Trabecular bone response to mechanical loading depends on trabecular type and is amplified at the cortical-trabecular bone junctions

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INTRODUCTION: Mechanical loading, or exercise, is an effective option for managing osteoporosis and reducing risks for fragility fractures as it has been shown to improve measures of bone strength. The *in vivo* murine axial tibial compression model is widely used to study bone mechanoadaptation, but the mechanisms for how bone responds at the microstructural level are still unclear. Studies have shown that in the trabecular bone under axial loading, the loading is primarily supported by axial plates and stabilized by transverse rods. In long bones, it is known that the mechanical function of trabecular bone is to transfer the load to the cortical bone. However, how this process occurs at the individual trabecular level and how the bone at the trabecular-cortical bone junctions respond to mechanical loading has yet to be established. To determine the role of individual trabecula under mechanical loading, we apply *in vivo* axial tibial compression to skeletally mature wild-type (WT) mice. Using *in vivo* µCT-based dynamic bone histomorphometry, individual trabecula segmentation (ITS), and finite element (FE) analysis, we quantify bone formation and resorption events in individual trabeculae at the trabecular-cortical bone junctions and analyze the microstructural strain.

METHODS: This study was approved by IACUC. WT C57BL/6J female mice (n = 11, JAX #000664) underwent weekly *in vivo* µCT scanning (vivaCT 80, SCANCO Medical) of both tibiae. Tibiae were scanned the same day and prior to the start of mechanical loading and then weekly until the end of the experiment. µCT images were reconstructed at 5 µm isotropic voxel size. At 16 weeks old, *in vivo* axial tibial compression (Bose ElectroForce, TA Instruments) was applied unilaterally to the right limb (loaded) and the contralateral non-loaded limb served as an internal control (control). The loading waveform was a haversine waveform between -1 N and -9 N at 2 Hz for 100 cycles, applied 5 consecutive days per week for 2 weeks. Animals were euthanized after the final µCT scan, 3 days after the final bout of tibial loading. The trabecular bone volume of interest (VOI) was 1.0 mm longitudinal length, beginning 0.15 mm distal to the proximal tibia growth plate. Weekly µCT images were then registered to the same position and instances of bone formation and resorption were then quantified by *in vivo* µCT-based dynamic bone histomorphometry. ITS labeled individual trabeculae by type (plates or rods) and orientation (axial, oblique, or transverse). For a subset of the animals (n = 6), ITS was used to label trabeculae that were connected to the cortical shell (junction trabecula) or not connected (inner trabecula). FE models were constructed from ITS-labeled µCT images, including the cortical shell, by converting each voxel to an eight-node brick element. The bone was modeled as an isotropic, linear elastic material with a Young's modulus of 15 GPa and a Poisson's ratio of 0.3. A 9 N distributed compressive load was applied and the peak principal compressive and tensile strains were computed (Abaqus, Dassault Systèmes Simulia). Data are presented as mean ± standard deviation. Statistical analyses were performed using paired t-tests to compare bone volume changes within groups, and a one-sample t-test was used to compare if the ratio between normalized loaded and normalized control bone volume changes was different from 1.0.

RESULTS SECTION: Mechanical loading significantly increased bone formation in trabecular plates of all orientations (axial, oblique, and transverse), and there was a trend for increased bone formation in transverse rods (Fig. 1A). Loading significantly decreased bone resorption in axial plates and axial rods (Fig. 1B). The magnitude of bone formation and resorption were greatest in axial plates and in transverse rods (Fig. 1A & 1B). Bone formation was increased in the loaded junction and inner trabeculae, with a greater effect in junction trabeculae (Fig. 1C). Bone resorption decreased under mechanical loading in the inner trabecula and there was a trend for decreased bone resorption in the junction trabecula (Fig. 1C). The average peak principal compressive strains were greater in inner trabeculae compared to junction trabeculae, while there was a trend for the average peak principal tensile strains being greater in the junction trabeculae (Fig. 1D).

DISCUSSION: With *in vivo* µCT-based dynamic bone histomorphometry, ITS, and FE analysis, we can quantify bone volume changes in individual trabeculae and the micromechanical environment. We find that over 2 weeks of mechanical loading, most of the bone formed and resorbed occurred on axial plates. Transverse rods showed the greatest bone formation and resorption, with mechanical loading decreasing resorption in axial rods and a trend for increasing formation in transverse rods. These results align with previous studies demonstrating that axial plates bear the load while transverse rods stabilize the structure. However, future work will include quantifying the micromechanical environment of the individual plates and rods under mechanical loading. We find that mechanical loading increases bone formation in junction and inner trabeculae; bone formation in junction trabeculae was increased to a greater degree. Though the junction trabeculae may be under greater average tensile strain than inner trabeculae, further analysis is needed. Future work will include utilizing sample-specific whole tibia FE models and quantifying the specific mechanical environment that formed and resorbed bone voxels are under at the individual trabecula level.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides insight into how the trabecular bone microarchitecture and individual trabeculae at the cortical-trabecular bone junctions respond to mechanical loading. It demonstrates that mechanical loading targets individual trabeculae at the cortical and trabecular bone interface. Additionally, the technique used in this study can map adaptations in bone volume to the corresponding micromechanical environment and the local cellular changes, which will be critical for understanding how bone adapts to exercise and/or pharmaceutical treatments.

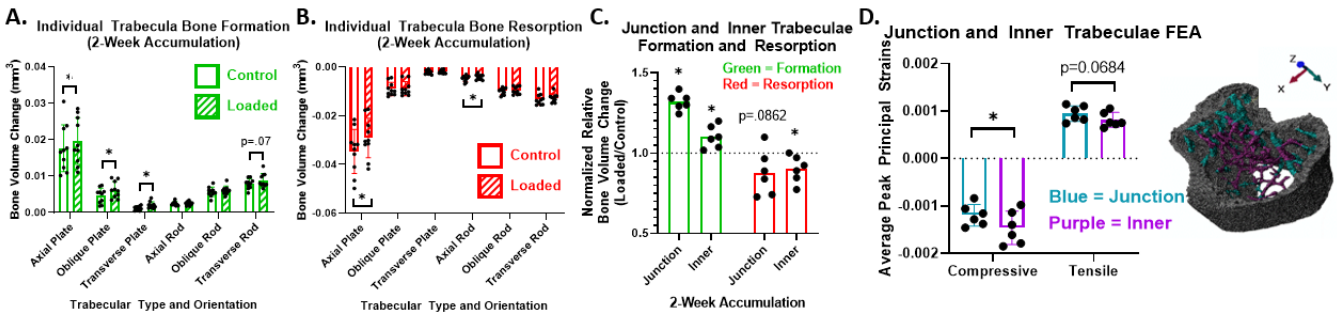

Figure 1. (A) Quantification of bone formation and (B) bone resorption in individual trabecular plates and rods in each orientation comparing control to loaded tibiae. (C) Quantification of bone formation and resorption in junction and inner trabeculae. (D) FE analysis comparing average peak principal strains in junction and inner trabeculae. * = P < 0.05. Vertical bars represent mean ± SD.