Early Rehabilitation Promotes Functional Restoration Of Hindlimbs Following Femoral Bone Defect Injury

Julia Andraca Harrer[1,2], Annemarie Lang[3], Sanique M. South[1], Kaitlyn Link[1], Madhura Nijsure[3], Joel D. Boerckel[3], Nick J. Willett[1]
[1]Knight Campus Department of Bioengineering, University of Oregon, Eugene, OR, [2]Wallace H. Coulter Department of Bioengineering, Emory University & Georgia Institute of Technology, Atlanta, GA, [3]Departments of Orthopaedic Surgery and Bioengineering, University of Pennsylvania, Philadelphia, PA
Email: jharrer@gatech.edu

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INTRODUCTION: Bone defect injuries pose a significant clinical challenge and often result in chronic pain and prolonged disability in patients. Clinically, these injuries are typically stabilized using load-shielding, titanium fixation plates to prevent mechanical loading across the defect. In addition, the prescribed rehabilitation regimens begin with extended periods of non-loading and wait to initiate active rehabilitation until healing has progressed for weeks. However, extended periods of non-loading have been shown to reduce bone mass, bone mineral density, muscle strength and volume; all of which likely contribute to the long-term functional deficits seen in patients. Pre-clinical studies have shown that load-sharing fixation coupled with early rehabilitation can improve bone healing. However, the effects of rehabilitation on functional restoration of the injured hindlimb, and the molecular mechanisms underlying these changes have not been fully identified. The objective of this study was to determine how early rehabilitation impacts pain sensitivity and hindlimb functionality and uncover the underlying mechanisms of mechanotransduction impacting these changes. We hypothesized that access to rehabilitation beginning at 7 days post injury would reduce hindlimb sensitivity and improve functional recovery of the injured limb.

METHODS: All procedures were conducted in accordance with IACUC protocol. Femoral defects ranging from 0.7-1.4mm were surgically-induced in the left hindlimb of 12-14 week old male (n=14) and female (n=12) C57CL/6 mice (Jackson Labs). Femurs were stabilized using an external fixation plate (RISystem). 14 days prior to surgery, mice were randomized into sedentary (n=6 female, n=6 male) and rehabilitation (n=6 female, n=8 male) groups and running wheels were introduced to the rehab mice for acclimation. Wheels were removed at surgery and reintroduced 7 days post injury (dpi) in the rehabilitation group. Daily distance on the running wheels was monitored and radiographs were used to evaluate bridging rates longitudinally. von Frey for pain sensitivity (males and females) and dynamic weight bearing (males only) were assessed at baseline and weeks 1, 2, 3, 4, 6, and 8 after injury, and gait (males only) was assessed at baseline and weeks 2, 4, 6 and 8. At the study endpoint (8 weeks), isometric torque for muscle strength and micro-computed tomography (μCT) scans for bone volume and mineralization were obtained. In addition, follow-up studies were performed on 18-week-old females with and without access to running wheels (n=16) and euthanized at 14 dpi for bulk RNAseq and immunostaining analyses. von Frey results were analyzed using a three-way ANOVA ($p<0.05$) and Bonferroni's post-hoc analysis (95% CI), other longitudinal results were analyzed using a two-way ANOVA ($p<0.05$) Tukey's post-hoc analysis (95% CI), and individual timepoints were analyzed with an unpaired t-test ($p<0.05$) using GraphPad Prism 9.

