# Ischemic Stroke Inhibits Exercise-Induced Bone Gains in the Distal Femur

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**INTRODUCTION:** Stroke sufferers fall more and experience hip fractures 2-4 times more frequently than with typical aging[1], yet little is known about underlying factors contributing to the rapid bone loss. Vascular function is vital for bone maintenance and is known to decline in the forearm following stroke[2,3] and thus may contribute to stroke-related bone loss. Because exercise stimulates vascular growth and can decrease fracture risk by improving balance, musculoskeletal strength, and bone turnover[4,5], it may help mitigate bone loss post-stroke. We hypothesized that stroke negatively impacts both bone microstructure and osteovasculature, and that exercise therapy during acute recovery can at least partially offset these effects.

**METHODS:** Under a protocol approved by the IACUC at North Carolina State University, 12-week-old, male C57Bl6/J mice received either a stroke (n=15) or sham (n=12) surgery. We induced ischemic stroke by inserting a thin silicone-coated filament into the middle cerebral artery (MCA), occluding blood flow for 30 minutes, and then removing the filament to allow reperfusion[6], closely mimicking the most common stroke conditions experienced by human patients. In the sham surgery, surgical incisions were made and left open for 30 minutes but the MCA was not occluded. Mice were further divided into exercise and sedentary groups after 4 days of recovery. Exercise mice (n=6 'sham ex', n=8 'stroke ex') performed treadmill exercise therapy (9 m/min, 37 min, 5 days/wk, 5° incline) every weekday, while sedentary mice were placed on a stationary treadmill for a matched time period (n=6 'sham sed', n=7 'stroke sed'). Tibial blood flow was monitored *in vivo* weekly during recovery using laser Doppler flowmetry (LDF)[7,8]. After 4 weeks of recovery, mice were sacrificed, and hindlimb bones were removed. Vascular microstructure was measured in the distal femoral metaphysis using contrast-enhanced, nano-computed tomography (nano-CT, 2 µm voxels) that allows for the visualization of vasculature within bone[9]. Bone microstructure was measured in the distal femoral metaphysis with micro-CT (10 µm voxels). Group differences were assessed using a mixed model, repeated measures ANOVA and two-way ANOVAs with Tukey's post-hoc comparisons (α=0.05, R Statistical Computing).

**RESULTS:** Compared with sham, stroke mice had increased blood vessel density (p=0.040) in the distal femur of the paretic limb four weeks after stroke (Figure 1). Exercise therapy increased bone microstructure relative to sedentary mice in sham but not stroke mice. In the affected limb, sham exercise mice had higher bone volume fraction (p=0.011 vs sham sed, p=0.049 vs stroke ex), trabecular thickness (p=0.009 vs sham sed, p=0.0045 vs stroke ex), and cortical area (p=0.0066 vs sham sed, p=0.0001 vs stroke ex) (Figure 2). No significant limb-to-limb differences were detected in bone microstructure for stroke or sham mice.

**DISCUSSION:** With ischemic stroke bone microstructure was not altered within 4 weeks recovery, but osteovascular structure was. In our previous work in these mice, stroke reduced tibial blood flow for the first two weeks of stroke recovery relative to sham, and exercise helped restore perfusion faster than sedentary[8]. Stroke also inhibited the exercise-induced microstructural bone gains seen in sham mice. High-speed video analysis showed no significant gait changes with stroke, indicating no limping or limb unloading during exercise. The early temporary perfusion decreases observed previously[8] may trigger an angiogenic response that could explain the increased vessel density after 4 weeks of recovery and also the lack of exercise-induced bone changes, since angiogenesis and osteogenesis are coupled and regulated by many of the same pathways[10]. Future work will characterize endothelial cell subtypes known to orchestrate bone remodeling and angiogenesis in bone[11] and examine material properties of bone with Raman spectroscopy and atomic force microscopy.

**SIGNIFICANCE:** We observed an absence of an exercise response in mice following ischemic stroke, which is indicative of declined bone health. Researching mechanisms responsible for these shifts may enable better therapies to prevent stroke-related bone fragility for human stroke patients.

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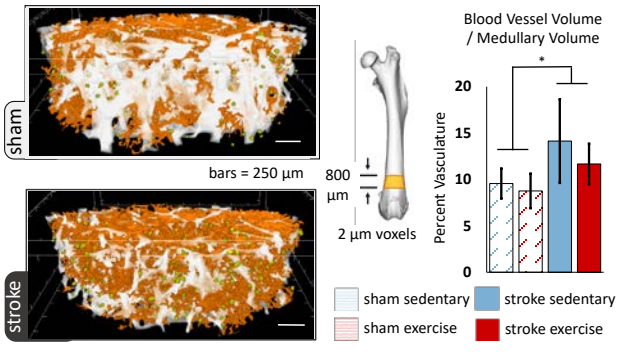


**Figure 1**: Left: Representative contrast enhanced nano-CT reconstructions of the distal metaphysis show more vasculature (orange) within the cancellous bone (white) for stroke vs. sham. Right: Vessel volume per medullary volume was greater for stroke than sham, based on two femurs scanned per group thus far. *p<0.05

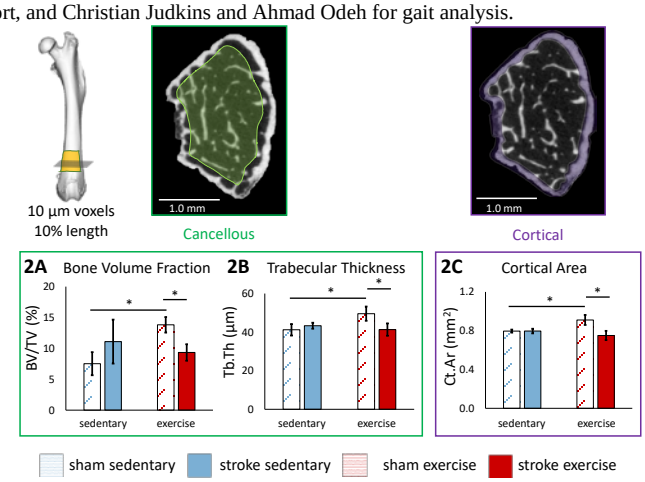


**Figure 2**: Cancellous bone: For sedentary, trabecular architecture was similar between stroke and sham mice. With exercise, BV/TV and Tb.Th increased for sham but not stroke mice (2A, 2B). Cortical shell around cancellous bone: Similarly, exercise-induced increases in Ct.Ar occurred in sham mice only (2C). *p<0.05