Evaluating Changes in Tendon Crimp with Fatigue Loading as an Ex Vivo Structural Assessment of Tendon Damage

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Introduction

Since many tendons operate at high and repeated loads, fatigue-induced damage is a likely contributing factor for tendon rupture. Previous studies have used various imaging modalities to study the accumulation and progression of fatigue-induced damage.[1,2] However, such methods have not been used to evaluate load and region dependence simultaneously on the entire tissue, and, in addition, can be costly and time consuming. We have developed a novel, ex vivo imaging method to study fascicle-level crimp using polarized light imaging. This technique has been used to directly measure tendon crimp (e.g., to assess the dynamics of collagen uncrimping and lateral contraction, and the effect of ionic concentration),[3] but has not yet been applied to the evaluation of structural damage via fatigue loading. Therefore, the objective of this study was to measure regionally-dependent, fatigue-induced changes in crimp frequency in the mouse patellar tendon using polarized light imaging. We hypothesized that crimp properties would show regional differences, increase with fatigue damage, and correlate with mechanical properties assessed during fatigue loading.

Methods

Ten patellar tendons from 10 C57BL/6 mice at P120 were used (IACUC approved). Following tissue harvest, surrounding musculature was removed and the patella-patellar tendon-tibia unit was carefully prepared for mechanical testing. Cross-sectional area was measured using a laser device.[4] Tendons were fatigue tested using a sinusoidal waveform, oscillating at 1Hz, between 2 and 4N (~30-75% of ultimate failure load) (Figure 1A), while being imaged with a crossed polarizer system.[3] After preconditioning, images were captured at 3 loads (0.1, 0.5, and 2.0 N) (Figure 1B), at 10, 25 and 50 cycles, and every subsequent 100 loading cycles. These 3 loads were chosen to approximate the toe, transition, and linear portions of the patellar tendon force-displacement curve (Figure 1B).

To quantify tendon crimp (Figure 1C), a region of interest (ROI) of visible tendon crimp was chosen. Since it was expected that crimp properties would show regional dependence,[5] two specific ROIs were chosen (tendon center (N=10) and lateral portions (N=9)) for analysis in this study. These same ROIs were used for the analysis of all images throughout

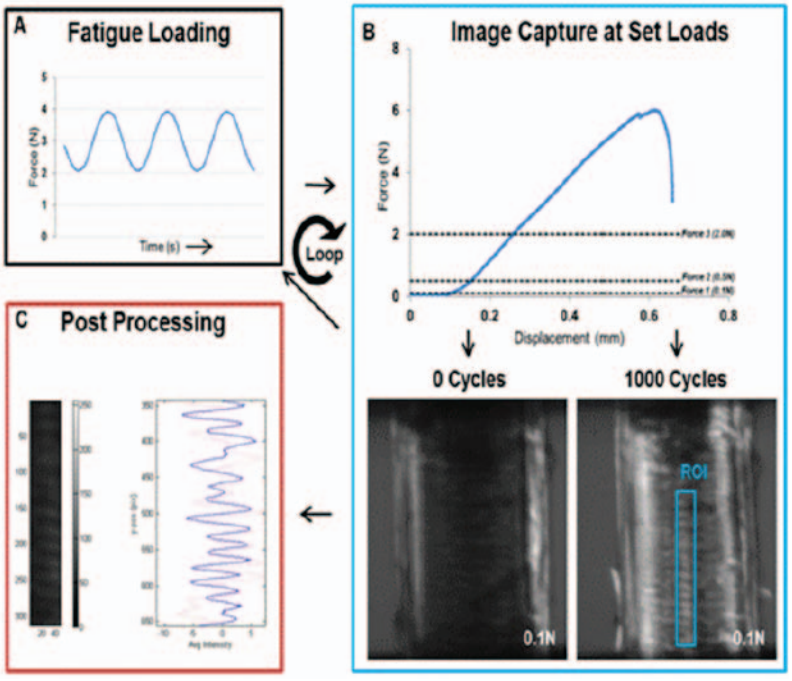

Figure 1. Mechanical testing and imaging capture flow chart: (A) Tendons were fatigue loaded between 2 and 4N with a sinusoidal waeform. (B) After 10, 25, 50, and 100 cycle intervals of fatigue loading, images were captured at threee loads (0.1, 0.5, and 2.0N) to quantify tendon crimp properties. This process was repeated until tendon failure or tendons reached 7500 cycles. (C) 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 frequency (F_{cr}) and cumulative spectral power (CSP) were computed.

