Published in final edited form as:

J Biomech. 2017 May 03; 56: 55–60. doi:10.1016/j.jbiomech.2017.02.030.

Mechanical, histological, and functional properties remain inferior in conservatively treated Achilles tendons in rodents: Long term evaluation

Benjamin R Freedman, George W Fryhofer, Nabeel S Salka, Harina A Raja, Cody D Hillin, Courtney A Nuss, Daniel C Farber, and Louis J Soslowsky

McKay Orthopedic Research Laboratory, Philadelphia, PA

Abstract

Conservative treatment (non-operative) of Achilles tendon ruptures is suggested to produce equivalent capacity for return to function; however, long term results and the role of return to activity (RTA) for this treatment paradigm remain unclear. Therefore, the objective of this study was to evaluate the long term response of conservatively treated Achilles tendons in rodents with varied RTA. Sprague Dawley rats (n=32) received unilateral blunt transection of the Achilles tendon followed by randomization into groups that returned to activity after 1-week (RTA1) or 3-weeks (RTA3) of limb casting in plantarflexion, before being sacrificed at 16-weeks post-injury. Uninjured age-matched control animals were used as a control group (N=10). Limb function, passive joint mechanics, tendon properties (mechanical, histological), and muscle properties (histological, immunohistochemical) were evaluated. Results showed that although hindlimb ground reaction forces and range of motion returned to baseline levels by 16-weeks regardless of RTA, ankle stiffness remained altered. RTA1 and RTA3 groups both exhibited no differences in fatigue properties; however, the secant modulus, hysteresis, and laxity were inferior compared to uninjured age-matched control tendons. Despite these changes, tendons 16-weeks post-injury achieved secant stiffness levels of uninjured tendons. RTA1 and RTA3 groups had no differences in histological properties, but had higher cell numbers compared to control tendons. No changes in gastrocnemius fiber size or type in the superficial or deep regions were detected, except for type 2× fiber fraction. Together, this work highlights RTA-dependent deficits in limb function and tissue-level properties in long-term Achilles tendon and muscle healing.

Keywords

Fatigue; Mechanics; Collagen; Function; Extracellular matrix

Corresponding Author: Louis J. Soslowsky, PhD, McKay Orthopaedic Research Laboratory, University of Pennsylvania, 424 Stemmler Hall; 3450 Hamilton Walk, Philadelphia, PA 19104-6081, T: 215-898-8653; F: 215-573-2133, soslowsk@upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Contributions: All authors were fully involved in the study and preparation of the manuscript. The manuscript has been read and approved by all of the authors.

Conflicts of Interest

The authors have no conflicts of interest to report.

Introduction

Achilles tendon ruptures are common and devastating injuries that affect 15 to 55 per 100,000 individuals yearly (Huttunen et al., 2014). Conservative treatment (non-operative) is suggested to produce equivalent capacity for return to function compared to surgical repair (Freedman et al., 2014; Lantto et al., 2015). Long term results of conservative management, however, remain unclear. Although modern functional conservative treatment may achieve favorable Achilles tendon total rupture scores (ATRS) and a return to pre-injury activity levels (Ecker et al., 2016; Hufner et al., 2006; Karkhanis et al., 2010; Young et al., 2014), other studies examining ankle torque, 3D gait, and muscle strength have suggested that functional deficits persist from months (Brorsson et al., 2016; Metz et al., 2009) to years (Olsson et al., 2011; Rosso et al., 2015; Tengman and Riad, 2013) post-injury. Additionally, early functional weight-bearing may be beneficial compared to non-weight-bearing rehabilitation protocols (McNair et al., 2013) and may create superior tendon material properties in the short term (Freedman et al., 2016a).

