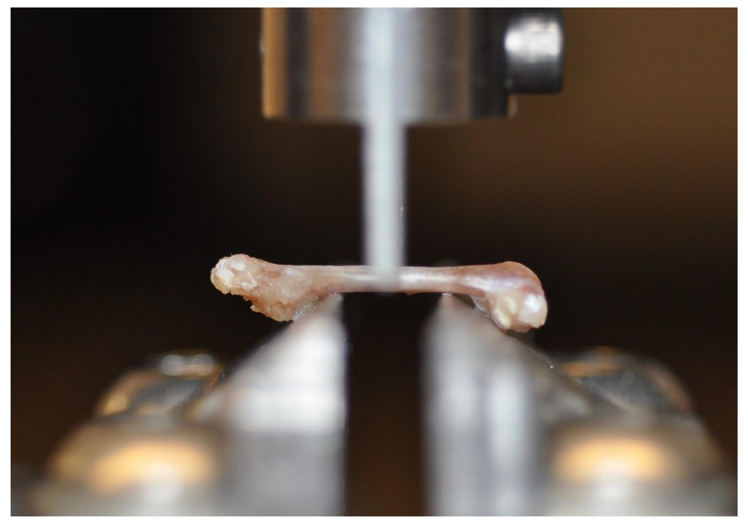


Figure 2: Three-point bending of mice specimen across a fixed, 10 mm span. Supports located at the beginning and end of the shaft to allow specimen to lie flat for loading duration. Load applied from the top by Instron at constant rate of 0.05 mm/s.

Statistical Analysis

Statistical significance for post-treatment FEA and mechanical values between treatment groups was determined by a one way ANOVA and post-hoc Tukey test using Origin Pro 2015 (64-bit). Regression analysis was also conducted to assess correlations for mechanical strength data to FEA analysis. Alpha values of 0.05 was used for all statistical analysis. Data is presented as means ± SD.

Results

FEA and Mechanical Analysis

Significant differences were evaluated for percent difference between pre and post scans for all treatment groups. Post-hoc Tukey tests showed no significant difference however between HLS and HLS+IMM groups.

Post-treatment FEA data was used as a terminal surrogate to be analyzed directly with mechanical data of corresponding specimen. Individual parameter analysis indicated significant differences between treatment groups for FEA stiffness, maximum load, and mechanical stiffness. Post-hoc analysis showed significant differences only with respect to control.

Regression Analysis

There were several general trends present amongst the correlations. FEA stiffness was most strongly correlated to mechanical strength on average, as opposed to structural efficiency, which was most weakly correlated. The only significant correlation between mechanical and FEA values was maximum load. Ground control mechanical parameters were most strongly correlated to their FEA values. HLS and HLS+IMM treatment groups showed some instances of negative correlations between parameters, however these trends were only observed within specific treatment groups and not on average. Lastly, ground control had the largest range of FEA and mechanical values, while treatment groups tended to cluster within this specific range toward the lower end (Figure 3).

