

Biological and Physiological Adaptations to Bone Injury Given a History of Prior Exercise

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INTRODUCTION: Exercise can help maintain healthy musculoskeletal tissue and regulate inflammation. The inflammatory response is a known, critical mediator of fracture healing, however little is known about how exercise affects the inflammatory response to fracture and healing outcomes. We sought to elucidate if regular exercise prior to injury would modulate inflammation and improve bone healing in a femoral defect model. We hypothesized that 4-weeks of treadmill running prior to injury, termed prehabilitation, would increase pre-injury bone volume and bone mineral density. We further hypothesized prehabilitating animals would decrease systemic inflammation and promote improved bone regeneration post-injury as compared to sedentary controls.

METHODS: To investigate the effects of prehabilitation on bone healing, nine-week-old female Wistar rats (n=7) were subjected to exercise training on a treadmill. Prehab animals acclimatized to treadmills for 10 days, with speed and duration gradually increased until reaching final exercise conditions of 18m/min with 5-degree inclination for 30min/day. This regimen corresponded to ~70-75% VO₂ max. Rats were then prehabilitated under these conditions 5days/wk for 2 weeks. Bone adaptations to prehab were evaluated using *in vivo* Micro-CT. At the conclusion of the prehabilitation period, prehab and sedentary rats (n=7 per group) underwent surgical creation of a 2mm femoral defect stabilized with internal fixation. All subjects remained sedentary for the following 8-weeks, over which bone regeneration was quantified via *in vivo* x-ray and Micro-CT at weeks 2, 4 and 8. Blood was drawn longitudinally at days 0, 3, 7, 14, 28 and 56 post-op to generate a systemic inflammatory profile and cell populations were quantified using flow cytometry. Electronic Von Frey was used to quantify mechanical sensitivity metrics including paw withdrawal threshold (PWT), with lower withdrawal forces interpreted as indicators of pain. All procedures were approved by University of Oregon's IACUC. Longitudinal data were analyzed with a two-way ANOVA with repeated measures or mixed-effects model to test for significant difference across time and prehab treatment (p<0.05). All data are displayed as mean ± sem.

RESULTS: Contrary to our hypothesis, prehab and sedentary groups did not exhibit significant differences in bone volume, bone mineral density, or other morphologic properties at the end of the prehabilitation period. Significant growth-associated changes in bone occurred in both groups, with bone volume, bone mineral density, cortical thickness and polar moment of inertia increasing significantly in both groups across four weeks of time (data not shown).

Following injury, the early systemic immune response was closely monitored. At day 7, we observed significant differences as compared to baseline in the circulating cell populations; sedentary subjects had a longitudinal increase in myeloid derived suppressor cells (MDSCs) (p=.0209), and concomitant longitudinal decrease in overall T cells (p=.0013), neither of which was not seen in the prehab animals. Rather in the prehab group at day 7 there was a significant increase in CD4+ T cells (p=.0183) as compared to sedentary counterparts. By day 14 there were no differences in immune cell profiles.

We hypothesized we would see improved healing in prehab animals. Unexpectedly, we observed bridging of the bone defect in only one prehab, and no sedentary subjects (n=7). However, average bone volume in the prehab group, although not statistically different, was 3.4X greater than sedentary after 8 weeks of recovery. By week 7, pain withdrawal threshold in the prehab group had returned to baseline and was significantly greater than the sedentary animals (p=.0259).

DISCUSSION: Given the well-known effects of loading on bone adaptation, we hypothesized that exposing subjects to load bearing treadmill exercise prior to injury may help bone's endogenous ability to regenerate. Unexpectedly, we found that prehab had no effect on bone structure. This lack of response to prehab may be partially due to the rats' skeletal immaturity during the prehab period. Several studies have elucidated differences in response to weight-bearing exercise in adolescent and aged rodents.

Despite lack of significant differences in bone adaptation, prehab exercise did modulate the early systemic inflammatory response. In the week after injury, sedentary subjects had a significant increase in MDSCs, whose elevated levels have been previously correlated with reduced bone formation and chronic immunosuppression. Prehab rats did not see a significant increase in MDSCs or decrease in T cells, rather they exhibited significantly more CD4+ T cells. Without elevated MDSC levels and an increase in pro-osteogenic CD4+ T cells, the prehab animals had a more pro-regenerative early inflammatory profile. However, this pro-regenerative profile was not sustained and by 14-days there were no differences between sedentary and prehab groups. Recent studies in mouse models have shown metabolic alterations in macrophages for only 2 weeks suggesting a limited chronic effect of exercise on immune populations, which may provide an explanation for the duration of the pro-regenerative profile seen in the prehab group.

Counter to the pro-osteogenic early increase in CD4+ T cells, only one prehab subjects defect bridged. Although not significant, prehab animals had a 3.4x greater final bone volume over sedentary subjects. Prehab animals also had significantly reduced limb sensitivity to stimulation, with an increased PWT equivalent to their baseline levels in Von Frey testing, whereas sedentary animals did not return to baseline levels indicating sustained pain.

Overall, these data suggest that although a history of prior exercise can modulate components of the initial immune response to be pro-regenerative, the early biological response alone is insufficient to orchestrate significantly improved healing. Prior work in our group revealed improved bone healing with exercise introduced one week after injury. Taken together, these studies demonstrate the importance of both biological and mechanical cues at the regenerative niche, with the potential to tune pre- and post-surgical rehabilitation protocols to beneficially modulate immune response to injury and promote functional regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Improving our understanding of how regular exercise modulates the systemic immune response to bone injury may contribute to our ability to generate patient-specific treatment plans for those who have a history of physical activity.

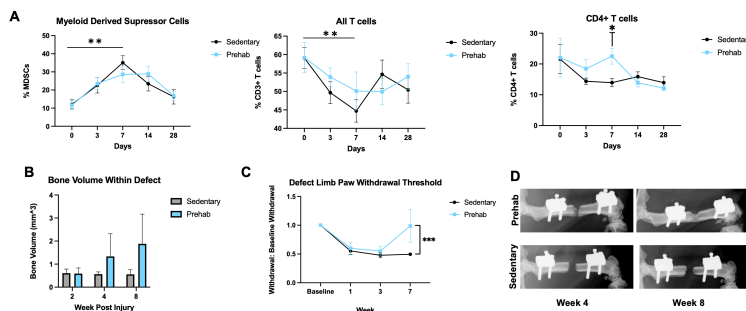


Fig. 1: Prehabilitation resulted in an early pro-regenerative immune profile and decreased pain but did not influence healing outcomes. (A) Changes in immune populations post-op. (B) Bone volume quantification within the center 1.5mm of the 2mm defects. (C) Longitudinal paw withdrawal force normalized to baseline withdrawal force. (D) X-ray images of bone regeneration.