Although clinical studies provide insight into functional deficits that may remain following long durations post-injury, they are unable to provide detailed analysis of tissue quality beyond approximations of tendon modulus. Functional assessment such as the hop test (Chiodo et al., 2010) and gait analysis only approximate tendon strength and are unable to fully capture the load bearing potential of healing tendon in a manner that is both sensitive and specific. To overcome these limitations and to standardize the injury and post-injury rehabilitation, we developed a rat animal model of Achilles tendon injury (Freedman et al., 2016a; Freedman et al., 2016b). Combining conservative treatment with early return to activity (RTA) resulted in increased injured limb weight-bearing, improved range of motion and ankle stiffness, better fatigue resilience, and greater tissue cross sectional area at both 3- and 6-weeks post-injury (Freedman et al., 2016a). However, it remained unknown whether the early benefits of activity would persist throughout long-term healing or prove detrimental to limb function, Achilles tendon properties, and gastrocnemius/soleus muscle properties. Understanding the long-term biomechanical effects of conservative treatment and RTA on joint and tissue properties is essential to fully understand the effects of this treatment paradigm.

The purpose of this study was to evaluate the long term response of conservatively treated Achilles tendons in rodents with varied RTA. We hypothesized that tendon and muscle properties would be superior with earlier RTA compared to delayed RTA, but both injured groups would remain inferior to uninjured controls 16-weeks post-injury. We also hypothesized that no differences in limb functional outcomes would exist between groups by 16-weeks post-injury, but early RTA would have accelerated return to normal limb function.

Methods

Study Design

Male Sprague Dawley rats (n=42) at 16-weeks of age were used (IACUC approved). Animals were housed in a conventional facility (12 hour light/dark cycles), fed standard

chow, and provided water ad libitum. Animals had two weeks of treadmill training (Freedman et al., 2016a) prior to surgical intervention using sterile techniques under anesthesia (Isoflurane) to bluntly transect the right Achilles tendon midsubstance and resect the plantaris longus tendon. Animals were given a single dose of buprenorphine (0.08 mL, 0.3mg/mL) prior to surgery, which was repeated every 12-hours until 3-days post-surgery. Animals were randomized into groups that returned to activity after 1-week (RTA1) or 3-weeks (RTA3) post-injury. Hind limb immobilization in plantarflexion was achieved using stirrups, a 3-D printed splint, Webril padding, CoFlex, and poly(methyl-methacrylate), with all casts checked daily and replaced weekly at minimum (Freedman et al., 2016a). Uninjured age-matched animals (n=10) allowed only cage activity were used as controls. Animals were euthanized 16-weeks post-injury (mass: 618±59g) (CO₂ inhalation), and tissues were harvested for ex vivo assays as described previously (Freedman et al., 2016a; Freedman et al., 2016b; Pardes et al., 2016).

In vivo Functional Assays

Ground reaction forces and temporal patterns for the right hind limb were used to evaluate active limb function prior to surgery, and at 4-, 6-, 10-, and 16-weeks post-injury (Freedman et al., 2016a; Freedman et al., 2016b; Fryhofer et al., 2016). Briefly, animals ambulated across an instrumented walkway containing load/torque cells and a camera, and all animals were acclimated four times prior to formal data collection. Ground reaction forces (lateral, braking, propulsion, vertical) and spatiotemporal properties (step width, stride length, velocity) were computed using MATLAB (Mathworks; Natick, MA). Ground reaction force measurements were calculated as percent animal body-weight (%BW), and animals were weighed prior to each functional assessment time point.

Following functional ambulatory assessment, passive ankle joint range of motion (ROM) and stiffness (n=10–16/group) were assessed on anesthetized animals prior to injury using a custom torque cell and orientation device (Freedman et al., 2016a). Ankle measurements consisted of two sets of three plantarflexion/dorsiflexion cycles from which the average total ROM from plantarflexion to dorsiflexion was computed. Additionally, due to nonlinearity in the torque-angle response, bilinear curve fitting was used to quantify the toe and linear joint stiffness into dorsiflexion and plantarflexion (Freedman et al., 2016b).

Mechanical Testing

Tendons (n=10/group) were fine dissected, measured for cross sectional area with a laser-based device (Favata, 2006), and prepared for viscoelastic, quasi-static, and fatigue testing, as described (Freedman et al., 2016a). Briefly, custom fixtures were used to grip the bone-tendon unit to maintain the Achilles tendon-foot complex in a perpendicular orientation during loading. These fixtures were mounted to a testing frame (Electropuls E3000, Instron, Norwood, MA) while specimens were submerged in a 1×, 37°C PBS bath. Mounted specimens were mechanically loaded, and force and displacement data were acquired using WaveMatrix (Instron, Norwood, MA), and processed using MATLAB. Achilles tendon percent relaxation, dynamic modulus ($|E^*|$), $\tan\delta$, optical toe- and linear- modulus, hysteresis, and laxity were evaluated (Freedman et al., 2016a; Freedman et al., 2013; Freedman et al., 2015b; Gordon et al., 2014).

