Muscle Contraction Contributes to the Recovery of Tendon Length and Mechanical Strength and Is Accompanied by Accelerated Neovascularization /Vascular Regression Process After Surgery for Achilles Tendon Rupture.

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INTRODUCTION: Lack of muscle strength recovery after Achilles tendon rupture (ATR) includes reduced force transfer function due to tendon scar healing[1,2] and reduced muscle tension associated with tendon elongation.[3] Tensile loading can stimulate and hasten the tendon healing process.[4,5] However, there are few standard benchmarks for when and what type of exercise should be performed for clinical application that have been uncovered. Weight-bearing and walking, conventionally defined as mechanical stimuli, are combinations of joint movements and muscle contractions that limit our understanding of what type of exercise actually activates the cellular and molecular mechanisms that contribute to healing. In the present study, we employed a combination of joint immobilization and muscle paralysis in a mouse model of tendon rupture suture, allowing us to control mechanical stress on tendons and elucidate the roles of muscle contraction and joint motion in tendon healing. Furthermore, angiogenesis is essential for cell migration to the injured area, oxygen supply, and the transport of nutritional factors during the early phase of tendon healing. We focused on the fact that blood vessels are susceptible to mechanical stress and hypothesized that exercise plays a role in angiogenesis and regression in the complex process of restoring tendon mechanical properties. The purpose of this study was to clarify the effects of passive joint motion and muscle contraction on tendon elongation, strength recovery, collagen fiber alignment, and angiogenesis and regression in and around tendons after ATR surgical repair.

METHODS: All animal works were approved by IACUC. Eighty-eight male C57BL/6J mice (10 weeks old) were surgically dissected to expose their left Achilles tendon (AT) and repaired by Kessler's method. Only males were included because ATR occurs more frequently in males. We used a left ATR model in mice, which was repaired using the Kessler method. Mice were randomly divided into four groups for evaluation at 2, 3, and 4 weeks post-surgery: the Immobilization (IM) group, where the ankle joint was fixed with a brace immediately after surgery; the Denervation (DN) group, where the sciatic nerve was resected to inhibit gastrocnemius muscle contraction; the IM+DN group, which combined both treatments; and the Intact group, using the contralateral limb as a control. [Evaluation items] Tendon Elongation: Tendon length was measured using a stereomicroscope. Mechanical Strength: Evaluated the maximum failure force, stiffness, and stress by ex vivo tensile testing. Histological evaluation: Collagen fiber alignment was evaluated with picrosirius red staining (n = 3/ group), and vascular density was assessed with CD31 staining (n = 3/ group). Volume of angiogenesis (n = 2-3/group): The vessel volume was calculated using micro-CT angiography. Statistical analysis was conducted using the Kruskal-Wallis test ($p < 0.05$).

RESULTS SECTION: Muscle contraction altered tendon length. At PO2w, tendons were significantly longer in the IM than in the IM + DN. At PO4w, the results were reversed, with only the IM recovering to the same length as the Intact group, while the IM+DN and DN tendons were longer than the Intact group (**Fig. 1A**). Preservation of muscle contraction significantly increased AT cross-sectional area (CSA). Joint motion alone did not contribute to increased CSA in tendons. Tendon maximum force and stiffness were significantly recovered in the IM with preserved muscle contraction compared to the denervated groups at PO3 and 4 weeks (**Fig. 1B-D**). We performed Histological analyses to investigate the factors contributing to the differences in mechanical strength. There was no significant difference in collagen fiber arrangement among the groups (**Fig. 2)**. Interestingly, the CD31-positive area in the IM tended to increase at PO2 weeks and decrease at PO4 weeks compared to the denervated groups (**Fig. 3A, B**). However, the volume of blood vessels around the tendon showed no significant differences among groups (**Fig. 3C**).

