

Search for mechanical stresses that promote tendon healing and prevent tendon lengthening after Achilles tendon rupture and surgical repair.

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INTRODUCTION: Tendons have low intrinsic healing capacity and do not recover to normal levels after rupture. Tendon lengthening after surgical repair is the main factor of the ankle plantar flexor muscle weakness. While it has been hypothesized that excessive stretching load on the tendon leads to elongation, the exact relationship remains insufficiently elucidated. Previous studies have reported that mechanical stress promotes tendon healing modulated gene expression, but the impact on tendon elongation has received limited attention. This study aims to investigate how mechanical stress induced by muscle contraction and passive motion in mice after Achilles tendon suture surgery influences tendon healing, elongation, and mechanical properties. Understanding the relationship between mechanical stress and tendon elongation is crucial for optimizing comprehensive rehabilitation protocols to prevent excessive elongation and enhance the quality of healing tendons.

METHODS: 48 male C57/BL6 mice (10 weeks old) were surgically dissected their left Achilles tendon and repaired by Kessler's method. They were randomly divided into three groups in combination with ankle immobilization and planter flexor paralysis: Immobilization (IM) group, in which ankle joint motion was inhibited immediately after surgery with a brace; Denervation (DN) group, in which the sciatic nerve was transected 3 mm at the same time as tendon suture and contraction of the triceps muscle was inhibited; and IM + DN group, which was a combination of both IM and DN. Analysis was performed at 2 and 3 weeks after surgery. To evaluate the tendon elongation, the Achilles tendon length was measured with a stereomicroscope. Then, those tendons were analyzed for ex vivo tension testing as an analysis of mechanical strength, and those gastrocnemius muscles were analyzed for wet weight as the analysis of muscle atrophy ($n=5/\text{group}$ at each time point). Tendons were also stained with picrosirius red, and the collagen fiber arrangements were analyzed ($n=3/\text{group}$ at each time point). Statistical analysis was conducted using the Kruskal-Wallis test, with a significance level set at $p < 0.05$. The Ethical Committee of Saitama Prefectural University approved this study (#2020-9).

RESULTS SECTION: Through the toe spreading tests and gait analysis, the denervation model showed no neuromuscular reconnection within the sciatic nerve innervation area up to the third week postoperatively during this experimental period. The results of muscle wet weight and minimal ferret diameter of gastrocnemius muscle fibers also showed predominant atrophy in the IM+DN and DN groups compared to the IM group (Fig 1). Significant tendon lengthening occurred in the IM group at 2 weeks compared with IM+DN ($p = 0.0245$), but there were no significant differences between the 3 groups at 3 weeks (Fig 2-A). Tendon CSA in the IM group was significantly bigger than in the IM+DN group only at 3 weeks ($p = 0.0245$) (Fig 2-B). The tendon maximum force was significantly higher in the IM group than in the IM+DN and DN group at 3 weeks ($p = 0.0245$), but there were no significant differences between the stress (Fig 2-C). Picrosirius red staining at 2 weeks showed less extension between the tendon ends in the IM+DN group, in which mechanical stress was completely removed, but the collagen fibers between the tendon ends were thin and indistinct. At 3 weeks, red mature collagen fibers were extensively observed in the IM and DN groups, while less mature green and yellow fibers were observed in the IM+DN group (Fig 3).

DISCUSSION: Manipulation of mechanical stress on the tendon with or without joint motion and muscle contraction after Achilles tendon suture repair showed different results. At 2 weeks post-surgery, a significant elongation of the tendon was observed in the IM group compared to the IM+DN group, in which mechanical stress was completely removed and a widened gap at the tendon stump was observed. This suggests that mechanical stress from muscle contraction contributes to tendon elongation until two weeks post-surgery. At 3 weeks post-surgery, there was no significant difference in tendon length among the three groups (IM, IM+DN, and DN). The increase in CSA and the images of picrosirius red staining of the tendons in the IM group at 3 weeks post-surgery, as well as the increase in maximum force, indicated that the tendons remodeled into thicker and stronger tendons due to the retention of muscle contraction. Insufficient tendon strength recovery, as shown in the IM+DN and DN groups, subsequently resulted in tendon elongation, which may be more likely to occur. These findings suggest that mechanical stress applied to healing tendons induces detrimental elongation, contributes to beneficial tendon strength recovery, and may prevent tendon lengthening in the later healing phase. Therefore, the mechanism of tendon elongation appears to vary based on the healing stage of the tendon.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlighted the importance of timing in applying effective muscle contraction and contributed to the timing and establishing novel rehabilitation protocols that result in better ankle joint functional recovery after Achilles tendon rupture.

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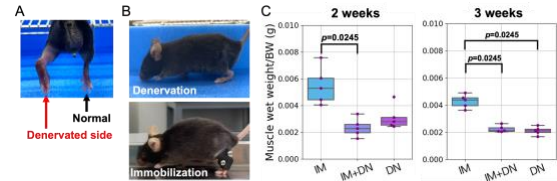


Fig 1. (A) Toe spreading test. In the lower extremity of the IM+DN and DN groups that underwent sciatic nerve transection, active spreading movements of the toes were inhibited, which confirming paralysis in the innervated area. (B) Ankle dorsiflexion was observed during walking in the DN group, while the IM group kept their ankle in the middle position. (C) Comparison of the muscle atrophy by normalizing muscle wet weight by body weight.

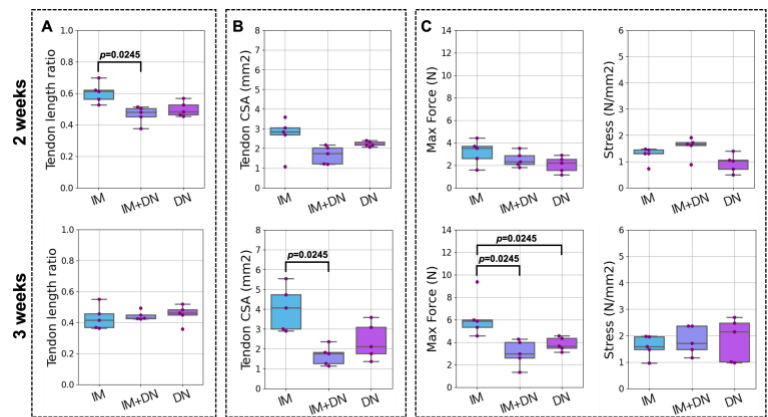


Fig 2. Evaluation results of tendon structure and mechanical properties at 2 and 3 weeks after surgery. Only those with significant differences are shown the P-values (<0.05) in the respective graphs. (A) Tendon length ratio calculated by Achilles tendon length/ Gastrocnemius muscle and Achilles tendon length. (B) Tendon cross-sectional area (CSA) calculated by width \times thickness. (C) Tendon tension testing results. Maximum force to failure and stress (Max force per unit of CSA).

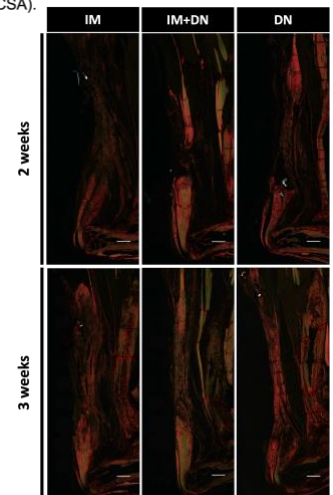


Fig 3. Picrosirius red stained section of Achilles tendon. Images viewed with circularly polarized light. Scale bar = 500 μm .