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Evaluating Changes in Tendon Crimp with Fatigue Loading as an *ex vivo* Structural Assessment of Tendon Damage

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Abstract

The complex structure of tendons relates to their mechanical properties. Previous research has associated the waviness of collagen fibers (crimp) during quasi-static tensile loading to tensile mechanics, but less is known about the role of fatigue loading on crimp properties. In this study (IACUC approved), mouse patellar tendons were fatigue loaded while an integrated plane polariscope simultaneously assessed crimp properties. We demonstrate a novel structural mechanism whereby tendon crimp amplitude and frequency are altered with fatigue loading. In particular, fatigue loading increased the crimp amplitude across the tendon width and length, and these structural alterations were shown to be both region and load dependent. The change in crimp amplitude was strongly correlated to mechanical tissue laxity (defined as the ratio of displacement and gauge length relative to the first cycle of fatigue loading assessed at constant load throughout testing), at all loads and regions evaluated. Together, this study highlights the role of fatigue loading on tendon crimp properties as a function of load applied and region evaluated, and offers an additional structural mechanism for mechanical alterations that may lead to ultimate tendon failure.

Keywords

imaging; patellar tendon; ligament; collagen; polarized light

Introduction

Tendons have a complex hierarchical structure that relates to their mechanical properties. For example, during mechanical loading a distinct nonlinearity or “toe” region is present that is often attributed to collagen fiber straightening or “uncrimping.”^{1–4} Alterations in tendon crimp may portend changes in mechanical properties. Structure-function

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relationships are therefore important to both provide an understanding of healthy tendon properties and serve as a guideline for injury assessment and treatment.

Although much research has investigated structure-function relationships during quasi-static tensile loading (e.g.,^{5; 6}), evaluation during dynamic loading has been limited.^{7; 8} Recently, crimp was visualized with polarized light imaging during dynamic loading.⁹ This technique, which consists of two polarizing sheets on either side of the tendon, depicts its birefringence, as a crimped waveform containing alternating patterns of bright and dark bands (Fig. 1).^{2-4; 9} This innovative approach allows whole tissue, region specific, and load dependent crimp properties, to be inferred simultaneously during a mechanical test without destructively altering the tendon. Crimp is believed to be ubiquitous in all tendons,¹⁰ and as a property, is strain dependent² and planar at this hierarchical level.^{4; 11} Understanding the process of uncrimping is significant as many tendons are believed to operate within the toe region of the stress-strain curve¹² and experience a wide range of *in vivo* strains and strain rates.

Studying structure-function relationships during dynamic loading at *high loads* is also important for elucidating potential mechanical mechanisms governing *fatigue-induced* damage and injury. Initially, mechanical fatigue properties in tendon highlighted the three-phase pattern of fatigue damage accumulation,¹³ but no structural evaluation was completed.¹³⁻¹⁵ Questions regarding the structural alterations contributing to the monotonic increases in peak cyclic strain during fatigue loading led to work utilizing *in vivo* and *ex vivo* animal models with structural image-based measures.¹⁶⁻²⁰ Although these previous studies have used various imaging modalities to study the accumulation and progression of fatigue induced structural alterations, such studies were not designed to simultaneously evaluate the load and region dependence of tendon crimp in the entire tissue. In addition, the imaging techniques utilized may be costly, time consuming, or destructive to the tissue.^{18; 21; 22}

The purpose of this study was to determine if tendon crimp could serve as an *ex vivo* structural and region dependent metric of fatigue-induced structural alteration. Specifically, we investigated fatigue-induced changes in crimp frequency and amplitude in the mouse patellar tendon using polarized light imaging. We hypothesized that crimp properties would increase with fatigue loading, show regional differences, and correlate with mechanical properties assessed during fatigue loading.

