MECHANICAL LOADING CAUSES AN ACUTE AND TEMPORARY DECREASE IN THE STIFFNESS OF MOUSE TIBIAE

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INTRODUCTION

Bone is a dynamic tissue that adapts to best resist its loading environment by remodeling, the process by which micro-damage is repaired and micro and macro structure may be altered. Animal models have been valuable tools for understanding this process and have been studied with the long-range goal of developing mechanical loading-based interventions to improve bone strength in aging humans.

Our previous work with a human model of bone adaptation has shown that young women experience an acute and temporary decrease in bone mineral content (BMC) approximately three months after initiating a regular novel mechanical loading regime [1]. We believe this may be a reflection of increased remodeling space within the bone. The degree to which bone strength may be decreased due to this change in BMC is unknown. The purpose of this study is to quantify the changes in BMC and mechanical stiffness during the initial remodeling phase in a mouse tibia loading model. Our hypothesis is that, similar to humans, mice will show a temporary decrease in BMC. Along with this decrease in BMC, we also hypothesize that a decrease in mechanical stiffness and strength of the bone will occur.

METHODS

With protocols approved by the Institutional Animal Care and Use Committee, 26 female C57BL/6J mice (age: 18 weeks) were divided into five loading groups: 3-day (6 mice), 7-day (4 mice), 10-day (6 mice), 14-day (6 mice) and 21-day (4 mice). Prior to any mechanical loading and after sacrifice, both tibiae were scanned using a small animal DEXA (pDXA, Norland Stratec, Ft. Atkinson, WI) to measure the BMC. Prior to the start of the experiment, a strain gage was affixed to the mid-diaphysis of the tibiae of two euthanized mice and used to identify the force required to produce surface strains of 1000-1200με. Based on the strain gage data a peak compressive load of 8N was chosen.

A custom fabricated loading apparatus was used to axially compress the left tibia of each mouse for 50 cycles a day at 0.5Hz (Figure 1), three days per week (Monday, Wednesday, Friday) for the respective number of days. For example, the 10-day group was loaded on Mon/Weds/Fri/Mon and sacrificed on the following Wednesday. Based on previous data indicating that a rest period between loading cycles can be osteogenic [2], a 15 second rest period was inserted between each loading cycle. The right tibia acted as a within-subject control.

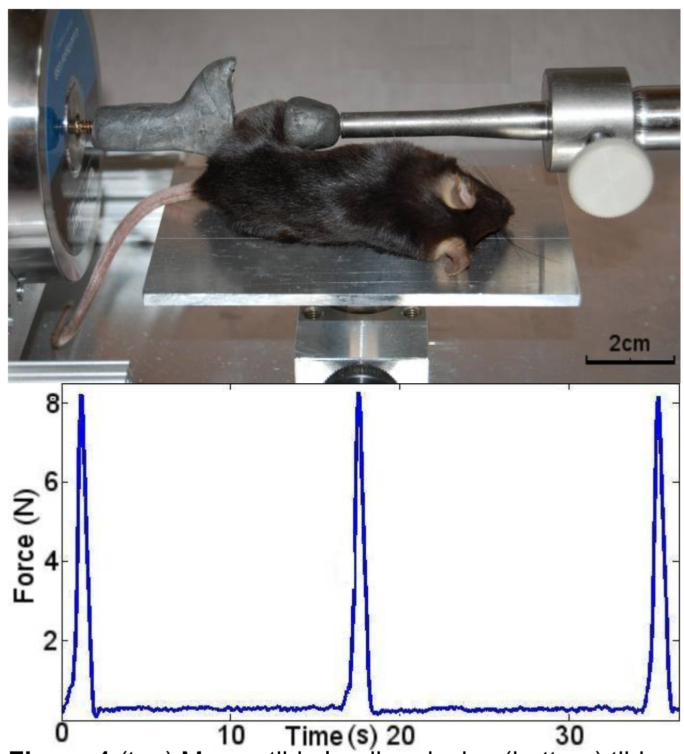

Figure 1 (top) Mouse tibia loading device (bottom) tibia loading wave form, recorded during an experimental trial.

After sacrifice, the loaded and the contra-lateral tibiae were dissected out for mechanical testing. Whole-bone stiffness was measured in axial compression by applying a ramped load up to 12 N at about 400 με/s. Stiffness was calculated as the average slope of 6 cycles. Tibiae from one mouse per group were reserved for histological analysis and the remaining bones were tested to failure at the same strain rate to determine the yield and ultimate force.