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the fatigue life from mechanical testing of the particular specimen. A Gaussian low-pass filter was applied to the image within the ROI to enhance the visibility of light and dark bands (Figure 1C) using a custom MATLAB program.[3] 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 in turn was integrated to determine the cumulative spectral power (CSP). Finally, the crimp frequency (Fcr) was determined by taking the frequency at mean spectral power. Throughout specimen fatigue life, the CSP was evaluated at the Fcr to provide a measure of average crimp amplitude. All post-processing procedures were completed for all images acquired throughout specimen mechanical fatigue testing. Repeated measures ANOVAs followed by paired T-tests with Bonferroni corrections ($p<0.05$) were used to evaluate the effects of the change in CSP and Fcr (ΔCSP and ΔFcr) following fatigue loading. Single linear regressions were evaluated to determine if mechanical fatigue properties (peak cyclic strain, tangent stiffness, hysteresis, modulus, and damage (defined previously[6] 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)) were correlated to ΔCSP or ΔFcr.

Results

As hypothesized, fatigue loading resulted in increased regional dependent crimp property differences. In particular, the lateral region of the tendon demonstrated a larger increase in ΔCSP after 10, 100, and 1000 cycles of fatigue life at both 0.1N and 0.5N than the center region ($p<0.001$), but not at 2.0N (Figure 2). ΔFcr only demonstrated regional differences after 1000 cycles at 0.1N and after 100 and 1000 cycles at 0.5N ($p<0.01$) (Figure 3). Both the center and lateral regions of the tendon showed a dramatic increase in ΔCSP with fatigue loading and at all loads evaluated (Figure 2) ($p<0.006$). ΔFcr decreased with fatigue loading at both 0.1N and 0.5N, but only in the center region (Figure 3) ($p<0.001$). In the center region, ΔCSP was moderately correlated ($r= 0.68$-0.75 ($p<0.001$)) to tendon mechanical damage at all loads. In the lateral region, ΔCSP was moderately to strongly correlated ($r=0.68$-0.86