Methods

Experimental Design

Patellar tendons from C57BL/6 mice at 150 days of age (IACUC approved) were used (N=10–11) (*Level of Evidence: Basic Science Study*). While hydrated in phosphate buffered saline (PBS), the surrounding musculature was removed, and the patella-patellar tendon-tibia unit was prepared for mechanical testing.⁸ Cross sectional area was measured using a laser-based device.²³

Mechanical testing and image capture protocol

To test the effect of fatigue loading on tendon crimp properties, patellar tendons were secured in a mechanical testing setup while submerged in a PBS bath. Tendons were preloaded, preconditioned, imaged at three loads (representing the toe ($F=0.1\text{N}$), transition ($F=0.5\text{N}$), and linear regions ($F=2.0\text{N}$) of the force-displacement curve), and fatigue tested at 1Hz between 2 and 4N corresponding to 25–75% of the ultimate failure load using an Instron Microtester (Instron 5848, Norwood, MA) (Fig. 2A,B). A preliminary study determined that the mean failure load of mouse patellar tendons was approximately 5.5N, from which the necessary target loads were computed for the load controlled fatigue test. During loading, force and displacement data were acquired at 100 Hz and analyzed using custom MATLAB code (Mathworks, Natick, MA). Several post processing parameters were computed:¹⁷ 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); and 6) laxity (defined as the ratio of displacement and gauge length relative to the first cycle of fatigue loading, and assessed at constant load throughout fatigue testing).^{24; 25} Specifically, after each cycle of fatigue loading, the length (L_S) of the patellar tendon was measured at the load level of 2.5 N. The percent change in nonrecoverable length (laxity) relative to the tendon's original length (L_o) is therefore defined as $L = 100 * [(L_S - L_o) / L_o]$.^{17; 24–26}

For image capture of tendon crimp, a plane polariscope was integrated with the mechanical testing setup, consisting of a backlight, two linear polarizers surrounding the tendon, and a camera (Basler GigE aca2040gm camera (resolution: 2048x2048 pixels); Exton, PA) (Fig. 1). This provided a pixel size of 3 microns, and the total average length at baseline was 820 pixels (~2.4 mm). The polarizer (P) and analyzer (A) were crossed at 90° and were oriented at the angle θ at which maximal extinction in the dark crimp bands occurred at preload. In this setup, alternating light and dark bands appear in a crimped tendon. Images of the whole tendon were captured after 0, 10, 100, and 1000 fatigue loading cycles at loads described (Fig. 2A,B).

Image Processing

A custom program was created (MATLAB, Version: R2012a, Natick, MA) to quantify tendon crimp properties. Four regions of interest (ROIs) (200x30pixels) were chosen to represent the insertion and midsubstance, at the tendon center and lateral positions (Fig. 2C). These same ROIs were used for the analysis of all images throughout the fatigue test across all loads evaluated. A Gaussian low-pass filter was applied to the image within the ROI to enhance the visibility of light and dark bands. Next, intensity values were averaged across the ROI width to give an intensity profile as a function of the vertical axis of the region that was then high-pass filtered. The spectral power was determined using the Fast Fourier Transform (FFT), which was then integrated to determine the cumulative spectral power (CSP). Finally, the crimp frequency (F_{crimp}) was determined by taking the frequency at mean spectral power. Throughout specimen fatigue life, the CSP was evaluated at the F_{crimp}

to provide an optical measure of average crimp amplitude (A_{crimp}). All post-processing procedures were completed for all images acquired throughout specimen testing.

Statistical Evaluation

For statistical evaluation, one-way repeated measures ANOVAs followed by paired t-tests with Bonferroni corrections were used to evaluate the effect of fatigue loading on the change in crimp amplitude (A_{crimp}) and frequency (F_{crimp}) (SPSS, IBM SPSS Inc. Version 20, Armonk, New York). Two-way repeated measures ANOVAs were used to evaluate changes in region dependent crimp properties for each load and fatigue loading cycle evaluated. Significant relationships were assessed using post hoc paired T-tests (SPSS, IBM SPSS Inc. Version 20, Armonk, New York). One-way repeated measures ANOVAs were also used to determine if cycle number was a significant factor for the measured peak strain, tangent stiffness, hysteresis, and laxity. Pearson's correlation coefficients were used to determine if fatigue properties (i.e., peak strain, laxity, stiffness, hysteresis, and modulus) were correlated to the A_{crimp} and F_{crimp} . Significant relationships were defined as $\alpha = 0.05$ and trends $\alpha < 0.1$ after applying the appropriate Bonferroni correction factor.

