Quantitative Comparison of Three Rat Models of Achilles Tendon Injury: A Multidisciplinary Approach

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Introduction

The Achilles tendon, while the strongest and largest tendon in the body, is frequently injured. Even after surgical repair, patients risk re-rupture and can have long-term deficits in function, with the rate of return to pre-injury level of activity reported to be as low as 16%[1]. Animal models of tendon injury are essential for understanding physiological processes of tendon repair and for testing the effects of potential therapeutics[2]. We have adapted and utilized three rat models of Achilles tendon injury (complete, full-thickness tear with post-operative immobilization, partial tear with post-operative immobilization, and a partial tear without post-operative immobilization). However, comparisons of the effects of these injuries on tendon mechanics and ankle joint function have not previously been made. Therefore, the objective of this study was to quantitatively define and compare the effects and relative impact on tendon properties and ankle function of the three Achilles tendon injury models. We hypothesized that animals receiving a complete tear would have inferior mechanical properties and ankle function compared to those receiving a partial tear, and that immediate loading after a partial tear would improve post-operative mechanical properties and ankle function compared to immobilized tendons.

Methods

144 adult male Sprague-Dawley rats (400-450 g) were used (IACUC approved). Animals underwent either full-thickness, blunt complete transection and repair of the right Achilles tendon[3] (with one week of post-operative plantarflexion immobilization (CT+IM, n = 48) or full-thickness, partial-width transection (1.5 mm biopsy punch in center of tendon) without repair[4] (with one week of post-operative plantarflexion, PT+IM, n=48, or without IM, PT-IM, n = 48). Animals were sacrificed at 1, 3, or 6 weeks (n = 16/group/time point). Animals in 6 week groups underwent longitudinal in vivo ambulatory and passive ankle joint mechanics assessments[3]. At sacrifice, the Achilles-calcaneus complex was dissected out (n = 6/group/time point) and processed for histological analysis. All other animals (n = 10/group/time point) were frozen at -20°C and thawed for dissection prior to cross-sectional area measurement using a custom laser device and mechanical testing using a load controlled fatigue testing protocol (including frequency sweeps at 0.1Hz, 1Hz, 5Hz, and 10Hz, and fatigue cycling from 5 to 35N cycles at2 Hz until failure)[3]. Post-test, tendons were scanned using μCT at a 21μm resolution to assess for presence of heterotopic ossification (HO) within the healing tendon. Statistical comparisons were made between the CT+IM and PT+IM group and between the PT+IM and PT-IM group at each time point. Comparisons for mechanics, functional assessments, collagen fiber organization, and μCT metrics were made using one way ANOVAs with Bonferroni post-hoc tests. Histological comparisons were made using Kruskal-Wallis tests.

Results

Mechanical Properties

At 3 and 6 weeks post-injury, cross-sectional area was larger for CT+IM tendons compared to PT+IM (Figure 1A). PT+IM modulus was significantly greater than CT+IM at 1 and 6 weeks, but was significantly lower than PT-IM at 3 weeks (Figure 1B). Similar differences were also seen in stiffness (Figure 1C). Dynamic frequency sweeps at 0.125% strain also determined similar differences in dynamic modulus at all tested frequencies (data not shown). PT-IM tendons withstood significantly more fatigue cycles before failing than PT+IM tendons at 3 and 6 weeks, and only PT−IM tendons were able to produce a reliable fatigue response at 3 weeks (Figure 1D,E). Tissue modulus (Figure 1E) and both secant and tangent stiffness (data not shown) measured during fatigue testing were greater in PT+IM tendons than in CT+IM at 6 weeks, but there was no difference between PT groups in these metrics at this time (Figure 1E).

Histology

No differences were determined in cell number (cellularity), nuclear shape, or collagen organization. μCT: The presence of heterotopic ossification was observed in almost all samples in all groups at all time points (no differences between models, data not shown). Bone volume was significantly higher in CT+IM tendons than PT+IM tendons at six weeks (Figure 2A);

