Muscle Adapts Dynamically Following Acute Achilles Tendon Rupture in a Rat Model

Nabeel Salka, BS
Pankti Bhatt, BS
Benjamin Freedman, PhD
Tyler Morris, MD
Zakary Beach, BS
Joshua Gordon, MD
Louis Soslowsky, PhD

McKay Orthopedic Research Laboratory, Philadelphia, PA

Introduction

Achilles tendon ruptures are common musculoskeletal injuries with an incidence of 15 to 55 per 100,000 person-years[1]. Although substantial research has evaluated Achilles tendon properties following injury[2], a paucity of data exists on the effects of Achilles surgical repair and return to activity timing on gastrocnemius and soleus muscle properties. Previous studies found that rats experiencing longer immobilization times and surgical treatment exhibit lower muscle TNF-alpha and a higher collagen 1:3 ratio at 3 weeks post injury[3]. However, early changes in muscle that lead to this finding, as well as later changes in muscle architecture, remain unknown. Therefore, the objective of this study was to evaluate muscle fiber size and remodeling for up to 6 weeks post injury. We hypothesized that at early time points, Achilles tendon repair would result in increased muscle MMP activity, and at later time points, delayed return to activity would result in reduced muscle fiber size and increased MMP activity.

Materials and Methods

Male Sprague-Dawley rats (n = 222) at 16-weeks of age were used (IACUC approved). Animals received 2 weeks of treadmill training (up to 60 min at 10 m/min) followed by surgical removal of the right plantaris longus tendon and blunt transection of the right Achilles tendon. Tendons were either repaired (modified Kessler) (R) or non-repaired (NR). All right ankle joints were subsequently immobilized in plantar flexion. Rats experienced immobilization for different lengths of time, returning to activity 1 week (RTA1), 3 weeks (RTA3), or 6 weeks (RTA6) after injury (Figure 1). Animals were sacrificed prior to injury (n = 7), as well as at 1 (n = 33), 3 (n = 74), and 6 weeks (n = 108) post-injury. Upon sacrifice, the right gastrocnemius-soleus muscle complex was harvested, embedded in optimum cutting temperature (OCT) compound, flash frozen, and sectioned transversely at 10 μm. Sections were stained with Laminin and Dapi, imaged, and analyzed for fiber size using the SMASH application[4]. Nuclear number was measured using Image J (NIH, v1.48). Muscle tissue was also excised from the gastrocnemius, flash frozen, and quantitatively evaluated for MMP activity (Sensolyte 520 Generic MMP Assay Kit) using a human MMP-13 standard. Prior to MMP activity quantification, samples were normalized for protein concentration using a bicinchoninic acid (BCA) assay (Pierce BCA Protein Assay, Fisher Scientific). Two-way ANOVAs with Fisher's post-hoc tests were performed for multiple comparisons, while Student's t-tests were performed for pairwise comparisons.

Results

At 1 and 3 weeks post-injury, fiber size was unaffected by repair or RTA. However, at 6 weeks post-injury, delayed return to activity (RTA6) resulted in a significantly smaller muscle fiber size when compared to RTA1 and RTA3 groups (Figure 2). No differences in nuclear number were found. MMP activity was significantly elevated in the muscles of repaired tendons as early as 1 week post-injury (Figure 3A). However, by 3 weeks, no significant effects were seen (Figure 3B). Interestingly, at 6 weeks, RTA1 showed decreased MMP levels regardless of surgical treatment (Figure 3C). Between 1 and 6 weeks post-injury, MMP activity increased significantly more in RTA3 and RTA6 when compared to RTA1, while also increasing more with non-repair in RTA3 (Figure 3D).

Discussion

The decrease in muscle fiber size following 6 weeks of immobilization is consistent with

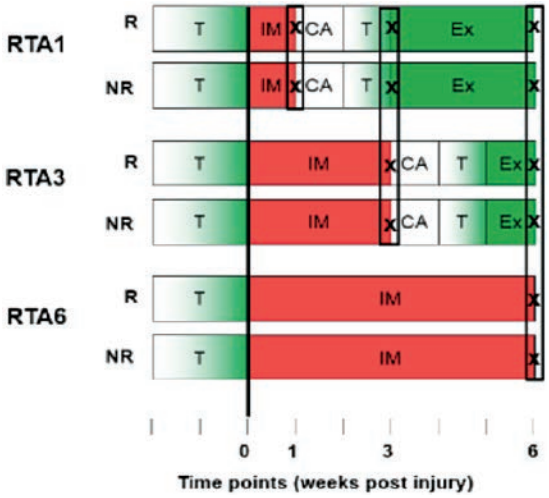

Figure. 1. Study Design. R: Repaired, NR: Non-Repaired, T: Treadmill training, IM: Immobilization, CA: Cage Activity, Ex: Exercise, X: Sacrifice of 18 animals. Boxes indicate the time points post injury evaluated.