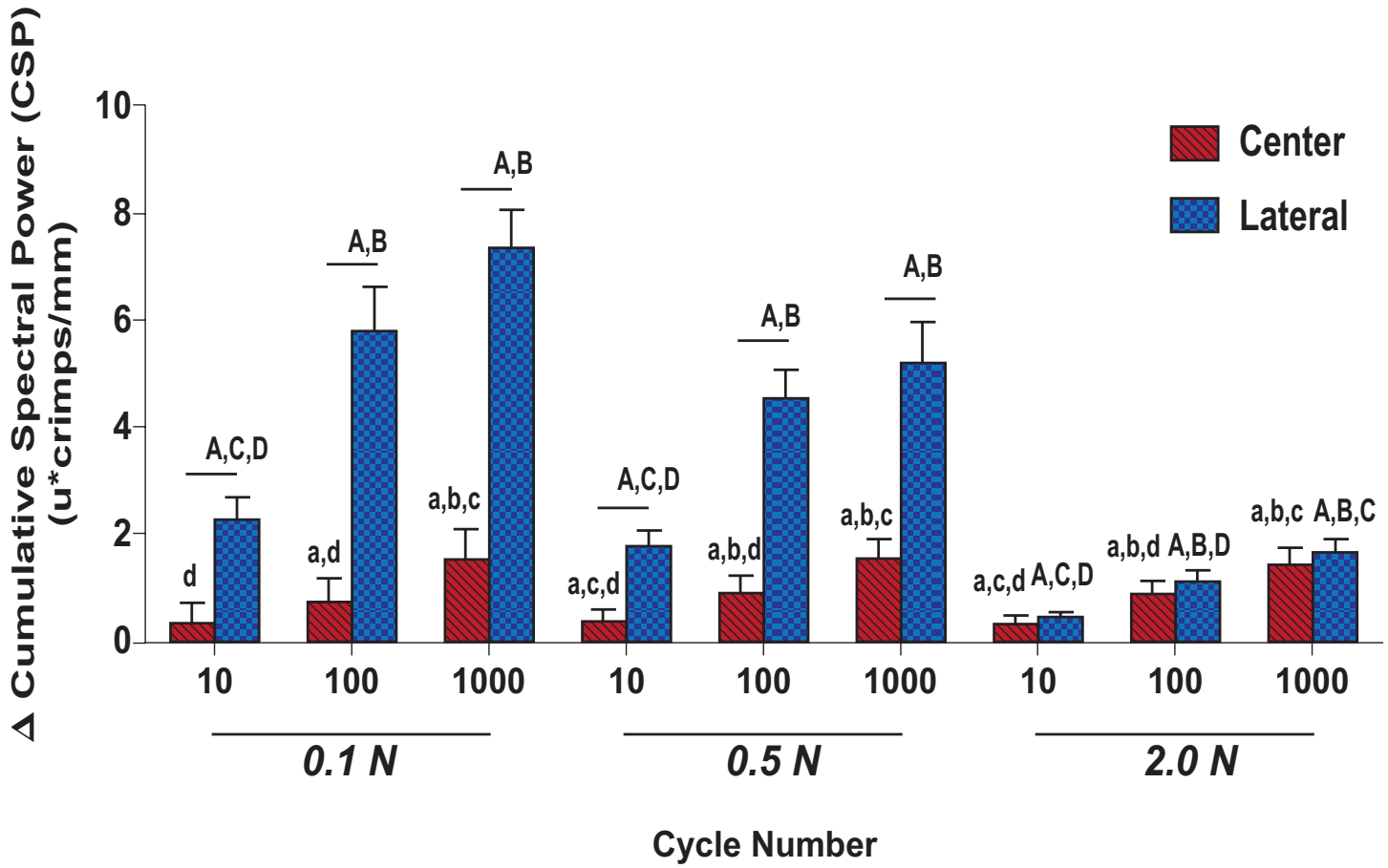

Figure 2. Δ cumulative spectral power (ΔCSP) increased with fatigue loading when assessed at three different loads (0.1, 0.5 and 2.0N) representative of the toe, transition and linear portions of the force-displacement mechanical testing curve. Bars indicate significant paired differences between the center and lateral ROIs for a tendon after 10, 100 or 1000 cycles of fatigue loading "u" indicates and intensity unit ranging between 1 and 256, "a, b, c, d" indicate significant differences in the center ROI when compared to 0,10, 100 and 1000 cycles respectively. "A, B, C, D" indicate significant differences in the lateral ROI when compared to 0, 10, 100, and 1000 cycles, respectively.