RESULTS SECTION: Daily running (Fig. 1) increased during acclimation until reaching an average of 17.9 km/day for the females (n=6) and 5.2 km/day for the males (n=8). Running distance was significantly less after surgery and increased through week 8 until reaching an average of 12.3 km/day for the females and 9.5 km/day for the males. von Frey analysis for mechanical allodynia (Fig. 2 A,B) demonstrated increased sensitivity at 7 dpi. Female animals undergoing rehabilitation (n=6) had lower withdrawal response at 14 dpi compared to sedentary animals (n=6) ($p=0.049$), and the male rehabilitation group (n=8) also had lower withdrawal response compared to the sedentary group (n=6) at 14- and 21-dpi ($p = 0.0082$, $p = 0.0109$). Dynamic weight bearing analysis (Fig. 2 C,D) revealed injured animals increased their weight distribution to their uninjured right hindlimb and reduced their weight distribution on the injured left hindlimb at 10 dpi in both groups. Animals undergoing rehabilitation supported more of their weight on their injured left limb compared to sedentary animals on 10 and 14 dpi ($p = 0.042$, $p = 0.043$). The rehabilitation group also off-loaded onto their uninjured right limb less than the sedentary group at day 28 ($p = 0.0185$). There was no effect of rehabilitation on bridging rates or bone volume (Fig. 3).

DISCUSSION: We hypothesized that early rehabilitation would improve functional outcomes following bone defect injury. Daily running revealed sex-based differences, with females running more throughout the study compared to the males, though overall trends were the same. von Frey analysis also showed sex-based differences, with increased baseline sensitivity in the males compared to the females. Sedentary animals from both sexes took longer to return to baseline levels and had significantly elevated withdrawal responses at 14 dpi for both sexes and 21 dpi in the males. Dynamic weight bearing analysis in males demonstrated that sedentary animals had a less equal distribution of weight on injured and uninjured paws compared to the rehabilitation animals on 10, 14 and 28 dpi. Both von Frey and dynamic weight bearing analyses show that early initiation of rehabilitation reduces sensitivity and regains functional use of the injured limb sooner than conservative non-loading regimens. Forthcoming data will further elucidate the effects of rehabilitation on gait. Ongoing bulk RNAseq and immunostaining analyses of femora at the 14-day timepoint to assess the underlying molecular mechanisms driving these differences. Bridging and bone volume at 56 dpi showed no differences between the sedentary and rehabilitation groups, indicating no negative effect of early rehab. This could indicate that this bone defect model requires additional treatment to promote bone formation to supplement the functional effects seen by rehabilitation. One of the limitations of this study was the wide range of defect sizes induced, which could also explain the variability in healing. Another limitation was that dynamic weight bearing and gait were not evaluated in female mice.

SIGNIFICANCE/CLINICAL RELEVANCE: This research demonstrates that early rehabilitation promotes earlier hindlimb functional recovery and reduces sensitivity following bone defect injury, with no significant effect on the bone healing response. This indicates that early rehabilitation may improve bone defect injury treatment outcomes and has the potential to alleviate long-term functional deficits seen in patients suffering from these injuries.

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IMAGES AND TABLES:

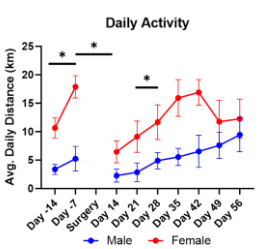

Figure 1. Daily running activity.

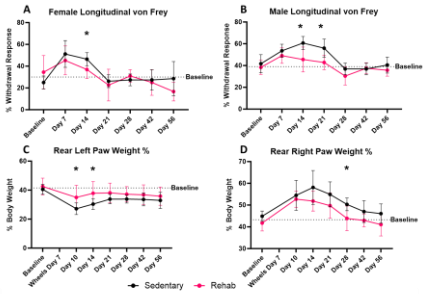

Figure 2. von Frey for (A) female and (B) male mice, and weight % on (C) left and (D) right rear paws.

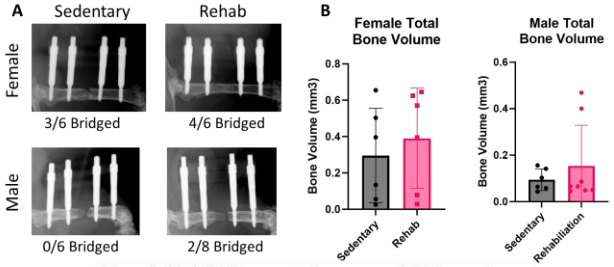

Figure 3. Week 8 (A) representative x-rays and (B) bone volume.