Results

After 1000 fatigue loading cycles, all tendons attained the secondary phase of fatigue loading¹⁶ without rupture. Cycle number was a significant ($p < 0.001$) factor for peak strain, tangent stiffness, hysteresis, and laxity (Fig. 3). At baseline, there were 14–18 crimps per mm, and the crimp amplitude ranged from 2.9–4.2 a.u. at 0.1N, 2.2–3.5 a.u. at 0.5N, and 1.8–2.4 a.u. at 2.0N depending on the ROI chosen. As hypothesized, fatigue loading resulted in crimp properties that were dependent on cycle number and the region of interest evaluated. The A_{crimp} increased with fatigue loading (Fig. 4), and demonstrated load and region dependence. At low loads (0.1N), the A_{crimp} was greatest (Fig. 4A). Additionally, the A_{crimp} decreased at the higher loads evaluated (Fig. 4B,C). In contrast, the F_{crimp} decreased with fatigue loading, but this was region dependent (Fig. 5). Regional variation was also evident in A_{crimp} with fatigue loading (Fig. 4). The lateral region of the tendon demonstrated a larger increase in A_{crimp} after 10, 100, and 1000 cycles of fatigue life at both lower loads (0.1 and 0.5N) when compared to the center region ($p < 0.001$), but not at the higher load (2.0N) (Fig. 4C). Differences between the insertion and midsubstance regions were evident, but this response was load and cycle dependent.

Single linear regression analysis revealed that A_{crimp} was strongly correlated to laxity at all loads and regions evaluated ($r = 0.72$ – 0.89) (Fig. 6). In addition, A_{crimp} was correlated to tendon strain, but only at the higher load ($r = 0.53$) at both the center midsubstance and lateral insertion regions. No other fatigue mechanical properties correlated with A_{crimp} ($p > 0.05$).

F_{crimp} was not related to any mechanical property measured ($p > 0.05$).

Discussion

This study evaluated real-time tendon crimp and mechanical properties nondestructively during tendon fatigue life using a plane polariscope. Results demonstrated that fatigue loading altered crimp properties and that this response was both region and load dependent.

Such work is novel since it evaluated the effects of fatigue loading on tendon structure at several regions and loads simultaneously. This methodology provides further support for potential translation of other real-time structure-based assays, such as ultrasound,²⁷ into *in vivo* systems to assess tendon mechanics.

The A_{crimp} was region dependent, but region specific differences were muted at high loads. This supports the concept that crimp remains a primary factor at lower loads in the toe and transition regions of mechanical loading, but this response may be altered with fatigue loading. Furthermore, the regional difference in uncrimping across the tendon width and length, supports the observation that the structural response of collagen fibrils to loading is non-uniform.² Regional differences in crimp properties across the patellar tendon width may be expected as a result of physiologic *in vivo* function. Variation in fibril area fraction has been demonstrated across the patellar tendon thickness,²⁸ which may be due to the six degrees-of-freedom motion the patella²⁹ and adjacent patellar tendon experiences during knee flexion/extension. Changes in crimp properties across the tendon length may be explained by the transition in mechanical properties from the tendon midsubstance to its fibrocartilagenous insertion.³⁰ Similar changes in crimp properties with load have been observed in the supraspinatus.²

Several studies have measured structural changes in tendon following fatigue loading.^{16; 17; 21; 22; 31–33} Structural assessment following fatigue loading has ranged from histology,¹⁶ second harmonic generation,^{18–20; 31} polarized light imaging,¹⁷ scanning electron microscopy,³² and confocal imaging using photo bleaching.³⁴ However, few measures of tendon fatigue mechanics have been reported,^{22; 32; 34} and only one study reported correlations between structure changes in tendon and fatigue loading.¹⁷ Further, our observed changes in crimp properties throughout fatigue life were more dramatic than previous reports assessing disorganization with fatigue loading.^{20; 35; 36} It is possible that evaluation of tendon crimp alterations with fatigue loading provides a more sensitive and mechanistic description than other metrics, such as the damage area fraction.²⁰ In addition, recent studies have suggested that repeated subrupture loading results in fibril kinks that occur at distinct spacing intervals.³³ Such changes have been shown to primarily occur early during repeated loading at the nanostructural level,³² which was also observed in this study for A_{crimp} at the fascicle level. Thus, this provides support that fatigue loading alters collagen structure at several hierarchical levels of the tendon.

