Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury ☆

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ABSTRACT

Achilles tendon injuries affect both athletes and the general population, and their incidence is rising. In particular, the Achilles tendon is subject to dynamic loading at or near failure loads during activity, and fatigue induced damage is likely a contributing factor to ultimate tendon failure. Unfortunately, little is known about how injured Achilles tendons respond mechanically and structurally to fatigue loading during healing. Knowledge of these properties remains critical to best evaluate tendon damage induction and the ability of the tendon to maintain mechanical properties with repeated loading. Thus, this study investigated the mechanical and structural changes in healing mouse Achilles tendons during fatigue loading. Twenty four mice received bilateral full thickness, partial width excisional injuries to their Achilles tendons (IACUC approved) and twelve tendons from six uninjured mice were used as controls. Tendons were fatigue loaded to assess mechanical and structural properties simultaneously after 0, 1, 3, and 6 weeks of healing using an integrated polarized light system. Results showed that the number of cycles to failure decreased dramatically (37-fold, $p < 0.005$) due to injury, but increased throughout healing, ultimately recovering after 6 weeks. The tangent stiffness, hysteresis, and dynamic modulus did not improve with healing ($p < 0.005$). Linear regression analysis was used to determine relationships between mechanical and structural properties. Of tendon structural properties, the apparent birefringence was able to best predict dynamic modulus ($R^2 = 0.88$–0.92) throughout healing and fatigue life. This study reinforces the concept that fatigue loading is a sensitive metric to assess tendon healing and demonstrates potential structural metrics to predict mechanical properties.

1. Introduction

Achilles tendon ruptures result in significant pain and disability with long recovery times and affect between 5.5 and 9.9 per 100,000 individuals each year in North America (Suchak et al., 2005), and the incidence of these acute and chronic injuries is rising (Maffulli et al., 1999; Suchak et al., 2005). Although previous studies have aimed to elucidate the mechanisms of injury and healing, the basic science and efficacy behind many treatments and rehabilitation protocols for Achilles tendon ruptures remains "weak" or "inconclusive" as cited by a recent comprehensive review by the American Academy of Orthopaedic Surgeons (AAOS, 2009). Current clinical tests evaluating Achilles tendon injuries cannot rigorously evaluate tendon mechanical properties directly *in vivo*, and thus rely on surrogate measures such as the hop test to approximate tendon strength (Chiodo et al., 2010). Given that the Achilles tendon typically performs at high and repetitive loads at or near failure (Fukashiro et al., 1995; Komi et al., 1992), the clinical importance of utilizing methods to test this tendon at physiological levels via fatigue loading becomes increasingly evident (Dourte et al., 2012; Dunkman, 2012; Fessel and Snedeker, 2009; Fung et al., 2009; Ikoma et al., 2013; Schechtman and Bader, 1997, 2002; Wang et al., 1995; Wren et al., 2003).

Cadaveric and animal studies have shown that the response of tendon to fatigue loading is marked by changes in stiffness and deformation (among other properties) that consists of three phases (Fig. 1A) (Fung et al., 2009; Wren et al., 2003). Specifically, tendon stiffness increases initially, reaches a maximum, and then gradually decreases. This gradual decrease in stiffness is attributed to accumulated sub-rupture damage, which ultimately leads to the dramatic increase in peak deformation and decrease in stiffness
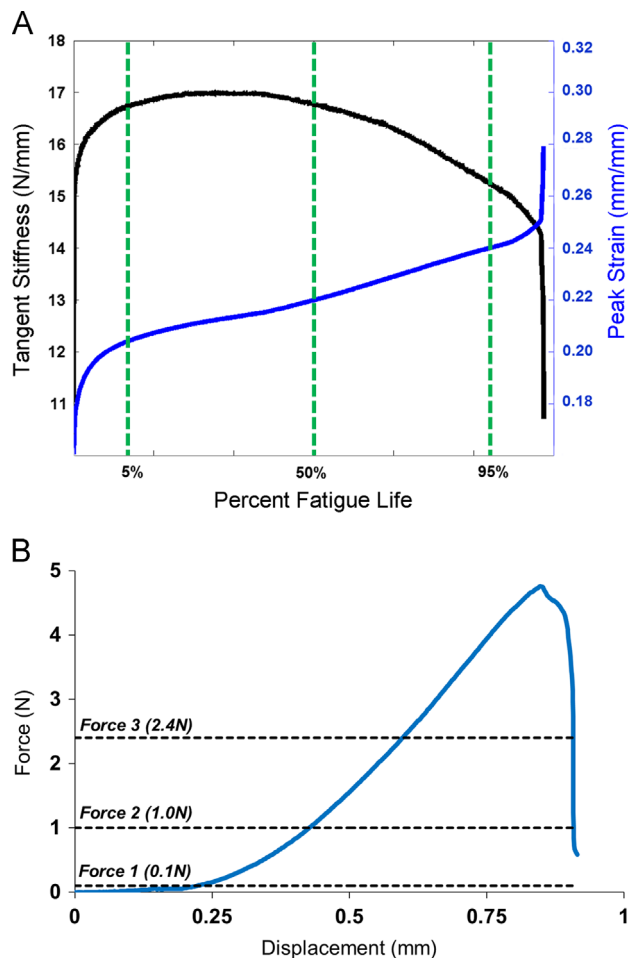
Fig. 1. (A) Typical mechanical response of a tendon undergoing fatigue loading. Briefly, changes in peak strain and stiffness undergo a three phase series of property changes leading to tendon rupture. 5%, 50%, and 95% indicate the time points of fatigue life from which mechanical parameters were evaluated. (B) Typical load displacement curve for an Achilles tendon during a ramp to failure. Dashed lines indicate the three loads that alignment maps were taken that approximate the toe, transition, and linear portions of the load displacement curve.

