Immobilization Angle Effects on Tendon Healing in Achilles Tendon Rupture

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Introduction

Management of Achilles tendon ruptures in the elderly and overweight population is most commonly nonoperative[1] given their higher surgical complication rate. Nonoperative treatment includes immobilizing the ankle in plantarflexion, which is thought to improve ruptured tendon end apposition, and has been shown to produce the best outcomes when combined with early physical therapy.[2] However, the effect of ankle position during immobilization on Achilles tendon healing and limb function is unknown. Therefore, the objective of this study was to investigate the effects of the ankle immobilization angle, with or without angle manipulation, as well as immobilization time on hindlimb function and Achilles tendon mechanical, structural and histological properties 6 weeks after injury in a rat model. We hypothesized that a more dorsiflexed immobilization would result in inferior tendon properties and function compared to full ankle plantarflexion immobilization, and that this effect would be exacerbated with increased time spent immobilized. Additionally, we hypothesized that manipulating the ankle into a more dorsiflexed position during immobilization would improve tendon properties and function.

Methods

Study Design

Male Sprague-Dawley rats (n = 128) received 2 weeks of increasing treadmill acclimation (at 10m/min, up to 60min/day, 5 days/week)[3](IACUC approved) prior to surgical blunt midsubstance transection of the right Achilles tendon and plantaris tendon resection. Animals were then assigned to one of eight hindlimb immobilization groups (n = 16/group) before being sacrificed at 6 weeks post-injury. Immobilization was performed for 1 or 3 weeks at 160°, 90°, or 20° (Figure 1). Two additional groups were manipulated halfway through a 3-week immobilization period, from either 160° to 90° or from 90° to 20°.

In vivo Assays (Pre-injury, 4 & 6 weeks post-injury)

Hindlimb ground reaction forces and gait metrics were quantified using an instrumented walkway.[4]

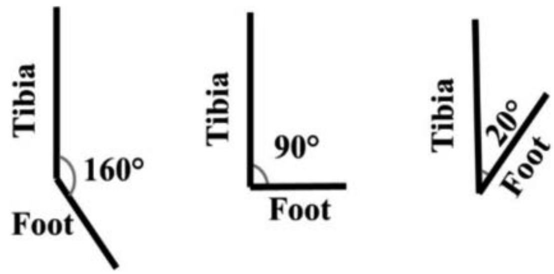

Figure 1. Immobilization position. Tibiopedal angle used to define ankle position. Rat ankle range of motion (10-160deg), was divided into three positions for immobilization.

Ex vivo Assays (6 weeks post-injury)

After sacrifice, the Achilles tendon-foot complex was carefully dissected (n = 10/group), measured for cross-sectional area, and secured in testing fixtures. Tendons were assessed for collagen fiber organization (circular standard deviation) and fiber density (echogenicity) using high frequency ultrasound while loaded at 1N in a PBS bath.[5] These same tendons were mechanically tested (n = 10/group) to evaluate their relaxation, low-strain frequency response (0.1 to 10Hz), and fatigue properties using mechanical and optical testing data.[6] Additional tendon samples were harvested at time of sacrifice, processed by paraffin procedures (n = 5-6/group) and stained with Hematoxylin-Eosin and Safranin-O/Fast Green to measure cell density, nuclear shape, glycosaminoglycan content, and tendon length. *Analysis:* Data was analyzed in a blinded fashion, with comparisons to the clinical standard of full plantarflexion at 160°. Manipulated immobilization was compared to non-manipulated immobilization of the same starting angle and duration. Normally distributed data was evaluated with Student's t-test or one-way ANOVAs, and functional data that was collected over time was assessed using two-way ANOVAs. Significant relationships ($p < 0.05$) were analyzed with post-hoc Student's t-tests with Bonferroni correction for multiple comparisons.

Results

90º Immobilization

There was no difference between 90° and 160° immobilization with regard to Achilles tendon length, histology, or echogenicity following 1 or 3 weeks of immobilization. With 1 week of 90°

immobilization, there was increased collagen fiber alignment (decreased CSD) (Figure 2) and stiffness (5% fatigue life), with decreased hysteresis (5 & 50% fatigue life), laxity (95% fatigue life) and stride length as compared to 160° (full plantarflexed) immobilization. With 3 weeks of 90° immobilization there was also increased alignment (Figure 2) and decreased hysteresis (5% fatigue life) compared to 160° immobilization. Additionally, 3 weeks of 90° immobilization had increased stiffness (95% fatigue life) (Figure 3) and stride length with decreased laxity (5, 50 & 95% fatigue life) and cycles to failure compared to 160° immobilization. Increasing the time immobilized at 90° from 1 to 3 weeks resulted in increased cycles to failure, stride length and echogenicity and decreased propulsion force, but no changes in other mechanical properties, cross-sectional area, tendon length, alignment, or histology metrics.