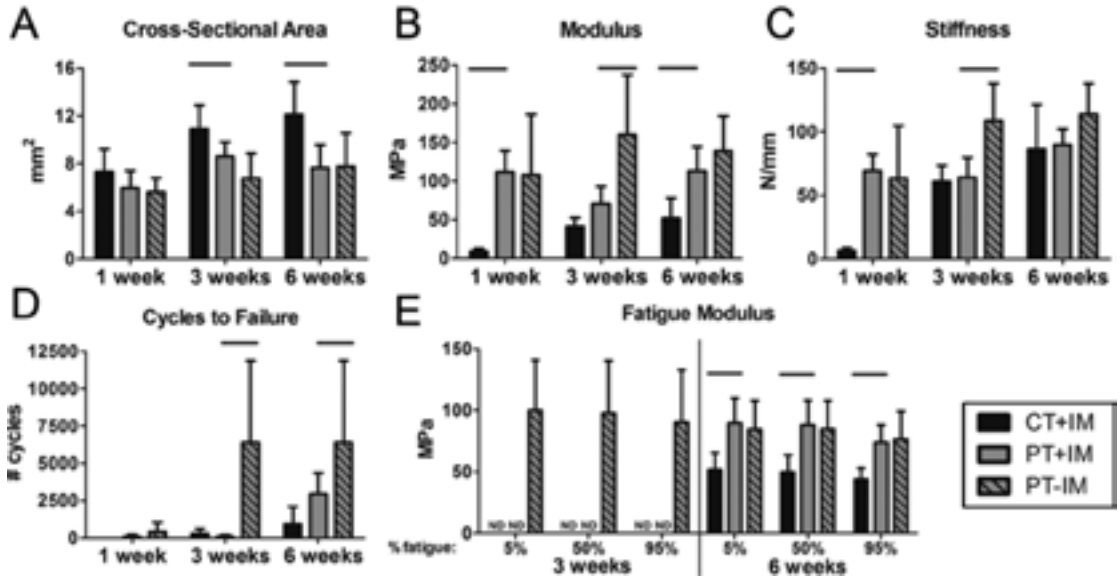

Figure 1. Mechanical Properties**.** Injury model affects **(A)** tendon cross-sectional area at 3 and 6 weeks; both injury mode and IM alter **(B)** tissue modulus and **(C)** tendon stiffness; immediate load bearing improves **(D)** cycles to failure at 3 and 6 weeks; and CT decreases **(E)** fatigue modulus at 6 weeks. ND: data was not able to be collected. Bars: $p < 0.025$.

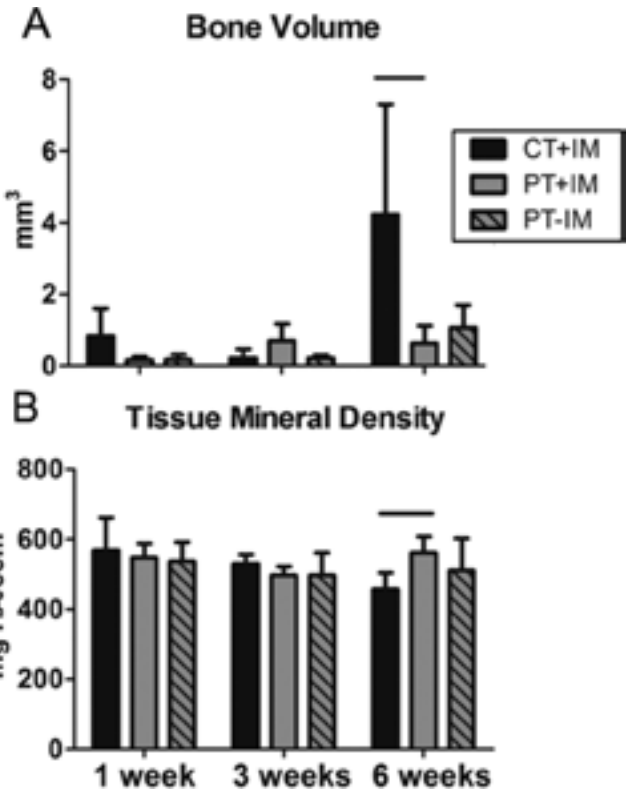

Figure 2. uCT Properties. **(A**) CT+IM showed **(A)** increased heterotopic bone volume but **(B)** decreased tissue mineral density at 6 weeks post-injury. Bars: $p < 0.025$.

however, this mineralized tissue had decreased tissue mineral density (Figure 2B).

