

In Sequence Anti-Fibrotic Treatment and Rehabilitation for Volumetric Muscle Loss Injury
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INTRODUCTION: A lack of spontaneous skeletal muscle regeneration underpins the sustained functional impairment that characterizes volumetric muscle loss (VML) injuries. The underlying pathophysiology of impediments to muscular regeneration after VML remain under investigation, but treatments that broadly address functional rehabilitation of remaining muscle may provide significant benefit. Mitigation of local pathologic fibrotic tissue deposition following VML is a target area of interest for functional rehabilitative treatments. Nintedanib, a tyrosine kinase inhibitor that functions as an anti-fibrotic, has shown promise for reduction of fibrosis following VML. Previous studies have analyzed the efficacy of nintedanib in mouse and pig models for reducing post-injury fibrosis. Additionally, various studies have utilized wheel running as a model for rehabilitative exercise in mice and have introduced wheel running in concordance with, or shortly after, beginning pharmaceutical interventions. This research seeks to investigate whether a delayed time frame for introduction of wheel running following early pharmaceutical treatment of nintedanib will attenuate post-injury fibrosis and improve functional recovery.

METHODS: Adult (12-week-old; n=52) male C57BL/6 mice were injury naïve or underwent unilateral VML injury and were randomly assigned to receive nintedanib (6 mg/kg/day) treatment for two weeks or were untreated; additionally, mice were either sedentary or given free access to a running wheel beginning 2 weeks following injury until the terminal 8-week timepoint. At 8 weeks, mice underwent maximal *in vivo* functional testing and terminal harvest. A subset of mice (n=4/group) was assigned to no treatment or nintedanib treatment following VML, or were injury naïve, and were sacrificed at 2 weeks post-injury following muscle function testing. This 2-week subset was used to evaluate early anti-fibrotic signaling and mitigation with nintedanib treatment. Biochemically, muscle collagen content and fibrotic and myogenic signaling related markers were evaluated in muscle and serum, and histological analyses assessed fibrotic tissue accumulation in the mid-belly of the gastrocnemius muscle, corresponding with the VML defect. One-way ANOVA with Tukey's HSD *post hoc* evaluated outcomes across groups within each terminal time point. All experiment protocols and animal care guidelines were approved by the Institutional Animal Care and Use Committee (#2008-38365A); in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

RESULTS: Eight weeks following injury, all mice assigned to 6 weeks of running had similar running distances (6.1 ± 2.6 km daily; $p=0.939$) across groups regardless of injury or treatment status. As expected, there was a slight decrease in body mass up to 2 weeks following VML injury, independent of treatment, and body mass was lower up to 8 weeks for all delayed running groups, independent of injury and treatment ($p<0.001$). At 2 weeks, VML injury significantly reduced gastrocnemius mass normalized by body mass, regardless of treatment ($p<0.001$), corresponding with a significantly lower maximal isometric torque than injury naïve controls ($p<0.001$). By 8 weeks, the injured gastrocnemius mass of mice that ran, independent of treatment status, was similar to sedentary injury naïve mice but smaller than injury naïve mice that ran ($p<0.001$). Similarly, VML-injured mice that ran, independent of treatment status, produced lower maximal isometric torque than injury naïve mice that also ran ($p=0.001$). Passive torque about the ankle was also evaluated terminally. It is expected that treatment with the anti-fibrotic nintedanib will decrease passive stiffness, compared to leaving the VML injury untreated, by attenuating fibrotic tissue deposition, and rehabilitation may further help decrease stiffness. Passive torque at 20° of dorsiflexion was higher for VML-injured mice that received combined nintedanib and rehabilitative (i.e., wheel running) treatment compared with sedentary injury naïve controls, indicating increased stiffness above no injury; however, passive torque was similar between the combined nintedanib/running group and injury naïve controls that ran, suggesting an effect of running on increased stiffness that is independent of injury and treatment status ($p<0.001$).

DISCUSSION: It has been suggested that a combined regenerative and rehabilitative treatment regimen following VML injury may address the lack of functional muscle regeneration when these treatments are used individually. It is notable that early prevention of fibrotic tissue deposition may improve the microenvironment of the remaining muscle and provide a conducive environment for myofibers to regenerate. Treatment with an anti-fibrotic may inhibit early fibrotic signaling to mitigate long-term fibrotic tissue deposition, and delayed rehabilitation may promote myogenic signaling, resulting in myofiber regeneration and improved functional recovery. Delayed rehabilitation improved gastrocnemius mass across groups in addition to enhancing the capacity of the muscle to produce force, independent of treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: Regenerative rehabilitative solutions that could be implemented readily in the clinic need to be evaluated for the VML-injured patient population. The ability to translate current FDA-approved pharmacologic treatments, in a repurposing approach, is critical to mitigate the pathophysiologic consequences of VML to support clinical functional recovery. Additionally, rehabilitation implemented in a delayed manner is more practical following traumatic musculoskeletal injury such as VML and may have a protective and positive impact on improving the muscle's functional capacity following injury.

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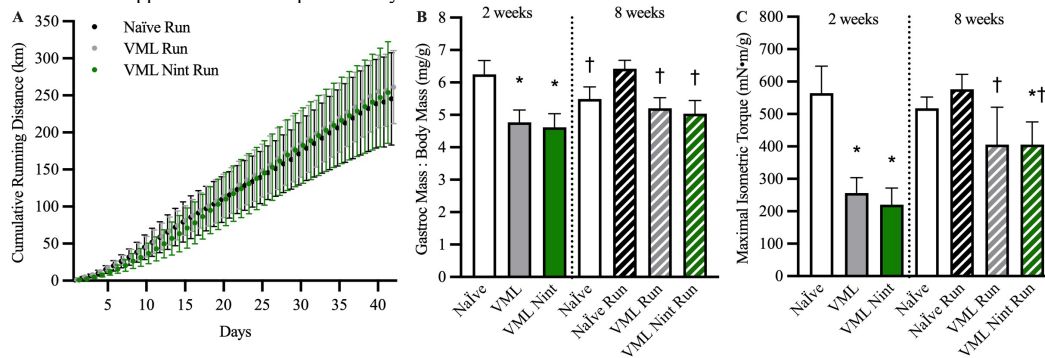


Figure 1. A) Similar cumulative running distances were noted across running groups over 6 weeks ($p=0.939$). B) The VML-injured gastrocnemius muscles, normalized by body mass, were significantly lower at 2 weeks following injury compared to naïve, while running mitigated this difference by 8 weeks following injury; though, running did not mitigate the discrepancy in VML-injured muscles compared to the naïve run group. C) At 2 weeks following VML, both untreated and nintedanib treated posterior hindlimb compartment muscles produced substantially lower maximal isometric torque, normalized by body mass, than injury naïve. By 8 weeks, VML-injured mice that ran produced lower maximal isometric torque than injury naïve mice that ran. Significantly different than *Naïve, † Naïve Run.