Multidisciplinary Evaluation of Treatments for Achilles Tendon Ruptures During Early Healing in an Animal Model

**Introduction**

Achilles tendon ruptures are common and devastating injuries, resulting in significant pain, disability, and healthcare costs. Despite the higher risk for complications and increased costs, operative treatment is often assumed to provide superior outcomes compared to non-operative treatment. However, recent reviews have suggested that outcomes for Achilles ruptures are not necessarily superior with surgical treatment, depending on the rehabilitation protocol (e.g., duration of immobilization and exercise). To elucidate the basic mechanical, structural, and biological mechanisms governing these clinical outcomes, it is necessary to evaluate the role of surgical intervention and rehabilitation strategies throughout the healing process. Therefore, the objective of this study was to investigate the effects of surgical repair and hind limb immobilization on joint function and early Achilles tendon healing following complete transection. We hypothesized that repaired tendons with more aggressive rehabilitation would have superior mechanical, structural, histological, and functional properties compared to non-repaired tendons and compared to those with moderate rehabilitation.

**Methods**

Sprague Dawley rats (N = 100) received two weeks of treadmill training (up to 60 minutes at 10m/min) (IACUC approved). All animals then underwent surgery to remove the central plantaris longus tendon and received blunt midsubstance transection to their right Achilles tendon. Animals were then randomized into repaired (R) (Modified Kessler approach) (n = 50) or non-repaired (NR) (n = 50) groups, and all hind limbs were immobilized in plantar flexion. These groups were further divided into aggressive (1 week immobilization (IM1), 1 week cage activity, 1 week exercise) or moderate (3 weeks immobilization (IM3)) rehabilitation (n = 25/group). Functional evaluation (n = 10-24/group) of passive ankle joint range of motion (ROM) and stiffness was completed prior to surgery and after 3 weeks of healing using a custom torque cell and accelerometer-based device on anesthetized animals. All ex vivo assays were performed after 3 weeks of healing.

After sacrifice, the Achilles tendon-foot complex was carefully removed en bloc, fine dissected, measured for cross sectional area (CSA), and gripped. Tendons were then loaded at 1N in a PBS bath while a series of sagittal B-mode high frequency ultrasound images (HFUS) were acquired using a 40MHz scanner (MS550D; VisualSonics, CA) (n = 10-11/group). Tendons were then mechanically tested (n = 10-11/group) with a protocol consisting of stress relaxation (6% strain), a low-load dynamic frequency sweep (ranging from 0.1 to 10 Hz), and fatigue testing (~10-75% of ultimate failure load) at 2Hz using a sinusoidal waveform until failure (Instron Electropuls 3000). For histological analysis, Achilles samples were processed using standard paraffin procedures (n = 8/group). Sagittal sections (7 µm) were collected and tendon samples were stained with hematoxylin and eosin (H&E).

Functional ankle joint properties (i.e., resting joint angle at zero torque, toe and linear ankle stiffness, and ankle ROM) for both dorsiflexion and plantar flexion were evaluated. Achilles tendon percent relaxation, dynamic modulus (E’), tanδ, hysteresis, and cycles to failure were computed from mechanical testing data. Echogenicity mean and standard deviation were evaluated from the HFUS images for the injury region. H&E stained sections were imaged at the injury site of each tendon at 200X and were graded by three blinded investigators for cellularity and cell shape. One-way ANOVAs were used to evaluate the effects of treatment on mechanical and structural properties. Significant relationships (α = 0.05) were analyzed with post hoc Student’sT-tests with Bonferroni corrections. For histological grading, non-parametric Kruskal-Wallis tests were performed.

**Results**

After three weeks of healing, the R,IM1 group achieved a resting joint angle position closest to pre-surgery values (Figure 1A). Decreased dorsiflexion ROM (Figure 1B) and increased ankle stiffness (Figure 1C,D) was observed in
moderate rehabilitation groups. Tendon CSA was increased in the R tendons compared to NR tendons, but IM3 decreased the CSA in these groups. Quasi-static tendon mechanical testing revealed a decreased percent relaxation in the R groups (Figure 2A). No differences in toe-region dynamic modulus or tan δ were observed. Interestingly, more than 70% of the IM3 tendons, though none of the IM1 tendons, failed while ramping to the fatigue loading protocol. NR tendons demonstrated increased cycles to failure, |E*|, and hysteresis during fatigue loading compared to R tendons (Figure 2B-D). Ultrasonic tendon evaluation revealed that the IM3 groups had increased echogenicity mean compared to IM1 groups (Figure 3). Surprisingly, there were no significant differences found in cellularity and cell shape between groups.

Discussion

Early Achilles tendon healing following a variety of common clinical treatment methods was evaluated. Joint IM had a larger effect on ankle stiffness and ROM in dorsiflexion than repair strategy. Tissue echogenicity, a surrogate of tendon structure, indicated that longer IM duration resulted in more hyperechoic tendon. It is possible that IM promotes deposition of a stiffer matrix, but a return to exercise is necessary to allow full recovery of fatigue resistance. Superior fatigue mechanics in the IM1 group is in agreement with the suggested benefits of early rehabilitation for optimal Achilles tendon healing. Future work will relate organizational measures from HFUS to tendon fatigue mechanical properties. Additional studies will evaluate the optimal treatment combinations for moderate and late healing, and relate functional and structural measures acquired to ex vivo mechanical properties.

Significance

This study begins to define the scientific basis for treatment methods already used clinically, but not yet optimized for early Achilles tendon healing. Specifically, we evaluated the 1) role
of repair and specific IM protocols and 2) use of functional measures for return to activity criteria following Achilles tendon rupture. Taken together, this study demonstrated that aggressive rehabilitation leads to improved ankle function and tendon mechanics after early healing. Interestingly, within this aggressive rehabilitation group, R tendons demonstrated improved functional outcomes, but tendon mechanical integrity was better in NR tendons. Ultrasonic evaluation showed promise to detect changes in healing capacity between groups with different rehabilitation strategies.

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References