Functional Assessments

Ankle joint stiffness and range of motion (ROM) through dorsiflexion were significantly altered in CT+IM and PT+IM groups (Figure 3A,B).Ankles from complete tendon tears were stiffer than both partial tear groups at 14 days post-injury, but by 6 weeks, were only stiffer than the PT−IM group (Figure 3A). In contrast, CT+IM and PT+IM groups had similarly diminished dorsiflexion ROM (~60% decrease) at 14 days (Figure 3B). PT+IM joints regained significantly more ROM by 6 weeks, while CT+IM joints did not recover (Figure 3B). Few differences existed in plantarflexion parameters (data not shown). CT+IM animals also had significantly slower rate of loading (Figure 3C) and longer stance time (data not shown)

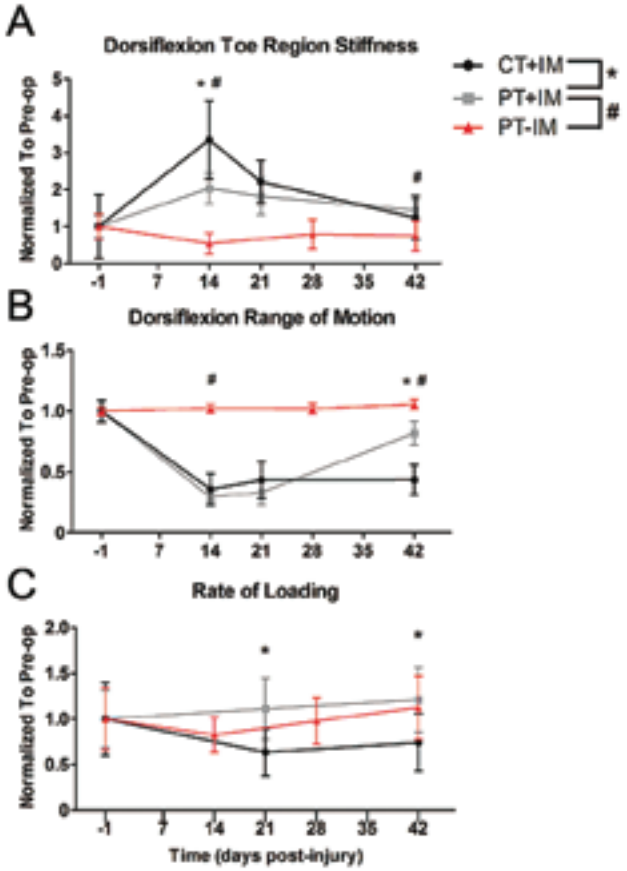

Figure 3. Functional Assessments. Passive joint testing demonstrated that **(A)** dorsiflexion stiffness was increased and **(B)** dorsiflexion ROM was decreased for CT+IM group post-injury. Ambulatory analysis showed that **(C)** rate of loading was decreased in the CT+IM group. post-injury. Sig difference notations labeled in legend ($p < 0.025$).

during ambulation than PT+IM, even though overall speed was increased at 6 weeks (data not shown).

Discussion

This study investigated differences in ankle function, tendon mechanics, and HO in three different models of Achilles injury. All models were reproducible and had distinct effects on measured parameters. Injury severity (CT vs PT) had a drastic influence on tendon healing, with complete tear causing diminished ankle mobility and decreased tendon mechanics throughout post-injury time points compared to partial tears. Changes in loading rate and stance time of the injured limb indicate that CT animals are altering ambulation patterns more severely, which may be due to loss of function or increased pain[5]. CT tendons also contained significantly more HO than PT tendons. However, differences in bone density between groups suggest that the mechanisms of HO development or maturation may vary between models. One week of plantarflexion IM had a strong effect on animals receiving a partial-width injury. Most notably, tendons in the PT+IM group failed extremely early during fatigue cycling 3 weeks post-injury (113 $\pm$ 85 cycles), prohibiting fatigue analysis. Surprisingly, a partial tear injury without immobilization had no effect on ankle range of motion through dorsiflexion at any time point, while PT+IM animals demonstrated diminished function at all post-injury time points. Together, these results indicate that even short-term immobilization may impair healing and increase ankle stiffness in partial Achilles tears in rats. Future studies will investigate long-term effects of these models.

All three models of Achilles injury could be useful for tendon healing investigations, chosen based on the prospective applications of a potential therapeutic. This work also sheds light on the universal occurrence of heterotopic ossification after surgically-induced injury in a rat Achilles tendon, as well as the potentially detrimental effects of complete immobilization/unloading on partial Achilles tears.

References

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