Effects of Combined Hind Limb Suspension and sRANKL on Bone Strength in Mice by Finite Element Analysis

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Abstract:

Crash tests performed in injury biomechanics research often involves elderly cadavers. As the elderly population often presents osteoporosis, it is critical to understand the associated reduction in bone strength with aging. Additionally, prolonged bed rest associated with hospitalization has been found cause severe bone loss. Mice injected with sRANKL have been used as an animal model for postmenopausal osteoporotic bone loss in humans. Also, Hind Limb Suspension (HLS) of mice is an established model for disuse. This study quantitatively compares bone strength in mouse models treated with sRANKL and/or HLS through high-resolution microCT imaging and FE Modeling.

Forty male, 16-week ± 3 day old, C57BL/6J mice were used in the study and were assigned to 4 treatment groups: HLS/PBS (n=3), HLS/RANKL (n=9), GC/RANKL (n=7), and GC/PBS (n=5). Mice received an intraperitoneal injection of either phosphate buffered saline (PBS) or human recombinant sRANKL (1 mg/kg body weight/day), for a total of three injections and HLS was maintained for 14 days. MicroCT scans were performed pre-treatment (day 0) (in vivo), and after tissue excision (day 14) (ex vivo). Image data was exported for 3D image processing to segment the bone regions, register and align the pre and post scans, and create a FE mesh of the proximal tibia region. Simulated axial compression was performed on the FE model and the volume, stiffness, and structural efficiency was calculated, which allows for a quantitative comparison between the effects of HLS and RANKL induced osteoporosis. Differences were assessed between groups using a two-way ANOVA.

HLS caused statistically significant decreases in bone volume ($p<0.05$), stiffness ($p<0.05$), and structural efficiency ($p<0.05$). RANKL did not cause statistically significant changes in bone volume ($p=0.056$), stiffness ($p=0.170$), or structural efficiency ($p=0.861$). Changes in microstructure arise from a variety of chemical and physical stresses. As bone is consistantly remodeling, decreases in load cause a loss of bone density. The present study provides information on bone strength changes beyond density. Though RANKL did not produce a statistically significant change in bone volume, stiffness, or structural efficiency, the changes in cortical bone and loss of trabecular bone were visible image processing. The statistically insignificant change due to RANKL may be attributed to low sample size. Sample size was limited since registration/rotation reduced the number of slices available in several models. A decrease in model size will allow for increase sample size as well as statistical power for the data set. However, this also reduces the region of analysis.

In conclusion, a novel approach was used in order to assess bone strength changes *in vivo* with pre-treatment and terminal microCT scans combined with FEA for animals treated with hindlimb suspension and sRANKL. HLS yields significantly greater reductions in bone strength in comparison to RANKL treatment. In order increase the number of viable specimens for modeling, a new set of smaller models are currently being processed. After those models are processed, models of the femurs will be analyzed.