

Exercise Therapy Mitigates Reductions in Tibial Blood Flow during Acute Stroke Recovery

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INTRODUCTION: Stroke sufferers fall more and experience hip fractures 2-4 times more frequently than with typical aging¹, yet bone health is not typically monitored during rehabilitation. Although immobility and bed rest may be largely responsible for bone loss following stroke, some research suggests that limb vasculature declines following stroke^{2,3} and thus may contribute to the bone loss. Since intraosseous circulation is vital for bone maintenance, improving vascular health within bone may help mitigate bone loss post-stroke. Exercise stimulates angiogenesis and can decrease fracture risk by improving balance and musculoskeletal strength^{4,5}, but little is known about its effects on bone vasculature in stroke patients. We hypothesize that stroke negatively impacts 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, male C57Bl6/J mice (Jackson Labs, Bar Harbor, ME) were given either a stroke (n=15) or sham (n=12) surgery at 12 weeks of age. We induced ischemic stroke with the middle cerebral artery occlusion (MCAO)⁶ method, in which a thin silicone-coated filament was inserted into the MCA for 30 minutes to occlude blood flow and then removed to allow reperfusion, closely mimicking human ischemic stroke. For the sham surgery, surgical incisions were made, but the occluding filament was not inserted. Stroke and sham mice were divided into exercise and sedentary groups. Exercise mice were subjected to treadmill exercise therapy (9 m/min, 37 min, 5 days/wk, 5° incline) from 4 days (baseline) to 4 weeks post-surgery (n=6 'sham ex', n=8 'stroke ex'), while sedentary mice were placed on a stationary treadmill for a matched time period (n=6 'sham sed', n=7 'stroke sed'). Motor and sensory deficits were assessed with weekly functional neurological scores (neuroscores) to track stroke recovery. Tibial blood flow was monitored *in vivo* for 4 weeks during recovery using laser Doppler flowmetry (LDF)⁷. LDF measurements were taken by making a 2-4 mm incision over the medial tibial metaphysis, scraping away the periosteum, and placing a 0.8-mm diameter LDF probe directly on the bone surface. Flow was recorded and averaged over approximately 30 seconds, and this measurement was performed twice to ensure proper probe placement. High speed video analysis was performed weekly to assess gait alterations due to the stroke or LDF procedures. Specifically, phase dispersion, the ratio of times between footfalls of linked limb pairs, was calculated to determine if mice were limping⁸. Group differences were assessed using two-way ANOVAs with Tukey's post-hoc comparisons and a significance level of 0.05 (GraphPad Prism).

RESULTS: All mice were able to perform the exercise treatments four days following the stroke surgery. Functional neuroscores in stroke mice tended to improve more quickly over time with exercise than when sedentary (Figure 1). Proximal tibial blood flow was reduced in stroke relative to sham mice at one (p=0.010 sed, p<0.0001 ex) and two (p=0.0057 sed, p<0.0001 ex) weeks post-stroke but was similar to sham in the final two weeks. Exercise therapy increased tibial flow by two weeks (p<0.0001 sham ex vs sham sed, p=0.013 stroke ex vs. stroke sed), but the effect diminished by three weeks. By two weeks post-stroke, exercise therapy in stroke mice restored the tibial blood flow to levels of sham sedentary mice (p>0.05 stroke ex vs. sham sed in weeks 2-4). High-speed video analysis did not yield any significant changes in gait due to the LDF procedure or stroke.

DISCUSSION: LDF measures of blood perfusion in the tibia were affected by both stroke and exercise during acute stroke recovery. The first two weeks following stroke have been suggested to be integral to preventing bone loss, mobility challenges, and neurological deficits in human patients⁹. The observed decrease in tibial blood flow for the first two weeks following stroke suggest that deficits in perfusion may play a role in bone health following stroke. Although stroke decreased tibial blood perfusion, mice that exercised had similar perfusion as sham sedentary mice by the second week of recovery and recovered neurological function slightly faster, indicating that exercise therapy restored circulation losses from stroke and could be used as a rehabilitation strategy in the future.

SIGNIFICANCE: We have directly measured temporal changes in blood flow in the proximal tibia following ischemic stroke. These results indicate that therapies targeting osteovascular health may mitigate bone loss post-stroke.

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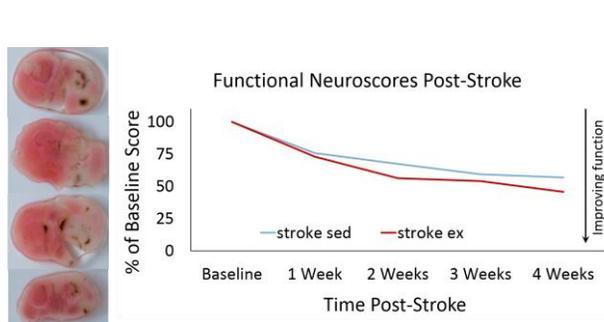


Figure 1: Left: 2,3,5-Triphenyltetrazolium chloride (TTC) staining of a brain 4 days post stroke (white = ischemic damage). Right: Neurological function tended to recover slightly faster with exercise vs. sedentary mice.

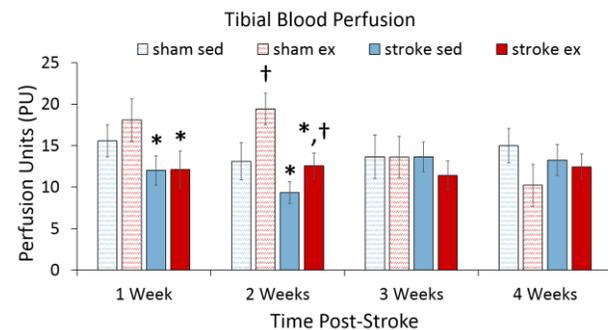


Figure 2: Stroke decreased tibial blood flow at 1-2 weeks post-stroke. Exercise therapy increased tibial flow relative to sedentary by 2 weeks post-stroke and offset deficits in the stroke mice through 4 weeks. *p<0.05 compared to sham, †p<0.05 compared to sedentary.