Tendon and Muscle Histology

After animal euthanasia, Achilles tendons (n=6/group) were immediately harvested and processed by standard paraffin procedures (Freedman et al., 2016a). Sagittal sections (7 μ m) were stained with Hematoxylin-Eosin (H&E) and Safranin-O and Fast Green (SAF-O) to evaluate cellularity, nuclear shape, and glycosaminoglycan (GAG) content. Images of the H&E-stained injury site (FOV: 0.87mm $\times$ 0.65mm) were evaluated by three blinded investigators using a scale of 1 to 3 for cellularity (1, low; 2, moderate; 3, high) and 1 to 3 for nuclei shape (1, spindle shaped; 2, mixed; 3, rounded). Full-length images of SAF-O stained tendon sections were obtained by stitching of several smaller images (FOV: 2.14 $\times$ 1.61mm) and were evaluated for GAG intensity (1, blue; 2, light red; 3, light to dark red). Additionally, the gastrocnemius/soleus muscle complex was harvested, sectioned axially (10 μ m), and stained for antibodies against laminin (L9393, Sigma Aldrich) and myosin heavy chain (MyHC) types 1, 2a, and 2b (type 1: BA-D5; type 2a: SC-71; type 2b: BF-F3), as described (Fryhofer et al., 2016; Pardes et al., 2016). The anti-MyHC antibodies were obtained from the Developmental Studies Hybridoma Bank, created by the National Institute of Child Health and Development of the National Institutes of Health (Schiaffino, S; University of Iowa, Department of Biology; Iowa City, IA).

Analysis

Functional ankle joint properties (ankle ROM and stiffness) for both dorsiflexion and plantarflexion were evaluated. Achilles tendon viscoelastic, quasi-static, and fatigue properties were computed (Freedman et al., 2016a). The tendon midsubstance was evaluated for cell density, nuclear shape, and GAG staining through grading by three independent and blinded investigators. Deep and superficial muscle regions were analyzed for fiber size (min Feret diameter) and fiber type distribution using the SMASH application (Smith and Barton, 2014). Two-way ANOVAs were used to evaluate the effects of time and group on functional properties, followed by post hoc t-tests with Bonferroni corrections. One-way ANOVAs followed by post hoc t-tests with Bonferroni corrections were used to compare mechanical and muscle properties between groups. Kruskal-Wallis tests with post hoc Dunn's tests were used to compare histological scoring between groups.

Results

Functional deficits due to injury were dependent on both time and RTA. At 4-weeks post-injury, normalized vertical ground reaction force was decreased in RTA1 and RTA3 groups (Figure 1A), but by 6-weeks post-injury had returned to age-matched control levels. In contrast, the normalized stride width in the RTA1 group was not different than uninjured control tendons at 4-weeks post-injury; however, by 10 weeks post-injury, both RTA1 and RTA3 groups exhibited increased stride width compared to control (Figure 1B). RTA was not a significant factor for the normalized propulsion force, braking force, or lateral force (Figure S1A–C). Although there were no differences in normalized stride length or step velocity between groups in early healing, by 16 weeks post-injury, RTA1 and RTA3 groups took larger steps (relative to pre-injury) compared to their age-matched controls (Figure S1D,E). Quantitative measures of ankle ROM and stiffness revealed that the total ROM was transiently decreased in both RTA1 and RTA3 groups compared to control animals at 4-

weeks post-injury (Figure 1C). Despite no differences in ROM, joint stiffness into dorsiflexion, remained greater in RTA3 compared to RTA1, and for both groups at 16 weeks was greater than control (Figure 1D, S2A). Stiffness into plantarflexion was also increased compared to control levels during early healing, but decreased for RTA3 animals by 10-weeks post-injury (Figure S2B,C).

