Muscle mechanical properties are maintained following focused ultrasound-driven muscle activation

Zoe M. Moore[1], Jake Elliott[2], Julianna C. Simon[1,2], Meghan E. Vidt[1,3]
[1]Biomedical Engineering, The Pennsylvania State University, University Park, PA, USA
[2]Graduate Program in Acoustics, The Pennsylvania State University, University Park, PA, USA
[3]Physical Medicine and Rehabilitation, Penn State College of Medicine, Hershey, PA, USA
Email of Presenting Author: zmm5238@psu.edu

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INTRODUCTION: Muscle disuse is common in adults, and often occurs as a result of prolonged bedrest[1]. Sustained muscle disuse causes a decrease in muscle mass due to unloading of the muscle and a decrease in neural activity[1,2]. These changes in muscle mass can affect muscle morphology and tissue mechanical properties[2]. Resistance exercise training is a common therapy for muscle disuse but can be difficult for individuals to perform, leading to low patient compliance[2,3]. Alternative therapies that promote development and maintenance of muscle mass without physical therapy do not currently exist[2]. Other treatments, such as electrical muscle stimulation, cannot target deep muscles and, due to the small sample size of previous research studies, there is insufficient evidence on the effectiveness of the therapies[4,5]. Focused ultrasound (fUS) is an emerging therapy that has been shown to activate skeletal muscle *in vivo* via peripheral nerve stimulation[6]. Prior work successfully applied fUS without inducing any tissue damage, but did not evaluate the effect of fUS on tissue mechanical properties. Therefore, this study aims to examine the mechanical properties of skeletal muscle following fUS muscle activation.

METHODS: The sciatic nerve of 19 Sprague-Dawley rats (IACUC #202101801; 9M/10F; 7 weeks old) was treated with fUS on a randomly selected side every other day for 2 weeks with a randomly selected treatment duration of either 30 s, 90 s, or 180 s. Indwelling electromyography (EMG) electrodes were inserted into the belly of the gastrocnemius muscle prior to the hind quarters of the rat being submerged in a body temperature water bath (~37°C). Prior to fUS application, EMG data were collected for 5s to acquire a baseline measure. All EMG was filtered using a 4th order Butterworth filter and then normalized to baseline. Following the final treatment, all animals were euthanized, and bilateral gastrocnemius muscles (treated side, contralateral control) were harvested for histology and mechanical testing. Eight samples were randomly selected for histological analysis (4 treated; 4 control) to determine whether tissue damage occurred due to the fUS treatment. Histology samples were fixed in formalin for 7 days, then processed using standard paraffin wax techniques. All samples were sectioned, stained using Hematoxylin and Eosin (H&E), and qualitatively analyzed. The remaining 30 tissue samples (15 treated; 15 control) underwent standard uniaxial tension testing using a previously reported protocol[7] with an 858 MiniBionix (MTS Systems). Briefly, the sample was preloaded at 0.08 N, followed by 10 cycles of preconditioning, then rested for 300 s. Next, a stress-relaxation test was performed for 600 s, followed by a load-to-failure test with a load application rate of 0.0015 mm/s. Percent relaxation was calculated using the peak and equilibrium stress from the stress relaxation test. An automated algorithm developed by our group[8] was used to calculate strain of the tissue. Stress was calculated using the experimental force values from the stress relaxation and load-to-failure tests and sample cross-sectional area measured with calipers. Young's modulus and stiffness were calculated using the slope of the stress-strain curves and force-displacement, respectively, from the load-to-failure test. Ultimate tensile stress (UTS) was calculated as the maximum stress achieved before sample failure. A Shapiro-Wilk test was used to test for data normality. Then, separate paired t-tests were used to evaluate differences within treatment groups, across treatment durations, and between treated and control groups. All analyses were performed with SAS software (v.9.4, SAS, Inc.) with significance set at $p<0.05$.

RESULTS SECTION: Muscle activation was observed in 15 of 19 rats in a total of 22 of 64 treatment attempts, as defined by a measurable EMG signal above baseline. The observed magnitudes and salient features of the EMG signal following each fUS pulse were consistent with previous work[6]. There was no difference in percent relaxation, stiffness, Young's modulus, or UTS between treatment durations within the separate treated and control groups (all $p>0.26$). Since there was no difference between treatment duration, all treatment durations were combined into single groups (treated, control) for each outcome measure. There was no difference in percent relaxation ($p=0.364$; Fig. 1A), Young's modulus ($p=0.231$; Fig. 1B), stiffness ($p=0.432$; Fig. 1C), or UTS ($p=0.744$; Fig. 1D) between treated and control groups. There was no evidence of cellular damage at the fUS target site or surrounding tissue (Fig. 2).

DISCUSSION: fUS is an emerging therapeutic that has been shown to activate skeletal muscle via nerve stimulation, which has clinical potential as a treatment for muscle disuse. This study confirmed the ability of fUS to elicit muscle activation, as measured with EMG, although there was no difference found in all mechanical variables following treatment every other day for 2 weeks. However, we also observed no damage to the tissue, which suggests tissue mechanical properties were maintained following treatment without creating damage at the treatment site. It is possible that longer bouts of treatment may produce more similar results to those expected following exercise. Ongoing work continues to examine these trends in a larger sample as we evaluate how fUS can be used to activate skeletal muscle in ways that improve mechanical properties of the tissue.

SIGNIFICANCE/ CLINICAL RELEVANCE: This study demonstrated that fUS can be used to activate skeletal muscle via peripheral nerve stimulation while maintaining tissue mechanical properties and without damaging the surrounding tissue. fUS has therapeutic potential for muscle disuse injuries, which motivates ongoing studies to determine the specific fUS parameters and intervention dosing needed to replicate the effects of exercise.

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IMAGES AND TABLES:

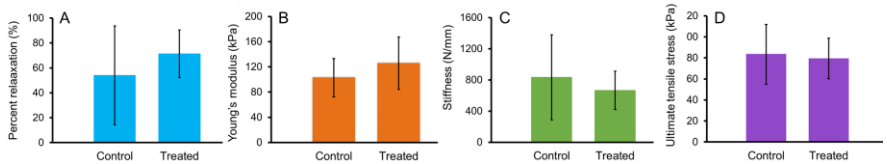

Figure 1: A) Percent relaxation ($p=0.364$) for the control (53±40%) and treated (71±19%) groups. B) Young's modulus ($p=0.231$) for the control (103±30 kPa) and treated (126±42 kPa) groups. C) Stiffness ($p=0.432$) for the control (833±546 N/mm) and treated (667±246 N/mm) groups. D) Ultimate tensile stress ($p=0.744$) for the control (83±28 kPa) and treated (79±19 kPa) groups.

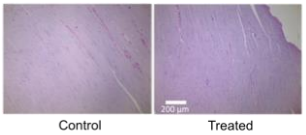

Figure 2: Hematoxylin and Eosin (H&E) stained images of muscle near the sciatic nerve in control and treated samples. No evidence of damage was observed on the treated tissue.