The strong relationship between A_{crimp} and tendon laxity demonstrates both the use of laxity²⁵ as a parameter for modeling the response of patellar tendon to fatigue loading, and A_{crimp} as an indicator of the progression of tendon laxity. The strong relationships between structural metrics and fatigue loading have not been reported previously, with only qualitative relationships presented.^{16; 18–21; 31; 34} Such changes may also be incorporated into structural fit fiber recruitment models³⁷ for the application of modeling tendon's response to fatigue loading.

The primary limitation of the study was that relationships linking the induction of fatigue loading with concurrent cellular mechanisms were not investigated.³⁸ Such effects likely have profound implications on the extracellular matrix, as well as the intracellular milieu in

the tendon that may promote matrix remodeling or degeneration. Another potential limitation is that tendon mechanical and structural recovery following fatigue loading was not investigated. However, all tendons followed the standard multiphase pattern of fatigue life.¹⁶ Pilot tests evaluating the effect of a 45 minute recovery period following 1000 cycles of our fatigue loading protocol showed that whole tendon strain recovered less than 5%, and that this recovery was muted within 25 cycles of fatigue loading (data not shown). Therefore, we believe our fatigue protocol to be mechanically damaging, and suggest that our measure of nonrecoverable laxity in this study can be considered metrics of mechanical damage.^{17; 24–26} Additionally, given that fluid comprises 70% of the tendon wet weight,^{39; 40} it is possible that the alterations in A_{crimp} observed were due to fluid exudation from the tendon.^{9; 41; 42} Although tensile stress can cause induced lateral contraction independent of uncrimping,⁹ this mechanism in the context of fatigue loading warrants future investigation. Finally, other factors may contribute to fatigue damage induction and progression, such as reductions in fibril continuity, and the role of glycosaminoglycans and other small matrix molecules.^{43; 44} Future studies will examine the effects of aging, additional tendon types,⁴⁵ pathology, and knockout models on fatigue mechanics and crimp properties.

In conclusion, this study revealed alterations in tendon crimp and mechanical properties nondestructively, and in real-time, during fatigue loading. This work may lead to improved diagnostic imaging methods based on tissue-level structural measures to assess injured and healing tendons, which may ultimately improve patient monitoring. Although periodic crimp in tendon has been studied using high-magnification optical microscopy,² electron microscopy,⁴⁶ optical coherence tomography,⁴⁷ and second harmonic generation,⁴⁸ its study using polarized light microscopy^{4; 9} has been limited. In light of our findings and the low cost of our system ($\$ < 1000$), this methodology presents both a robust and economic opportunity for understanding structure function relationships of tendons.

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References

1. Franchi M, Fini M, Quaranta M, et al. Crimp morphology in relaxed and stretched rat Achilles tendon. *J Anat.* 2007; 210:1–7. [PubMed: 17229278]
2. Miller KS, Connizzo BK, Feeney E, et al. Characterizing local collagen fiber realignment and crimp behavior throughout mechanical testing in a mature mouse supraspinatus tendon model. *J Biomech.* 2012; 45:2061–2065. [PubMed: 22776688]
3. Diamant J, Keller A, Baer E, et al. Collagen; ultrastructure and its relation to mechanical properties as a function of ageing. *Proc R Soc Lond B Biol Sci.* 1972; 180:293–315. [PubMed: 4402469]
4. Rigby BJ, Hirai N, Spikes JD, et al. The Mechanical Properties of Rat Tail Tendon. *J Gen Physiol.* 1959; 43:265–283. [PubMed: 19873525]
5. Soslowsky LJ, Thomopoulos S, Tun S, et al. Neer Award 1999. Overuse activity injures the supraspinatus tendon in an animal model: a histologic and biomechanical study. *J Shoulder Elbow Surg.* 2000; 9:79–84. [PubMed: 10810684]