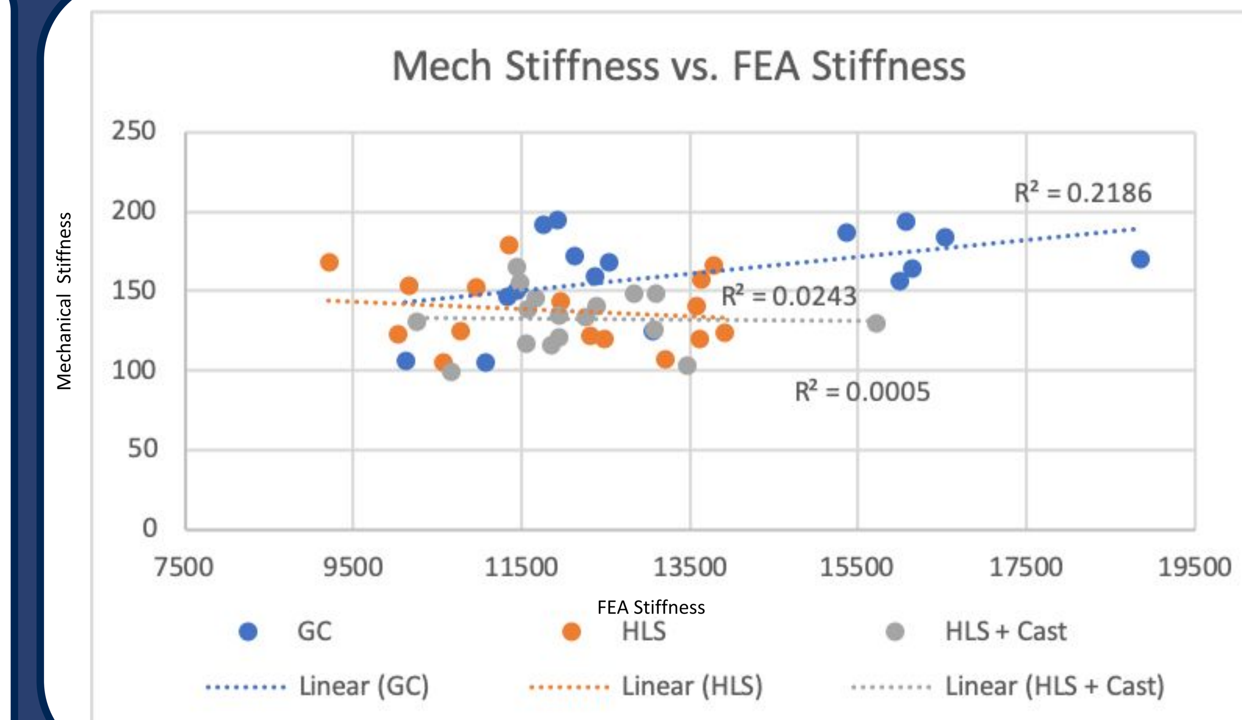


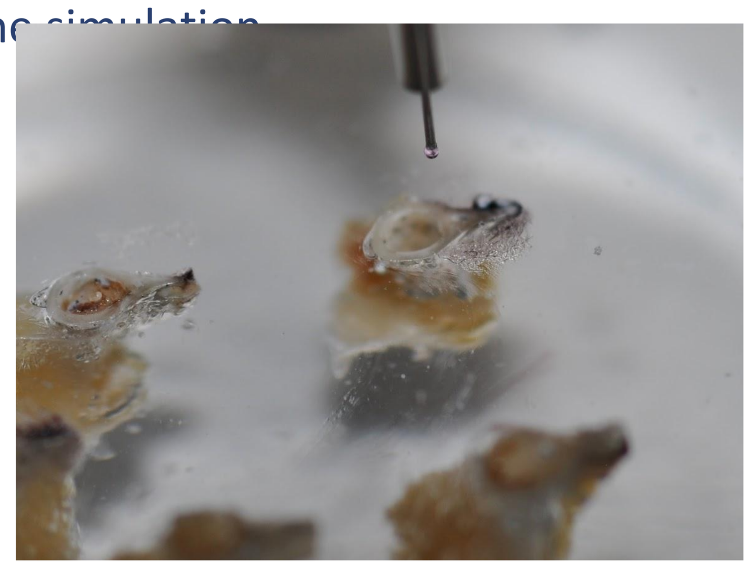
Figure 3: Regression analysis correlating FEA stiffness to mechanical stiffness. Non-significant, negative trends clustered within ground control (blue) seen for both HLS (orange) and HLS+IMM (gray) treatment groups.

Discussion

This study extended the work of previously conducted studies in an attempt to further understand the mechanical implications of microgravity environments present in space. It was the first to analyze the combined mechanical effects from HLS and IMM and to attempt to correlate both mechanical and simulated strength values. While preliminary work only detected minimal differences in FEA values, several significant changes were identified after the full cohort was analyzed, through increased statistical power from both pre and post scans. Additionally, comparing post-treatment scans to mechanical values further increased statistical power and identified correlations between the obtained values. Low strength for these correlations may be contributed to differences in loading methods and homogeneity assumptions made within the simulation.

Future material testing (Figure 4) will be conducted to better assess material changes as they related to overall strength of the bone.

Figure 4: Microindentation method to be used to assess material changes in mechanical tested bone.



Conclusion

The effects of microgravity, as modeled through disuse, shows significant declines in mechanical values, measured through FEA simulations and mechanical testing. These results can be weakly correlated to each other. Future analysis is to be conducted.

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