Although functional deficits were present for certain properties and RTA protocols at 16 weeks post-injury, mechanical properties remained inferior in both RTA1 and RTA3 groups. Compared to the uninjured control group, RTA1 and RTA3 groups had increased cross sectional area (Figure 2A) and decreased dynamic modulus ($|E^*|$) (Figure 2B). However, no differences in the viscoelastic properties percent relaxation or $\tan\delta$ were detected between groups following stress relaxation (Figure S3). The secant modulus (material property measured during fatigue loading) was decreased in RTA1 and RTA3 groups compared to control (not shown), while the secant stiffness (structural property) was not different between groups (Figure 2C). In addition, tendon laxity (a measure of tendon lengthening during fatigue loading) (Figure 2D) was elevated in RTA1 and RTA3 groups compared to control levels.

Histologically, RTA1 and RTA3 groups had increased cellularity compared to control tendons (Figure 3A) but exhibited no differences in cell shape or SAF-O staining (Figure 3B). Muscle staining revealed that RTA1 animals had decreased fraction of type 2x fibers compared to control and RTA3 animals, without differences in the fraction of type 1, type 2a, or type 2b fibers (Figure 4A,B). No changes in fiber size were detected (not shown).

Discussion

This study investigated whether the functional and mechanical benefits of conservative treatment with early return to activity would persist in the long-term or, alternatively, prove detrimental. We used an established animal model of Achilles tendon transection and post-injury hind limb immobilization (Freedman et al., 2016a; Freedman et al., 2016b; Fryhofer et al., 2016) to carefully control the activity paradigm of animals for longitudinal functional and tissue-level follow-up. As most studies examining long-term follow-up in Achilles tendon healing have been completed in humans, this work provides fundamental insight into the tissue-level mechanical and histological changes that exist following injury. This detail may provide a mechanical mechanism for potential deficits in limb function observed clinically in humans.

Our long-term functional outcomes for conservatively treated tendons were in general agreement with clinical literature in humans (Olsson et al., 2011; Young et al., 2014). Although many properties returned to control levels by 16-weeks post-injury (ground reaction forces, step width, velocity, total ROM, plantarflexion linear stiffness), other properties remained inferior (dorsiflexion toe and linear stiffness, plantarflexion toe stiffness, stride length) in either one or both RTA paradigms. Changes in dorsiflexion and plantarflexion stiffness reflect passive soft tissue resistance of the ankle joint (including during heel raise), which could affect gait. Additionally, our hypothesis that RTA1 animals would return to normal function faster than RTA3 animals was supported by measures in

stride length, dorsiflexion toe and linear stiffness, and plantarflexion toe stiffness. Sustained increases in ankle stiffness compared to control levels has also been shown due to non-weightbearing protocols 6 and 12-months post-injury in humans (Barfod et al., 2015). Further, these increases in ankle stiffness may contribute to the deficits in heel-rise (Brorsson et al., 2016) and reduced peak plantarflexion torque (Rosso et al., 2015) observed in humans. The improved hindlimb loading was better than that described in previous clinical literature (Olsson et al., 2011; Tengman and Riad, 2013), potentially due to the fact that an initial period of full plantarflexion casting was used to reduce tendon lengthening. Additionally, the function assessed was voluntary and not during high dynamic loading.

Mechanical properties improved compared to earlier time points in our rat model (Freedman et al., 2016a; Freedman et al., 2016b), similar to humans (Schepull et al., 2012). However, these mechanical properties remained significantly lower than controls. Interestingly, although material properties were inferior in RTA1 and RTA3 tendons, structural mechanical properties were similar to controls. This suggests that healing tendons can achieve mechanical stiffness values similar to healthy tissues, and may explain the lack of differences in muscle properties between groups. Histologically, RTA1 and RTA3 groups displayed a larger scar cross sectional area and higher cell counts compared to control tissues. The low GAG staining observed at 16-weeks post-injury contrasts to early RTA groups at 6-weeks (Freedman et al., 2016b), suggesting that a more tendon-like phenotype (Freedman et al., 2015a) may be achieved with additional recovery.