6. Fox AJ, Schar MO, Wanivenhaus F, et al. Fluoroquinolones Impair Tendon Healing in a Rat Rotator Cuff Repair Model: A Preliminary Study. *Am J Sports Med.* 2014
7. Lujan TJ, Underwood CJ, Jacobs NT, et al. Contribution of glycosaminoglycans to viscoelastic tensile behavior of human ligament. *J Appl Physiol* (1985). 2009; 106:423–431. [PubMed: 19074575]
8. Dunkman AA, Buckley MR, Mienaltowski MJ, et al. Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biol.* 2013; 32:3–13. [PubMed: 23178232]
9. Buckley MR, Sarver JJ, Freedman BR, et al. The dynamics of collagen uncrimping and lateral contraction in tendon and the effect of ionic concentration. *J Biomech.* 2013; 46:2242–2249. [PubMed: 23876711]
10. Dale WC, Baer E. Fiber-Buckling in Composite Systems - Model for Ultrastructure of Uncalcified Collagen Tissues. *J Mater Sci.* 1974; 9:369–382.
11. Elliott DH. Structure and Function of Mammalian Tendon. *Biol Rev Camb Philos Soc.* 1965; 40:392–421. [PubMed: 14340913]
12. Maganaris CN, Paul JP. In vivo human tendon mechanical properties. *J Physiol.* 1999; 521(Pt 1): 307–313. [PubMed: 10562354]
13. Wang XT, Ker RF, Alexander RM. Fatigue rupture of wallaby tail tendons. *J Exp Biol.* 1995; 198:847–852. [PubMed: 9244805]
14. Schechtman H, Bader DL. In vitro fatigue of human tendons. *J Biomech.* 1997; 30:829–835. [PubMed: 9239568]
15. Wren TA, Lindsey DP, Beaupre GS, et al. Effects of creep and cyclic loading on the mechanical properties and failure of human Achilles tendons. *Ann Biomed Eng.* 2003; 31:710–717. [PubMed: 12797621]
16. Fung DT, Wang VM, Laudier DM, et al. Subrupture tendon fatigue damage. *J Orthop Res.* 2009; 27:264–273. [PubMed: 18683881]
17. Freedman BR, Sarver JJ, Buckley MR, et al. Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury. *J Biomech.* 2013
18. Fung DT, Sereysky JB, Basta-Pljakic J, et al. Second harmonic generation imaging and Fourier transform spectral analysis reveal damage in fatigue-loaded tendons. *Ann Biomed Eng.* 2010; 38:1741–1751. [PubMed: 20232150]
19. Ros SJ, Andarawis-Puri N, Flatow EL. Tendon extracellular matrix damage detection and quantification using automated edge detection analysis. *J Biomech.* 2013; 46:2844–2847. [PubMed: 24112781]
20. Sereysky JB, Andarawis-Puri N, Ros SJ, et al. Automated image analysis method for quantifying damage accumulation in tendon. *J Biomech.* 2010; 43:2641–2644. [PubMed: 20627302]
21. Fung DT, Wang VM, Andarawis-Puri N, et al. Early response to tendon fatigue damage accumulation in a novel in vivo model. *J Biomech.* 2010; 43:274–279. [PubMed: 19939387]
22. Thorpe CT, Riley GP, Birch HL, et al. Effect of fatigue loading on structure and functional behaviour of fascicles from energy-storing tendons. *Acta Biomater.* 2014; 10:3217–3224. [PubMed: 24747261]
23. Favata, M. Implications and Strategies for Postnatal Tendon Repair. University of Pennsylvania; 2006. Scarless Healing in the Fetus.
24. Duenwald-Kuehl S, Kondratko J, Lakes RS, et al. Damage mechanics of porcine flexor tendon: mechanical evaluation and modeling. *Ann Biomed Eng.* 2012; 40:1692–1707. [PubMed: 22399329]
25. Provenzano PP, Heisey D, Hayashi K, et al. Subfailure damage in ligament: a structural and cellular evaluation. *J Appl Physiol.* 2002; 92:362–371. [PubMed: 11744679]
26. Quinn KP, Lee KE, Ahaghotu CC, et al. Structural changes in the cervical facet capsular ligament: potential contributions to pain following subfailure loading. *Stapp Car Crash J.* 2007; 51:169–187. [PubMed: 18278597]
27. Riggin CN, Sarver JJ, Freedman BR, et al. Analysis of Collagen Organization in Mouse Achilles Tendon Using High-Frequency Ultrasound Imaging. *J Biomech Eng.* 2013