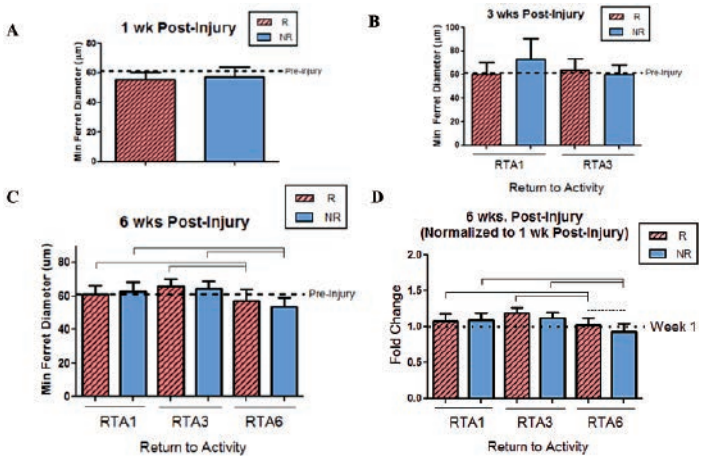

Figure 2. Muscle Fiber Size. R: Repair, NR: Non-Repair, RTA1,3,6: Return to activity after 1,3,6 weeks of cast immobilization respectively. sig. $p < 0.05$, trend $p < 0.1$. Data presented as means and standard deviations.

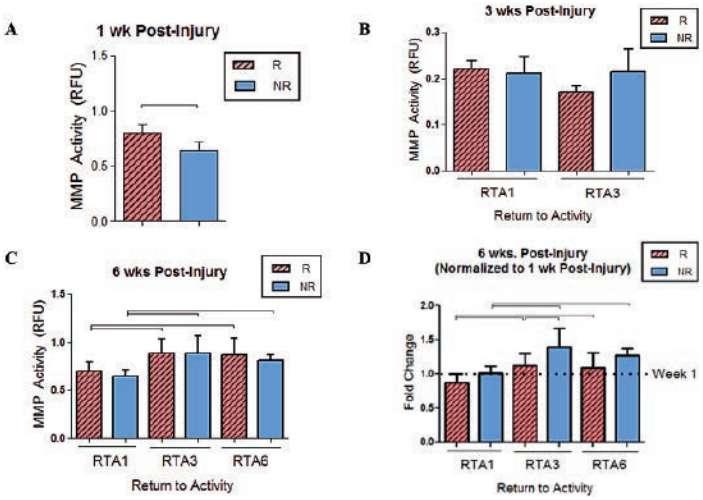

Figure 3. MMP Activity. R: Repair, NR: Non-Repair, RTA1,3,6: Return to activity after 1,3,6 weeks of cast immobilization respectively. sig. $p < 0.05$ Data presented as means and standard deviations.

clinical findings[5]. Prolonged immobilization also resulted in inferior mechanics in NR tendons[6], suggesting that an earlier return to activity provides advantages to both tendon and muscle health. Increased MMP activity in muscle of repaired tendons early in healing highlights the likely dependence of muscle architecture on tendon treatment. However, the absence of differences in muscle collagen content with repair 3 weeks post-injury[3] points to the potential involvement of regulating factors (e.g., TIMPs) and collagen synthesis between 1 and 3 weeks post-injury[7]. Interestingly, the current data suggests that RTA timing can significantly influence MMP activity at later time points. These changes may result from the new loading environment imposed by the injured tendon or additional MMP activity expected from longer periods of immobilization[7]. Either way, the role these differences in MMP activity have in influencing muscle health is unclear, because the extent and nature of matrix turnover caused by higher MMP levels is yet to be identified. Future work will continue to evaluate muscle remodeling by measuring muscle collagen content and uncovering the role of inflammation following Achilles tendon injury.

Conclusions

Although surgical repair initiates MMP activity early in Achilles tendon healing, return to activity time has a stronger effect on muscle properties at later time points by changing fiber size and regulating MMP activity.

Acknowledgements

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References

1. Huttunen TT, Kannus P, Rolf C, Felländer-Tsai L, Mattila VM. Acute achilles tendon ruptures: incidence of injury and surgery in Sweden between 2001 and 2012. *Am J Sports Med.* 2014 Oct;42(10):2419-23. doi: 10.1177/0363546514540599. Epub 2014 Jul 23. PubMed PMID: 25056989.

2. Freedman, BR *et al.,* *JOR,* 2016. In press.

3. Morris TM *et al.,* 2016. *ORS* Abstract.

4. Smith LR, Barton ER. SMASH—semi-automatic muscle analysis using segmentation of histology: a MATLAB application. *Skelet Muscle.* 2014 Nov 27;4:21. doi: 10.1186/2044-5040-4-21. eCollection 2014. PubMed PMID: 25937889; PubMed Central PMCID: PMC4417508.

5. Häggmark T, Eriksson E. Hypotrophy of the soleus muscle in man after achilles tendon rupture. Discussion of findings obtained by computed tomography and morphologic studies. *Am J Sports Med.* 1979 Mar-Apr;7(2):121-6. PubMed PMID: 434290.

6. Freedman, BR *et al.,* 2016. In review.

7. Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev.* 2004 Apr;84(2):649-98. Review. PubMed PMID: 15044685.