The majority of muscle properties assessed were not different from control levels 16-weeks post-injury, suggesting that conservative treatment does not have adverse effects on long-term muscle properties at the fiber level. Reduced activity is known to preferentially affect the slow oxidative type 1 (rather than fast type 2) fibers (Cho et al., 2016). In contrast, we found no differences in the percentages of type 1 and type 2a fibers in RTA1 and RTA3 animals compared to control, and a decrease in the fraction of type 2x fibers in RTA1 animals. Type 2x fibers are fast twitch glycolytic fibers that generate force/speed intermediate to type 2a and 2b fibers (Rockl et al., 2008). In general, the absence of major differences in the fiber type fractions and fiber size observed in our study was consistent with minimal differences in EMG readings that were observed several months following Achilles rupture in humans (Suydam et al., 2015).

A potential limitation of the study was that our injury model is surgical, which does not fully mimic *in vivo* injury onset. However, this model provides careful control of the injury, and allows evaluation of a host of *in vivo* and *ex vivo* assays not possible with humans. Additionally, this study did not investigate the specific biological mechanisms that prevented the treatment groups from regaining full functional, mechanical, or histological properties by 16-weeks post-injury. A previous study found decreased populations of stem/progenitor cells by 8-weeks post-injury in a similar rat model of Achilles injury (Runesson et al., 2015); thus, it is possible that healing rate may be reduced at a point, highlighting the need for potential therapeutics to continue to aid in longer-term healing. A final limitation is that the uninjured age-matched control animals did not undergo treadmill exercise. This control group was specifically chosen as a metric to compare to native untreated tendon. Future studies will examine structural properties and sex differences in long term Achilles healing.

In conclusion, as conservative management of Achilles tendon ruptures becomes more widely used, it is necessary to evaluate the long-term biomechanical effects of this treatment paradigm. Although hindlimb ground reaction forces and range of motion return to baseline levels by 16-weeks in this rodent model, ankle stiffness and tendon properties remain altered both mechanically and histologically, and gastrocnemius muscle properties may also be affected. Despite these changes, tendons 16-weeks post injury achieved many structural property characteristics (e.g., stiffness) of uninjured tendons, and overall differences created by early casting were minimal. This work aids evaluation of the scientific basis for Achilles tendon treatment methods used clinically following rupture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by NIH (R01AR064216, P30AR050950, T32AR007132, and TL1TR000138) and the NSF GRFP. We thank Pankti Bhatt, Adam Pardes, Ashley Rodriguez, and Cori Riggan for assistance.

Study approved by: University of Pennsylvania IACUC.

References

- Barfod KW, Bencke J, Lauridsen HB, Dippmann C, Ebskov L, Troelsen A. Nonoperative, dynamic treatment of acute achilles tendon rupture: influence of early weightbearing on biomechanical properties of the plantar flexor muscle-tendon complex-a blinded, randomized, controlled trial. *J Foot Ankle Surg.* 2015; 54:220–226. [PubMed: 25618802]
- Brorsson A, Olsson N, Nilsson-Helander K, Karlsson J, Eriksson BI, Silbernagel KG. Recovery of calf muscle endurance 3 months after an Achilles tendon rupture. *Scand J Med Sci Sports.* 2016; 26:844–853. [PubMed: 26283647]
- Chiodo CP, Glazebrook M, Bluman EM, Cohen BE, Femino JE, Giza E, Watters WC 3rd, Goldberg MJ, Keith M, Haralson RH 3rd, Turkelson CM, Wies JL, Raymond L, Anderson S, Boyer K, Sluka P, American Academy of Orthopaedic, S. Diagnosis and treatment of acute Achilles tendon rupture. *The Journal of the American Academy of Orthopaedic Surgeons.* 2010; 18:503–510. [PubMed: 20675643]
- Cho SH, Kim JH, Song W. In Vivo Rodent Models of Skeletal Muscle Adaptation to Decreased Use. *Endocrinol Metab (Seoul).* 2016; 31:31–37. [PubMed: 26996420]
- Ecker TM, Bremer AK, Krause FG, Muller T, Weber M. Prospective Use of a Standardized Nonoperative Early Weightbearing Protocol for Achilles Tendon Rupture: 17 Years of Experience. *Am J Sports Med.* 2016; 44:1004–1010. [PubMed: 26818449]
- Favata, M. Scarless healing in the fetus: Implications and strategies for postnatal tendon repair. University of Pennsylvania; Philadelphia: 2006.
- Freedman BR, Bade ND, Riggan CN, Zhang S, Haines PG, Ong KL, Janmey PA. The (dys) functional extracellular matrix. *Biochim Biophys Acta.* 2015a; 1853:3153–3164. [PubMed: 25930943]
- Freedman BR, Gordon JA, Bhatt PB, Pardes AM, Thomas SJ, Sarver JJ, Riggan CN, Tucker JJ, Williams AW, Zanes RC, Hast MW, Farber DC, Silbernagel KG, Soslowsky LJ. Nonsurgical treatment and early return to activity leads to improved Achilles tendon fatigue mechanics and functional outcomes during early healing in an animal model. *J Orthop Res.* 2016a
- Freedman BR, Gordon JA, Soslowsky LJ. The Achilles tendon: fundamental properties and mechanisms governing healing. *Muscles Ligaments Tendons J.* 2014; 4:245–255. [PubMed: 25332943]