28. Hansen P, Haraldsson BT, Aagaard P, et al. Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *J Appl Physiol* (1985). 2010; 108:47–52. [PubMed: 19892929]
29. Freedman BR, Sheehan FT. Predicting three-dimensional patellofemoral kinematics from static imaging-based alignment measures. *J Orthop Res*. 2013; 31:441–447. [PubMed: 23097251]
30. Thomopoulos S, Marquez JP, Weinberger B, et al. Collagen fiber orientation at the tendon to bone insertion and its influence on stress concentrations. *J Biomech*. 2006; 39:1842–1851. [PubMed: 16024026]
31. Sereysky JB, Andarawis-Puri N, Jepsen KJ, et al. Structural and mechanical effects of in vivo fatigue damage induction on murine tendon. *J Orthop Res*. 2012; 30:965–972. [PubMed: 22072573]
32. Veres SP, Harrison JM, Lee JM. Repeated subrupture overload causes progression of nanoscaled discrete plasticity damage in tendon collagen fibrils. *J Orthop Res*. 2013; 31:731–737. [PubMed: 23255142]
33. Veres SP, Lee JM. Designed to fail: a novel mode of collagen fibril disruption and its relevance to tissue toughness. *Biophys J*. 2012; 102:2876–2884. [PubMed: 22735538]
34. Thorpe CT, Riley GP, Birch HL, et al. Fascicles from energy-storing tendons show an age-specific response to cyclic fatigue loading. *J R Soc Interface*. 2014; 11:20131058. [PubMed: 24402919]
35. Shepherd JH, Screen HR. Fatigue loading of tendon. *Int J Exp Pathol*. 2013; 94:260–270. [PubMed: 23837793]
36. Parent G, Huppe N, Langelier E. Low stress tendon fatigue is a relatively rapid process in the context of overuse injuries. *Ann Biomed Eng*. 2011; 39:1535–1545. [PubMed: 21287276]
37. Peltz CD, Sarver JJ, Dourte LM, et al. Exercise following a short immobilization period is detrimental to tendon properties and joint mechanics in a rat rotator cuff injury model. *J Orthop Res*. 2010; 28:841–845. [PubMed: 20058271]
38. Andarawis-Puri N, Sereysky JB, Sun HB, et al. Molecular response of the patellar tendon to fatigue loading explained in the context of the initial induced damage and number of fatigue loading cycles. *J Orthop Res*. 2012; 30:1327–1334. [PubMed: 22227881]
39. Buckley MR, Sarver JJ, Freedman BR, et al. The dynamics of collagen uncrimping and lateral contraction in tendon and the effect of ionic concentration. *J Biomech*. 2013
40. Ethier C, Simmons C. *Introductory Biomechanics: From Cells to Organisms*. Cambridge Texts in Biomedical Engineering. 2007
41. Hannafin JA, Arnoczky SP. Effect of cyclic and static tensile loading on water content and solute diffusion in canine flexor tendons: an in vitro study. *J Orthop Res*. 1994; 12:350–356. [PubMed: 8207588]
42. Ahmadzadeh H, Freedman B, Connizzo B, et al. A micromechanical poroelastic finite element model of tendon that predicts large Poisson's ratio and water expulsion under tensile loading. *Acta Biomaterialia*. 2014 In review.
43. Provenzano PP, Vanderby R Jr. Collagen fibril morphology and organization: implications for force transmission in ligament and tendon. *Matrix Biol*. 2006; 25:71–84. [PubMed: 16271455]
44. Ahmadzadeh HCB, Freedman BR, Soslowsky LJ, Shenoy VB. Determining the Contribution of Glycosaminoglycans to Tendon Mechanical Properties with a Modified Shear-Lag Model. *Journal of Biomechanics*. 2013 in press.
45. Shepherd JH, Legerlotz K, Demirci T, et al. Functionally distinct tendon fascicles exhibit different creep and stress relaxation behaviour. *Proc Inst Mech Eng H*. 2014; 228:49–59. [PubMed: 24285289]
46. Dlugosz J, Gathercole LJ, Keller A. Transmission Electron-Microscope Studies and Their Relation to Polarizing Optical Microscopy in Rat Tail Tendon. *Micron*. 1978; 9:71–82.
47. Hansen KA, Weiss JA, Barton JK. Recruitment of tendon crimp with applied tensile strain. *J Biomech Eng*. 2002; 124:72–77. [PubMed: 11871607]
48. Goulam Houssen Y, Gusachenko I, Schanne-Klein MC, et al. Monitoring micrometer-scale collagen organization in rat-tail tendon upon mechanical strain using second harmonic microscopy. *J Biomech*. 2011; 44:2047–2052. [PubMed: 21636086]

