

## Eccentric Muscle Contraction Increases Trabecular and Cortical Bone Volume in Aged Mice

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**INTRODUCTION:** Osteosarcopenia is a geriatric syndrome characterized by the combined loss of bone density and strength (osteoporosis), and the decline in skeletal muscle mass and function (sarcopenia). During aging, sarcopenia and osteoporosis progress in a simultaneous and compounding manner, significantly increasing mortality and morbidity rates. In healthy individuals, bones respond to the mechanical loading from muscular contractions through a process known as mechanotransduction. During this process, mechanosensors within the bone (osteocytes) convert mechanical signals into biochemical signals, triggering intracellular pathways that promote osteogenesis. However, inactivity or disuse among the elderly leads to musculoskeletal unloading, which diminishes the transmission of mechanical signals across the muscle-bone unit, further decreasing anabolic potential. Consequently, considerable attention has been directed towards mechanical loading as a therapeutic intervention for osteosarcopenia. Resistance exercise is well-documented to increase muscle and bone mass in the elderly, but the type of muscle contraction required to produce optimal bone gain has yet to be explored. Neuromuscular electrical stimulation (NMES) protocols have been implemented in intensive care units, aging populations, and bedridden patients. Results from these studies reported increases in muscle strength, thigh circumference, and functional capacity, making it an effective therapeutic intervention for physical therapists. However, stimulation protocols typically mimic aerobic/endurance training due to the limitations imposed by painful maximal contractions, resulting in a gap in protocols and evidence on resistance training through NMES. To address this gap, we developed a resistance training program targeting a single hindlimb enabling contralateral comparisons within the same animal. This protocol also allowed for comparisons of maximal isometric (no change in muscle length), eccentric (lengthening contraction), and a combination of both types of muscle contraction needed for optimal bone gain. The goal of this study is to determine if isometric, eccentric or a combination of both types of muscle contraction produce optimal bone gain. Since eccentric muscle contractions have been shown to reduce bone loss in young adult rats following hindlimb suspension, we posit that eccentric contractions may also benefit aging bones. Specifically, we hypothesize that eccentric muscle contraction promotes greater bone accrual in aging compared to isometric contraction.

**METHODS:** C57Bl/6 male mice aged 6 and 20 months were obtained from the National Institute on Aging rodent colony. Only male mice were used due to the higher propensity of fractured tibias in female mice from sciatic nerve-stimulated muscle contractions. Each cage housed 4 mice and were stratified to 4 groups: 1) Isometric (Iso), 2) Eccentric (ECC), and Isometric+Eccentric (Iso+ECC) contractions, and 4) Non-stimulated (NS) cage controls exposed to equal amounts of isoflurane inhalation. Mice (n=9/age/group) were unilaterally stimulated using needle electrodes targeting the sciatic nerve, while the foot was taped to the foot pedal of a 300C dual-mode servomotor transducer (Aurora Scientific), with a fixed knee joint. Each stimulation was induced for 800 ms pulse duration with a frequency of 150 Hz and were repeated 4 times with 12 seconds rest in between each stimulation (1 set). Iso contraction was performed with a 90°-foot angle (neutral position) throughout the stimulation and ECC with 200 ms at 90°, 300 ms at 70° and 300 ms at 110°-foot angle (Figure 1). Each training session consisted of 4 sets of 5 stimulations with 3 minutes rest in between each set. Iso+ECC animals performed 2 sets of Iso and 2 sets of ECC contractions. Animals were trained for 3 sessions a week for 4 weeks, along with muscle peak torque measurements before and after training sessions. Animals were allowed to recover for 1 week after the last training session and hindlimb muscles and bones were harvested 10 days after the last session.

**RESULTS:** Body weight remained stable in young mice, whereas old mice showed progressive weight loss, consistent with osteosarcopenia. These findings indicate that stimulation effects were localized to the stimulated limb. Significant ( $p < 0.05$ ) decreases in dorsiflexion peak torque (-22%) were observed in young mice, and muscle mass (TA, EDL & soleus) decreased in all mice subjected to ECC, suggesting ECC-induced muscle damage. While all stimulated tibias had increased cortical thickness relative to contralateral unstimulated tibias, a divergent trabecular bone phenotype was observed between young and old mice. Young mice that performed Iso muscle contraction showed significant increases in tibial trabecular bone volume fraction (Tb. BV/TV: +32%) and thickness (Tb. Th: +12%) when compared to contralateral tibia. Intriguingly, old mice exhibited significant increases in tibial Tb. BV/TV (+53%) and Tb. Th (+22%) only after ECC muscle contraction compared with contralateral unstimulated tibia.

**DISCUSSION:** Eccentric muscle contraction produced a larger osteogenic effect in old mice, whereas isometric muscle contraction was more beneficial for young mice. We will further characterize the microstructural changes within the muscle (fiber type and cross-sectional area) and bone (dynamic histomorphometry). Importantly, the age-dependent differences in optimal muscle contraction type for bone gain will be explored using omics approaches in future studies.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Traditional methods, such as wheel running and treadmill training lack sufficient control to minimize inter-animal variability. This study utilized a novel NMES protocol involving unilateral sciatic nerve stimulation to simulate resistance training in mice, while allowing for within-animal comparisons between the stimulated and contralateral non-stimulated hindlimbs.

**ACKNOWLEDGEMENTS:** HJK is supported by NIH-NIA K00AG068438.

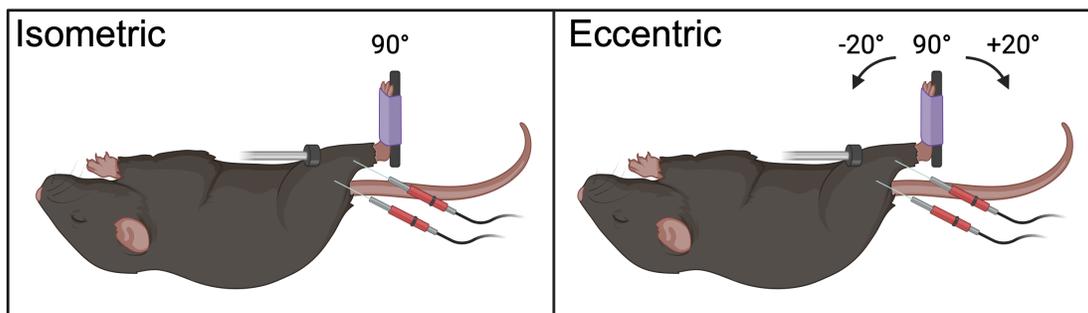


Figure 1: Illustration of NMES-induced isometric and eccentric muscle contractions used in this study. Mouse hindlimb were fixed with the knees clamped and the foot taped to a foot-pedal force transducer (Aurora 3-in-1 rig). *Left:* The foot was held in a neutral position (90°) during isometric contraction. *Right:* The foot pedal automatically moved through a 20° range of motion toward and away from the mouse, lengthening both the anterior and posterior hindlimb muscles to produce eccentric contraction.