- Freedman BR, Salka NS, Morris TR, Bhatt PR, Pardes AM, Gordon JA, Nuss CA, Riggan CN, Fryhofer GW, Farber DC, Soslowsky LJ. Temporal healing of Achilles tendons following injury depends on surgical treatment and return to activity time. 2016b In Review.
- Freedman BR, Sarver JJ, Buckley MR, Voleti PB, Soslowsky LJ. Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury. *J Biomech.* 2013
- Freedman BR, Zuskov A, Sarver JJ, Buckley MR, Soslowsky LJ. Evaluating changes in tendon crimp with fatigue loading as an ex vivo structural assessment of tendon damage. *J Orthop Res.* 2015b; 33:904–910. [PubMed: 25773654]
- Fryhofer GW, Freedman BR, Hillin CD, Salka NS, Pardes AM, Weiss SN, Farber DC, Soslowsky LJ. Post-Injury Biomechanics of Achilles Tendon Vary By Sex and Hormone Status. *J Appl Physiol* (1985). 2016 jap 00620 02016.
- Gordon J, Freedman B, Zuskov A, Iozzo R, Birk D, Soslowsky L. Achilles tendons from decorin- and biglycan-null mouse models have inferior mechanical and structural properties predicted by an image-based empirical damage model. *Journal of Biomechanics* In review. 2014
- Hufner TM, Brandes DB, Thermann H, Richter M, Knobloch K, Krettek C. Long-term results after functional nonoperative treatment of achilles tendon rupture. *Foot Ankle Int.* 2006; 27:167–171. [PubMed: 16539896]
- Huttunen TT, Kannus P, Rolf C, Fellander-Tsai L, Mattila VM. Acute achilles tendon ruptures: incidence of injury and surgery in Sweden between 2001 and 2012. *Am J Sports Med.* 2014; 42:2419–2423. [PubMed: 25056989]
- Karkhanis S, Mumtaz H, Kurdy N. Functional management of Achilles tendon rupture: A viable option for non-operative management. *Foot Ankle Surg.* 2010; 16:81–86. [PubMed: 20483140]
- Lantto I, Heikkinen J, Flinkkila T, Ohtonen P, Kangas J, Siira P, Leppilahti J. Early functional treatment versus cast immobilization in tension after achilles rupture repair: results of a prospective randomized trial with 10 or more years of follow-up. *Am J Sports Med.* 2015; 43:2302–2309. [PubMed: 26229048]
- McNair P, Nordez A, Olds M, Young SW, Cornu C. Biomechanical properties of the plantar flexor muscle-tendon complex 6 months post-rupture of the Achilles tendon. *J Orthop Res.* 2013; 31:1469–1474. [PubMed: 23649780]
- Metz R, van der Heijden GJ, Verleisdonk EJ, Tamminga R, van der Werken C. Recovery of calf muscle strength following acute achilles tendon rupture treatment: a comparison between minimally invasive surgery and conservative treatment. *Foot Ankle Spec.* 2009; 2:219–226. [PubMed: 19825777]
- Olsson N, Nilsson-Helander K, Karlsson J, Eriksson BI, Thomee R, Faxen E, Silbernagel KG. Major functional deficits persist 2 years after acute Achilles tendon rupture. *Knee Surg Sports Traumatol Arthrosc.* 2011; 19:1385–1393. [PubMed: 21533539]
- Pardes AM, Freedman BR, Fryhofer GW, Salka NS, Bhatt PR, Soslowsky LJ. Males have Inferior Achilles Tendon Material Properties Compared to Females in a Rodent Model. *Ann Biomed Eng.* 2016
- Rockl KS, Witczak CA, Goodyear LJ. Signaling mechanisms in skeletal muscle: acute responses and chronic adaptations to exercise. *IUBMB Life.* 2008; 60:145–153. [PubMed: 18380005]
- Rosso C, Buckland DM, Polzer C, Sadoghi P, Schuh R, Weisskopf L, Vavken P, Valderrabano V. Long-term biomechanical outcomes after Achilles tendon ruptures. *Knee Surg Sports Traumatol Arthrosc.* 2015; 23:890–898. [PubMed: 24162761]
- Runesson E, Ackermann P, Karlsson J, Eriksson BI. Nucleostemin- and Oct 3/4-positive stem/progenitor cells exhibit disparate anatomical and temporal expression during rat Achilles tendon healing. *BMC Musculoskelet Disord.* 2015; 16:212. [PubMed: 26290425]
- Schepull T, Kvist J, Aspenberg P. Early E-modulus of healing Achilles tendons correlates with late function: similar results with or without surgery. *Scand J Med Sci Sports.* 2012; 22:18–23. [PubMed: 20673250]
- Smith LR, Barton ER. SMASH - semi-automatic muscle analysis using segmentation of histology: a MATLAB application. *Skelet Muscle.* 2014; 4:21. [PubMed: 25937889]