prior to failure (Fung et al., 2009, 2010; Wren et al., 2003). This induction of subfailure damage accumulation with repetitive loading is suggested as the primary benefit of fatigue testing over conventional quasi-static methods. Following injury, quasi-static test methods have shown decreased tissue stiffness, modulus, failure load, and increased tendon cross sectional area with healing (Gimbel et al., 2007). However, no study has examined whether these mechanical and structural property changes are also evident during fatigue loading and whether such assessment may be more indicative of *in vivo* tissue healing. Further, an often unstated assumption in histological studies of tendon healing is that alterations in tendon structure are closely associated with changes in tendon mechanical properties and function. Understanding how mechanical properties in injured tissue relate to changes in structure during healing in response to fatigue loading is therefore an important step to elucidating the mechanical and structural mechanisms leading to specimen failure and to better define benchmarks for mechanically robust tissue engineering constructs.

Therefore, the objectives of this study were to examine the mechanical and structural properties throughout the fatigue life of healing Achilles tendons, and to investigate whether structural properties assessed during fatigue loading could predict fatigue properties throughout healing. A mouse model was used to

carefully control the injury and quantify tendon fatigue and structural properties using state-of-the-art mechanical testing techniques to evaluate fatigue mechanics and structure simultaneously. We hypothesized that fatigue loading would decrease mechanical properties (dynamic modulus, stiffness, hysteresis, peak strain, and damage) in conjunction with altered structure (fiber realignment and birefringence) that partially recover with healing. We speculate that the new extracellular matrix synthesized through healing will increase tendon stiffness during fatigue loading. Our results should demonstrate the importance for fatigue and structural property assessment to evaluate tendon healing that may provide further benchmarks for image-based methods to assess tendon mechanical integrity.

2. Methods

2.1. Study design

A total of 60 tendons from 30 C57BL/6 mice were used in this University of Pennsylvania IACUC approved study. At approximately 120 days of age, 6 uninjured mice were euthanized as controls and 24 mice received bilateral excisional injuries to the midsubstance of their Achilles tendons under aseptic conditions (Beason et al., 2012). Briefly, a single skin incision parallel to the tibia and lateral to the Achilles tendon was made. A rubber-coated backing was placed deep to the Achilles tendon to provide support while a full thickness, partial width (∼50%) region was excised using a 0.5 mm biopsy punch. Following the injury, the skin was closed with suture, and the animals returned to cage activity. To evaluate the effects of healing, 6 animals were euthanized at random after 0, 1, 3, and 6 weeks following injury and immediately frozen at −20 °C.

2.2. Specimen preparation and tendon mechanical testing with integrated polarized light imaging

At the time of testing, animals were thawed and the Achilles tendon and calcaneus were carefully harvested, removing all musculature and surrounding soft tissue, and were hydrated in phosphate buffered saline (PBS). A laser device was used to measure tendon cross sectional area (Favata, 2006), and tendons were placed in custom fixtures to grip the calcaneus and tendon ends. Tendons were then tensile tested according to the following protocol in a PBS bath. Zero strain was set as the strain at preload. First, specimens were preconditioned between 0.1 and 0.2 N at 0.5 Hz for 30 cycles using a sinusoidal waveform on an Instron 5848 universal testing system (Instron Corp., Norwood, MA). To determine the necessary target loads for the load controlled fatigue test to simulate *in vivo* conditions, a preliminary study was conducted that determined the mean failure load of mouse Achilles tendons to be approximately 5 N. Thus, during fatigue loading, specimens were cycled under load control between approximately 20 and 75% of their ultimate tensile strength (1–3.8 N) at 1 Hz until failure or 15,000 cycles. This upper cycle limit was determined from pilot tests to be an assumed theoretical endurance limit. During loading, force and displacement data were acquired at 100 Hz using the WaveMaker (Instron, Norwood, MA) data acquisition software and analyzed using custom MATLAB code (Mathworks, Natick, MA). Several post processing parameters were computed: (1) maximum/minimum cyclic displacement and strain, (2) tangent stiffness (calculated as the slope between the maximum and minimum force and displacements for each cycle), (3) stress (calculated as the force divided by the cross sectional area), (4) dynamic modulus (calculated as the slope between the maximum and minimum stress and strain for each cycle, (5) hysteresis (defined as the area enclosed by the stress–strain curve for a cycle), (6) damage (defined as the ratio of displacement and gauge length at a set threshold to the tissue displacement and displacement at a set threshold after the first cycle of fatigue loading) (Duenwald-Kuehl et al., 2012; Provenzano et al., 2002), and (6) cycles to failure (defined as the number of cycles until specimen failure).

Simultaneous evaluation of tendon structure via polarized light imaging was also conducted. This polarized light system (Lake et al., 2009) integrated with the mechanical testing system consisted of a backlight, 90° offset rotating polarizer sheets (Edmund Optics, Barrington, NJ) on both sides of the test sample (polarizer and analyzer), and a GigE aca2040gm camera (resolution: 2048 × 2048 pixels) (Basler, Exton, PA). Sets of alignment maps (15–18 images) were taken prior to fatigue loading, after 10, 25, 50 cycles of fatigue loading, and at intervals of 100 cycles at three distinct loads (0.1 N, 1.0 N, 2.4 N). These loads were chosen to be indicative of the toe, transition, and linear regions of the mouse Achilles load displacement curve (Fig. 1B), and were investigated to evaluate the sensitivity of structural properties to load. This protocol was fully automated during the fatigue tests using analog outputs acquired by a data acquisition device (USB 6008, National Instruments, Austin, TX) at 100 Hz that was monitored by a custom