Effects of Ultrasound Stimuli on Denervation-Induced Gastrocnemius Atrophy: An In-Vivo and In-Vitro Mouse Model Validation

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ABSTRACT INTRODUCTION: Loss of muscle mass and strength, known as muscle atrophy, is a common consequence of injury, disease, or extended periods of immobility, and it represents a major challenge for rehabilitation. Traditional therapies, such as physical training, may not be sufficient to fully prevent or reverse this deterioration, motivating the search for innovative, non-invasive approaches. Ultrasound, widely used in medical imaging, has recently gained attention for its therapeutic potential, particularly in promoting tissue repair and modulating cellular activity. Recent studies indicates that low-intensity pulsed ultrasound (LIPUS) can help reduce muscle atrophy by stimulating muscle regeneration [1]. For example, LIPUS has been shown to prevent muscle atrophy in diabetic rat models by enhancing protein synthesis and reducing proteolysis [2]. We hypothesized that LIPUS could help slow or counteract muscle atrophy by enhancing muscle regeneration. To address this, we investigated the effects of ultrasound stimulation on both animal models of muscle atrophy and cultured muscle cells, aiming to better understand its therapeutic potential and underlying mechanisms.

METHODS: We investigated LIPUS in a mouse model of denervation-induced gastrocnemius atrophy, induced by tibial nerve transection in male C57BL/6 mice (8 weeks old; n=8 per group; male sex used to avoid hormonal variations in females). Mice were divided into control and LIPUS group using focused ultrasound probe (1 MHz, 600 mVpp, 5% duty cycle, 5 min, I_{SATA} = 53.99 mW/cm²) , receiving treatment 3 times/week for 3 weeks. Muscle weight and cross-sectional area (CSA) were measured. In the *in vitro* study group, the C2C12 myoblasts (n=3 replicates) were treated with LIPUS (600 mVpp, 5% duty cycle, 1 MHz, 5 min, I_{SATA}=43.6 mW/cm²) to evaluate proliferation and differentiation (fusion index).

RESULTS: In the results of *in vivo* experiments (n = 8/group), the LIPUS treatment significantly preserved gastrocnemius weight following denervation. Muscle weight was normalized by calculating the ratio of the injured leg to the uninjured leg for each mouse, which allowed us to account for interindividual variation. The results demonstrated that LIPUS treatment maintained muscle mass with statistical significance ($p<0.05$) (Fig 1A). Further histological analysis of the gastrocnemius was preformed to assess structural preservation. CSA measurements were obtained from H&E-stained sections imaged at 40x magnification. Individual muscle fibers were carefully outlined and quantified, and the analysis confirmed that LIPUS preserved fiber size compared to untreated controls, with highly significant differences observed ($p<0.01$) (Fig 1B). These findings indicate that LIPUS treatment not only slows down muscle weight loss but also protects fiber morphology during atrophy progression. *In vitro*, LIPUS enhanced C2C12 myoblast proliferation and differentiation. Slow myosin heavy chains (sMHCs), a marker of mature myotubes, were immunostained (Fig 2). Cell nuclei were counterstained with DAPI to calculate the fusion index. The analysis revealed a significant increase with $p = 0.004$, indicating that LIPUS promotes myoblast fusion into myotubes and accelerates the maturation process.

DISCUSSION: LIPUS mitigate denervation-induced gastrocnemius atrophy by preserving muscle mass and CSA, consistent with mechanotransduction–mediated regeneration through MSTN/Akt/mTOR signaling and satellite cell activation. *In vitro*, C2C12 myoblasts demonstrated enhanced proliferation and myotube formation, with a significantly elevated fusion index, likely driven by inflammatory modulation (M1-to-M2 macrophage shift) and COX-2 upregulation. These findings support the role of LIPUS stimulation in muscle repair and address current gaps in targeted gastrocnemius therapy. Limitations include small sample sizes (n = 6 *in vivo*; n = 3 *in vitro*) and the use of male-only mice, which may limit generalizability; future studies should incorporate female cohorts and larger sample sizes. In addition, the relatively short treatment duration (3 weeks) and reliance on a single cell line highlight the need for longer-term studies and validation in human models. Conclusion: LIPUS represents a promising non-invasive strategy for muscle atrophy rehabilitation via satellite cell activation, though clinical trials are needed to establish its translational efficacy.

SIGNIFICANCE/CLINICAL RELEVANCE: This study advances the field by demonstrating the potential of LIPUS stimulation to mitigate muscle atrophy through mechanotransduction, addressing a critical barrier in non-invasive therapies for denervation-induced wasting that traditional rehabilitation often fails to prevent. Clinically, these findings could improve rehabilitation outcomes for patients with mobility-limiting injuries or sarcopenia, reducing muscle loss and enhancing recovery, thereby lowering healthcare burdens and improving quality of life in aging and immobilized populations.

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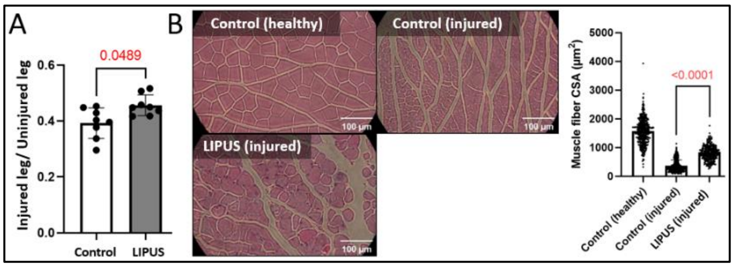

Fig.1: (A) Normalized data of the weight of the gastrocnemius muscle. (B) Cross-sectional area analysis of the gastrocnemius muscle fibers.

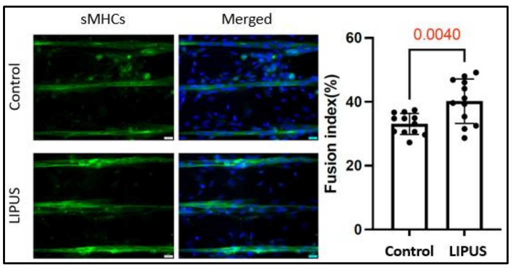

Fig.2: Immunofluorescence staining of slow myosin heavy chains and DAPI with analysis of fusion index of cell nuclei and myotubes.