

# Age-based differences in musculoskeletal adaptation to unloading in mice undergoing alendronate treatment

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**INTRODUCTION:** Mechanical unloading (disuse) leads to reductions in bone and muscle mass. During disuse, osteoclasts break down bone tissue, releasing osteokines such as transforming growth factor beta (TGF- $\beta$ ) that may contribute to muscle atrophy through different signaling pathways. However, the effects of osteokine release on muscle atrophy during periods of mechanical unloading, and how these effects changes throughout the lifespan have not been assessed. This study aimed to determine how mechanical unloading and anti-resorptive bisphosphonate treatment affect bone and muscle structure and function in young, middle-aged and old mice. We hypothesized that unloading would cause considerable bone loss in all mice, but that this loss would be prevented by bisphosphonate treatments in all age groups. Additionally, we expected to see a reduction in muscle mass and contraction force with unloading, but that these reductions would be partially mitigated by bisphosphonate treatment due to decreased release of osteokines.

**METHODS:** This study used young (3-month, n = 40), middle-aged (12-month, n=40), and old (20-month, n=40) male C57BL/6J mice. Mice received biweekly subcutaneous injections of alendronate (0.03 mg/mouse in phosphate buffer solution (PBS)) or PBS control injections starting one week prior to unloading. Mice underwent hindlimb unloading via tail suspension for 14 days. Maximum contraction force of the triceps surae (gastrocnemius and soleus muscle combined) and tibialis anterior were measured with a foot plate and electrical stimulation after 14 days of unloading, after which mice were euthanized and the triceps surae and tibialis anterior were removed and weighed. Femurs were imaged with micro-computed tomography ( $\mu$ CT 35, SCANCO Medical AG) with 6  $\mu$ m nominal voxel size; cortical bone was analyzed at the mid-diaphysis and trabecular bone was analyzed at the distal femur to determine bone microstructural outcomes.

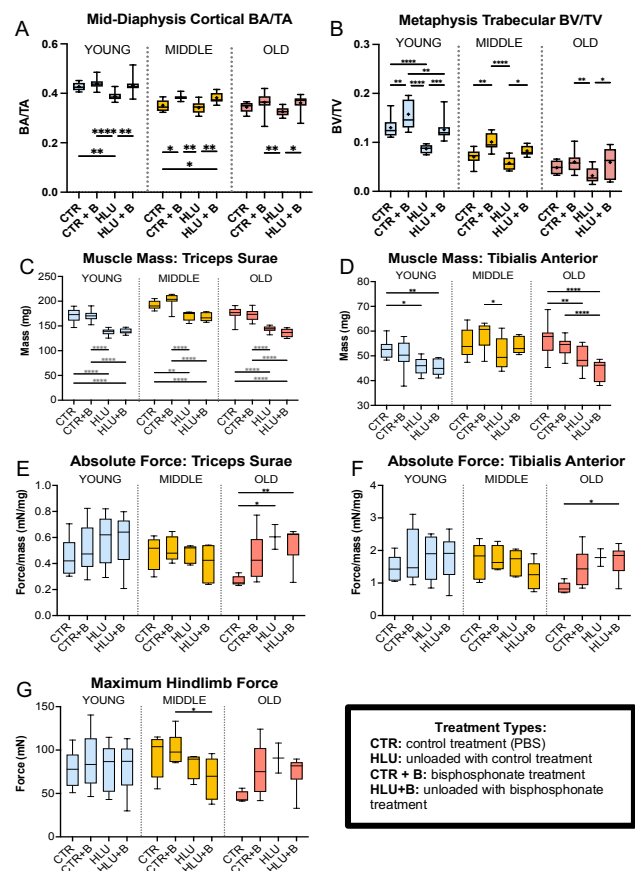
## RESULTS:

- Young mice had higher baseline cortical bone area than middle-aged or old mice (Fig 1A). BA/TA of the mid-diaphysis was reduced in young mice after HLU and maintained in mice undergoing treatment. BA/TA was not reduced in middle-aged mice or old mice, and bisphosphonate treatment further increased this percentage (Fig. 1A).
- Young mice had higher baseline trabecular bone volume than middle-aged and old mice (Fig. 1B). BV/TV of the distal metaphysis was reduced in young mice after HLU, with similar trends in middle-aged and old mice. BV/TV was maintained in all animals undergoing HLU with bisphosphonate treatments (Fig. 1B).
- Muscle mass of the triceps surae was reduced during HLU for all age groups and was not improved with alendronate treatment (Fig. 1C).
- Muscle mass of the tibialis anterior was reduced under HLU conditions for young mice and was not improved with treatment (Fig. 1D). Muscle mass of the tibialis anterior in middle-aged mice did not change with HLU or treatment (Fig. 1D). Muscle mass of the tibialis anterior was reduced with HLU and bisphosphonate treatment in old mice (Fig 1D).
- Absolute force of the triceps surae and tibialis anterior were not significantly different after HLU or alendronate treatment in young and middle-aged mice (Fig. 1E & 1F). Contraction forces increased with HLU and treatment in old mice (Fig. 1E & 1F).
- Maximum hindlimb force was unchanged in young mice, while middle-aged mice force reduced in HLU mice undergoing treatment (Fig 1G). Old mice Maximum hindlimb force trended towards increasing with treatment and under HLU (Fig. 1G).

**DISCUSSION:** These data establish age-related differences in bone atrophy during unloading and in response to bisphosphonate treatment. Trabecular and cortical bone volume were preserved during HLU with alendronate treatment as expected, and differences in bone adaptation between young and middle-aged/old mice highlight changes in bone remodeling across the lifespan. Additional data collection and analysis will establish specific mechanisms of these changes. While alendronate treatment was able to effectively maintain bone volume, changes in muscle mass and contraction force during HLU were generally not affected by alendronate treatment.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Bone and muscle adaptation to unloading are different across the lifespan, as are the effects of bisphosphonate treatments. The characterization of these changes and quantification of bone-muscle crosstalk are essential for understanding clinical outcomes related to periods of disuse and clinical bone and muscle-preserving treatments. Future research into these factors is needed to inform treatment strategies aimed at preserving musculoskeletal health and improving recovery from bedrest, wheelchair usage, or even spaceflight across different age groups.

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**Figure 1.** Bone and muscle changes in response to bisphosphonate treatment (+B) and HLU. \*:  $p < 0.05$ ; \*\*:  $p < 0.001$ ; \*\*\*\*:  $p < 0.0001$