- Suydam SM, Buchanan TS, Manal K, Silbernagel KG. Compensatory muscle activation caused by tendon lengthening post-Achilles tendon rupture. *Knee Surg Sports Traumatol Arthrosc.* 2015; 23:868–874. [PubMed: 23609529]
- Tengman T, Riad J. Three-Dimensional Gait Analysis Following Achilles Tendon Rupture With Nonsurgical Treatment Reveals Long-Term Deficiencies in Muscle Strength and Function. *Orthop J Sports Med.* 2013; 1 2325967113504734.
- Young SW, Patel A, Zhu M, van Dijk S, McNair P, Bevan WP, Tomlinson M. Weight-Bearing in the Nonoperative Treatment of Acute Achilles Tendon Ruptures: A Randomized Controlled Trial. *J Bone Joint Surg Am.* 2014; 96:1073–1079. [PubMed: 24990972]

Figure 1. Ambulatory Assessment

Group was a significant factor on the normalized vertical force, stride width, total ROM, and dorsiflexion linear stiffness. RTA1 and RTA3 groups had decreased vertical force 4-weeks post-injury compared to control levels and wider step widths that returned to control levels by 6-weeks post-injury. Additionally, although the total ROM returned to control levels by 10-weeks post-injury, stiffness into dorsiflexion remained elevated until 6-weeks post injury for RTA3 animals. Data shown as mean±standard deviation. Letters indicate significant differences ($p < 0.017$) between groups (a-CTRL v. RTA1; b-CTRL v. RTA3, c-RTA1 v. RTA3).

Figure 2. Mechanical Properties

RTA1 and RTA3 groups had increased (A) cross sectional area, decreased (B) $|E^*|$, and increased (D) laxity compared to uninjured control tendons 16-weeks post injury. $|E^*|$ was also increased in RTA1 tendons compared to RTA3 tendons. No differences in (C) secant stiffness were found between groups. Data shown as mean $\pm$ standard deviation. Lines indicate significant differences ($p < 0.017$).

Figure 3. Histological Evaluation

(A) Cellularity was increased in RTA1 and RTA3 tendons compared to uninjured control tendons when assessed at the midsubstance 16-weeks post-injury. No differences in (B) SAF-O intensity grade were found. Data shown as median $\pm$ interquartile range. Lines indicate significant differences ($p < 0.017$).

Figure 4. Muscle Immunofluorescence

(A) There were no differences in type 1, 2a, 2x, or 2b fibers between groups in the superficial region. (B) There was a lower percentage of type 2x fibers in RTA1 tendons compared to control and RTA3 groups. Data shown as mean $\pm$ standard deviation. Lines indicate significant differences ($p < 0.017$).