Planned comparisons included paired t-tests for post-loading BMC and stiffness (contra-lateral versus loaded) for each group and single sample t-tests of the percent change in BMC from pre-to post-loading for each group.

RESULTS AND DISCUSSION

The loaded limb of the 10-day group showed a 6.2% decrease in BMC compared to the contra-lateral limb (Table 1). This difference disappeared at 14 days and by 21 days the loaded limb's BMC was 3.8% larger than the contra-lateral limb.

The 3-day group's loaded limb stiffness was decreased 13.5% compared to the contra-lateral limb (Table 1). This difference disappeared by 10 days and at 21 days there was a trend towards the loaded limb being stiffer than the contra-lateral limb. Pre/post test comparisons for percent change BMC showed the same results, with a significant 6.5% decrease in BMC (p=0.007) in the loaded limb at 10 days.

Table 1: Mean (SD) of BMC and Stiffness of left and right tibiae of 3 day, 7 day, 10 day, 14 day and 21 day groups. L=loaded limb, R=contra-lateral limb

Group	BMC (mg)	Stiffness (N/mm)
3 day	**L** 26.8 (3.21) **R** 26.4 (1.39) p= 0.822	**L** 54.4 (4.7) **R** 63.8(4.3) p=0.003
7 day	**L** 21.67 (2.26) **R** 21.67 (1.53) p= 1.0	**L** 56.4 (9.9) **R** 60.8 (8.9) p=0.454
10 day	**L** 25.53 (1.51) **R** 27.22 (0.25) p= 0.035	**L** 58.3 (8.7) **R** 52.4 (10) p=0.051
14 day	**L** 25.28 (2.91) **R** 26.36 (1.82) p= 0.263	**L** 53 (8.9) **R** 52.5 (9.5) p=0.576
21 day	**L** 27.00 (1.83) **R** 26.00 (1.63) p= 0.092	**L** 54.8 (11.9) **R** 42.9 (4.3) p=0.276

The data support our hypotheses that both BMC and stiffness transiently decrease in response to mechanical loading. Interestingly, the decrease in stiffness preceded any detectable change in BMC (Figure 2). It is possible that the initial decrease in stiffness results from micro-damage to the bone (we would not expect the strains imposed here to cause gross damage). The micro-damage initiates a remodeling response that results in removal of damaged bone and apposition on the bone surfaces. Initially, this manifests as a decrease in BMC as bone is removed. Longer-term loading results in increased BMC and probably in geometrical changes in bone structure.

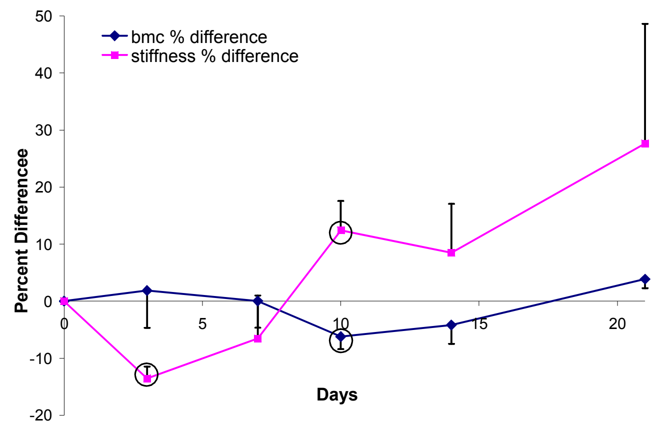

Figure 2: Percent difference in BMC and stiffness, between loaded and contra-lateral tibiae with respect to time. Error bars show standard error. 'O' indicates p<0.06. Zero percent difference is assumed at day zero.

At 6 weeks other groups have shown increases in BMC and in maximum cross sectional moment of inertia (I_{max}) [3]. The increase in I_{max} reflects increased strength. Though not statistically significant, the 21-day group shows a trend towards increased BMC and stiffness of the loaded limb. An increase in the number of mice in the group may result in statistical significance. Decreased stiffness may correspond to a decrease in failure strength of the bone. The decrease in BMC and stiffness in mice due to loading in the short term may have serious implications on fracture risks. However, the degree to which this phenomenon puts humans at risk for fracture after commencing a novel loading regime is yet to be determined.

REFERENCES

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