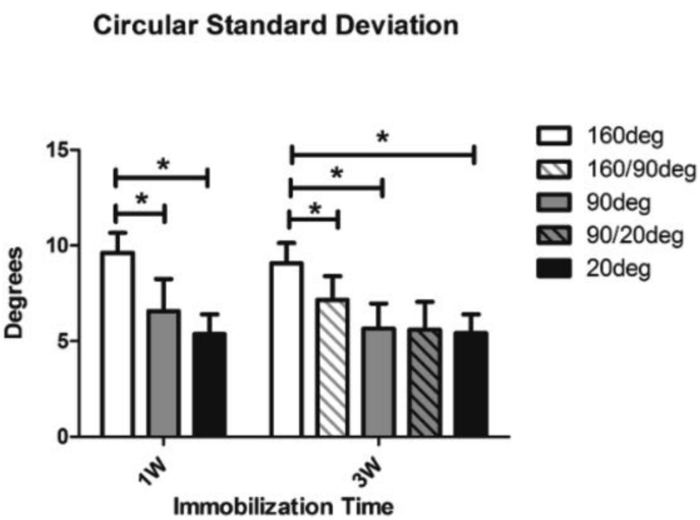

Figure 2. Tendon alignment. Using sequential HFUS images, tissue orientation is assessed. Decreased circular standard deviation indicates increased alignment. Asterisks indicate significant differences ($p < 0.05$).

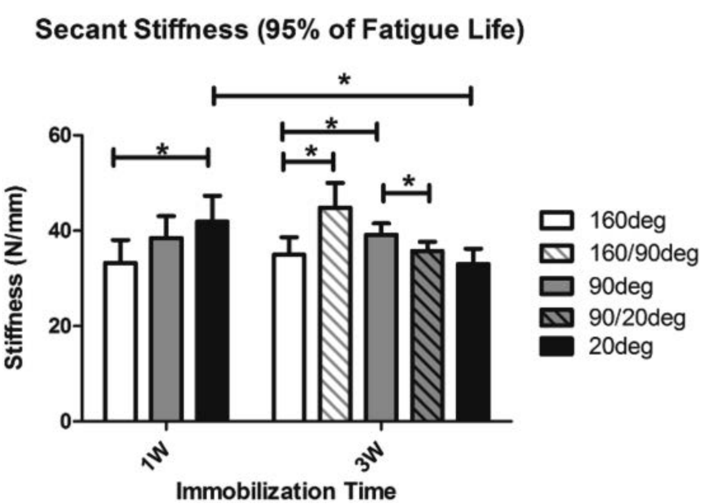

Figure 3. Late fatigue life stiffness. Calculated from force and displacement data at 95% fatigue life, where increased values indicate greater resistance to deformation. Asterisks indicate significant differences ($p < 0.05$).

20° Immobilization

Animals immobilized at 20° had increased tendon length, alignment (decreased CSD) (Figure 2) with decreased hysteresis (5% fatigue life) and laxity (50 & 95% fatigue life) compared to those immobilized at 160° regardless of immobilization duration. Additionally, with 1 week at 20° of immobilization there was increased stiffness (95% fatigue life) (Figure 3) and a sustained reduction in propulsion force measured at 4 (28% decrease) and 6 (22% decrease) weeks post-injury, compared to those in 160° immobilization. Increasing time immobilized at 20° from 1 to 3 weeks was associated with an increase in tendon cross sectional area and hysteresis (5% fatigue life) while also having a decrease in stiffness (5, 50 & 95% fatigue life) (Figure 3) and modulus (5, 50 & 95% fatigue life) but no differences in gait, histology, tendon length or HFUS parameters.

Angle Manipulation (160°-90°, 90°-20°)

Changing the ankle immobilization angle from 160° to 90° (increased dorsiflexion) midway through a 3-week immobilization showed increased stiffness (5, 50 & 95% fatigue life) (Figure 3), alignment (Figure 2) and Safranin-O staining, with decreased echogenicity, hysteresis (5% fatigue life) and cycles to failure but no change in tendon length or gait as compared to those that remained immobilized at 160°. Moving the ankle from 90° to 20° midway through the 3 week immobilization period resulted in increased tendon length, with decreased stiffness (50 & 95% fatigue life) (Figure 3) and stride length, but no changes in histology, cross sectional area, cycles to failure, laxity, modulus or HFUS parameters when compared to those that remained immobilized at 90° for 3 weeks.

Discussion

Achilles tendon properties and hindlimb function are influenced by the position and duration of immobilization. After just 1 week of immobilization at 20°, there was Achilles tendon lengthening and a consistent loss of propulsion force, perhaps due to early consolidation of scar tissue. This relation between Achilles tendon lengthening and reduction in plantarflexion strength and muscle activation has also been seen in humans, impacting function.[7,8] Dorsiflexing the ankle during the immobilization period from 160° to 90° produced a stiffer and more aligned tendon but no functional changes compared to remaining at 160°. However, immobilization angle manipulation from 90° to 20° caused a decrease in stiffness compared to remaining at 90°. These findings could be explained by the partially dorsiflexed foot providing the animal the ability to weight bear, without excess tendon end diastasis. Lastly, although increased tendon alignment at 90° or 20° immobilization resulted in decreased echogenicity, potentially reflecting changes in matrix density or collagen content, it also suggests improved collagen organization. Future work will investigate changes in muscle associated with immobilization angle following Achilles tendon rupture, given clinical changes seen in muscle activation[8] and volume [9] even with surgical repair.

Significance

Dorsiflexed immobilization can be used to enhance the tissue properties and function of healing Achilles tendon ruptures. However, excess dorsiflexion, even for short periods of time, can have lasting detrimental effects on the tendon and gait.

Acknowledgments

Aided by a grant from the Orthopaedic Research and Education Foundation with funding provided by the Dr. Dane and Mrs. Mary Louise Miller Endowment. This study was supported by NIH (R01AR064216, P30AR050950, T32AR007132, and TL1TR000138) and the NSF GRFP. We thank Courtney Nuss, Cori Riggin, Adam Pardes, Jessica Johnson, Zak Beach for their assistance.

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