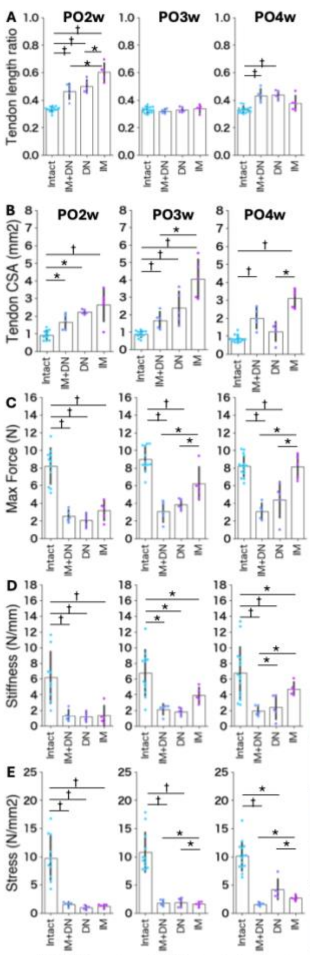

Fig 1 (A) Percentage of Achilles tendon length out of muscle-tendon complex length. (B) Tendon CSA. (C-E) Results of tendon mechanical testing. (mean ± SD, *P*-value; * <0.05, † <0.01)

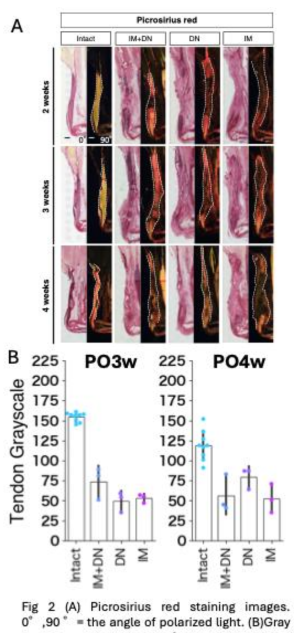

Fig 2 (A) Picrosirius red staining images. 0°, 90° = the angle of polarized light. (B)Gray scale calculated from 90° polarized images. (mean ± SD, *P*-value; * <0.05, † <0.01)

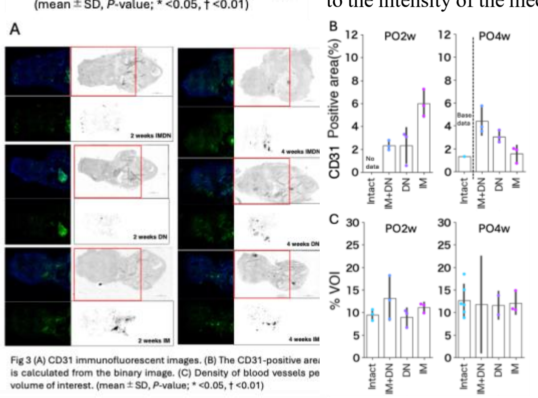

Fig 3 (A) CD31 immunofluorescent images. (B) The CD31-positive area is calculated from the binary image. (C) Density of blood vessels per volume of interest. (mean ± SD, *P*-value; * <0.05, † <0.01)

DISCUSSION: This study examined the role of mechanical stress from muscle contraction and joint motion in the healing of AT after surgery. We found that unloading by inhibiting muscle contraction up to 4 weeks resulted in a delay in the recovery of tensile strength and tendon elongation. This is consistent with previous studies [6, 7] that showed a nonlinear relationship between increased mechanical stimulus and tendon recovery. This result suggests that muscle contraction is crucial for tendons to regain proper tendon length as remodeling progresses. It was also observed that muscle contraction contributes to the increase in tendon CSA. Although there was no difference in collagen fiber arrangement among intervention groups, ECM synthesis may have been enhanced. Furthermore, muscle contraction tended to affect vascularization, increasing vessel density in the early stages of healing and promoting regression in the later stages of healing. These findings may be partially consistent with reports that the mechano-related expression of Angiopoietin-like 4 promotes angiogenesis [8] and tenomodulin regulates vascular invasion [9]. Importantly, joint motion alone did not affect any healing response outcome. Further study is required to determine whether muscle contraction produces a more significant tendon-healing effect than joint movement alone, due to the intensity of the mechanical stimulus, or whether it mediates endocrine effects.

SIGNIFICANCE/CLINICAL RELEVANCE: A basic understanding of the effects of muscle contraction on the structure and mechanical properties of tendons after rupture provides a foundation for clinical research to establish post-surgery rehabilitation protocols that promote functional recovery in patients.

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