Figure 1. Experimental setup of a plane polariscope

The setup (A) consists of a backlight, two linear polarizers on either side of the tendon(s), and a camera. (B) The polarizer (P) and analyzer (A) are crossed at 90° and are oriented at the angle θ at which maximal extinction in the dark crimp bands occurred at preload.

Figure 2. Mechanical testing and image capture protocol

(A) Tendons were preloaded (a), preconditioned (b), imaged at three loads (0.1N, 0.5N, and 2.0N) (c), and fatigue loaded (d). After 10, 100, and 1000 cycle intervals of fatigue loading, images were captured at these three loads to quantify tendon crimp properties in the toe, transition, and linear regions of a representative load-displacement curve (B). This process was repeated until tendons reached 1000 fatigue loading cycles. (C) Four ROIs were selected representing the midsubstance (orange), insertion (yellow), center (solid), and lateral (dashed) regions of the tendon. ROIs were low pass filtered to enhance the visibility of light and dark bands and intensities were averaged across the ROI width (red dashed line) before being highpass filtered (blue line). From these spectra, the crimp amplitude and frequency were computed.

Figure 3. Effect of fatigue loading on tendon mechanical properties

Cycle number was a significant ($p < 0.001$) factor for peak strain, tangent stiffness, hysteresis, and laxity. Individual lines indicate each specimen tested. With fatigue loading, peak strain, tangent stiffness, and laxity increased, whereas the hysteresis decreased.

Figure 4.

Crimp amplitude (A_{crimp}) increased with fatigue loading when assessed at (A) 0.1N (representative of the toe region of the force-displacement curve), (B) 0.5 N (representative of the transition region of the force-displacement curve), and (C) 2.0 N (representative of the linear region of the force-displacement curve). The A_{crimp} demonstrated a load-dependent response, with lower values at higher loads. Bars indicate significant paired differences ($p < 0.0125$) between the center and lateral ROIs and their corresponding insertion and midsubstances for a tendon after 10, 100, or 1000 cycles of fatigue loading. “u” indicates an intensity unit ranging between 1 and 256. *a,b,c,d indicates significant differences ($p < 0.0083$) in the ROI when compared to 0, 10, 100, and 1000 cycles, respectively. “#” indicates trends ($p < 0.017$).

Figure 5.

Crimp frequency (F_{crimp}) decreased with fatigue loading when assessed at 0.1N. Bars indicate significant paired differences ($p < 0.0125$) between the center and lateral ROIs and their corresponding insertion and midsubstances for a tendon after 10, 100, or 1000 cycles of fatigue loading. *a,b,c,d indicates significant differences ($p < 0.0083$) in the ROI when compared to 0, 10, 100, and 1000 cycles, respectively. “#” indicates trends ($p < 0.017$). Data for F_{crimp} at 0.5 and 2.0N are not shown since the power of crimp frequencies decreases to near the power of noise (high frequencies) at high loads. This is an unavoidable trade off with our high resolution images that does not exist in the evaluation of the A_{crimp} .

Figure 6.

Tendon laxity (defined as the ratio of displacement from gauge length at a set threshold to the tissue displacement and displacement at a set threshold after the first cycle of fatigue loading) was strongly correlated to the change in crimp amplitude at 0.1N as assessed at 0, 10, 100, and 1000 cycles of fatigue life. This same relationship held at both higher loads (0.5N and 2.0N). “u” indicates an intensity unit ranging between 1 and 256.