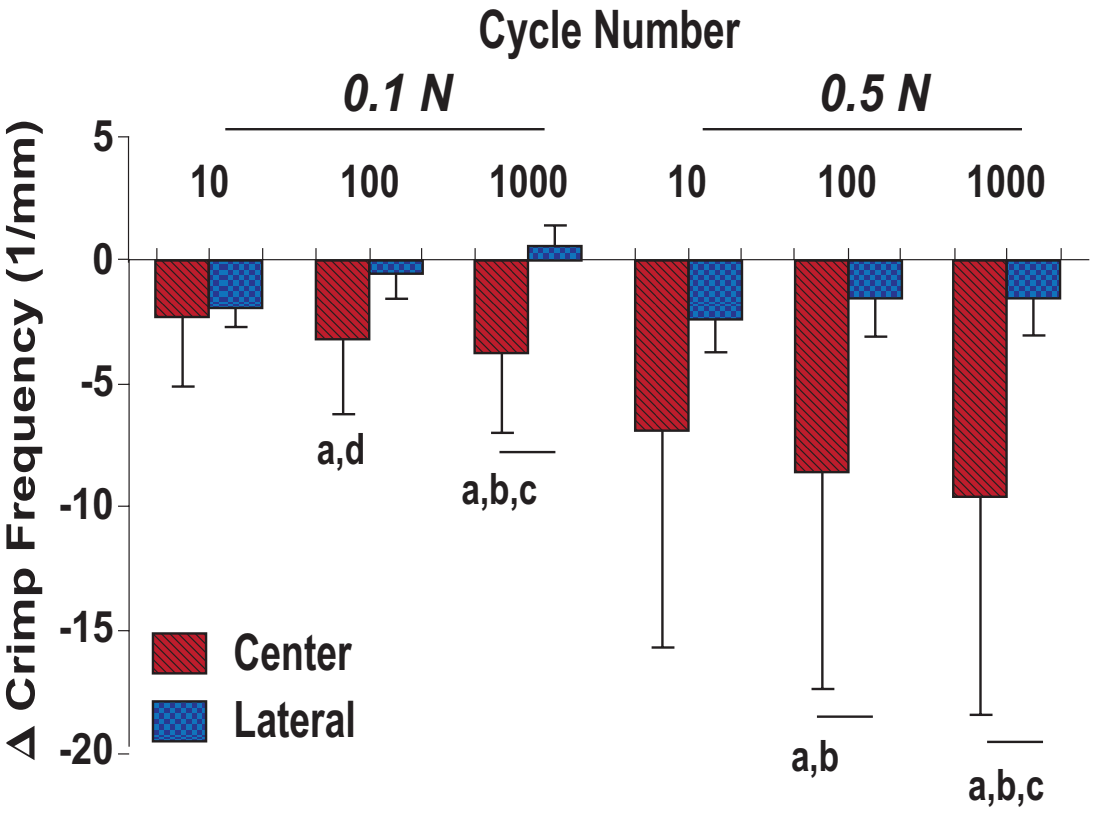

Figure 3. Δ Crimp frequency (ΔF_{cr}) decreased with fatigue loading when assessed at two different loads (0.1 and 0.5n) representative of the toe and transition potons of the force-displacement mechanical testing curve. Bars indicate significant paired differences between the center and lateral ROIs for a tendon after 10, 100, or 1000 cycles of fatigue loading. "a, b, c, d" indicate significant differences in the center ROI when compared to 0, 10, 100, and 1000 cycles, respectively. ΔF_{cr} was not significantly different in the lateral ROI when compared at 0, 10, 100 and 1000 cycles, respectively.

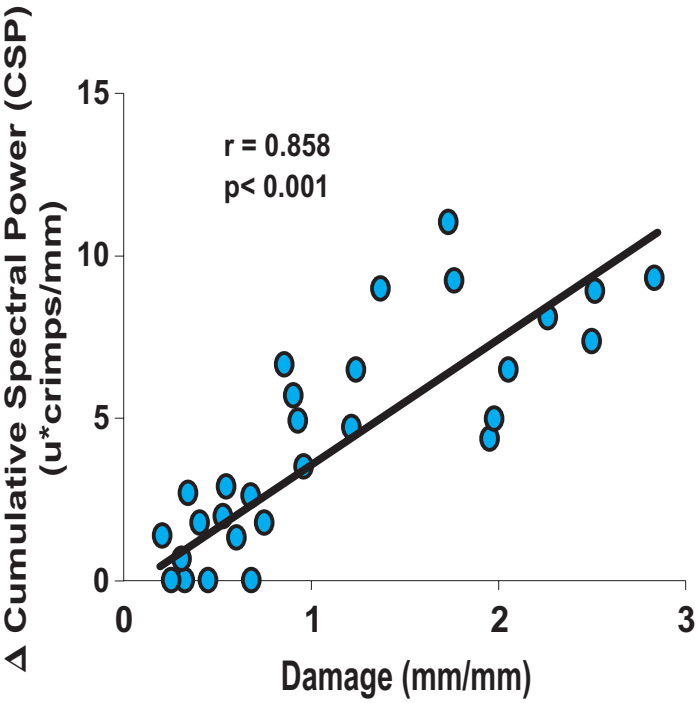

Figure 4. Tendon damage (defined as the ratio of displacement from gauge length at a set threshold to the tissue displacement and displacement at a set threshold after the first cycle of fatgue loading) was strongly correlated to the change in cumulative spectral power (ΔCSP) at 0.1N as assessed at 0, 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.

($p<0.001$) (Figure 4)) to tendon mechanical damage at all loads. In addition, ΔFcr was moderately correlated to tendon dynamic modulus, but only at 0.5N ($r= -0.55, p<0.001$).

Discussion

This study characterized patellar tendon crimp and mechanical properties during fatigue loading. The decrease in Fcr observed after fatigue loading may indicate the initial increase in stiffness observed during fatigue testing.[7] The ΔCSP showed region specific changes as it increased with induction of fatigue loading, 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/transition regions of mechanical loading, but this response may be altered with the induction of fatigue loading. Furthermore, the regional difference in uncrimping across the tendon width, supports the observation that the structural response of collagen fibrils to loading is non-uniform.[5] Although the specific structural mechanisms leading to failure were not investigated, recent studies have suggested that repeated subrupture loading results in fibril kinks that occur at distinct spacing intervals at the nanostructural level.[2,8] Such changes in nanostructure have been shown to primarily occur early during repeated loading,[2] which was also observed in this study for ΔCSP. The strong relationship between ΔCSP and damage with fatigue loading as assessed at multiple loads through mechanical testing demonstrated both the utility of damage[7] as a parameter for

modeling the response of patellar tendon fatigue loading and ΔCSP as a contributing factor to the mechanism governing the progression of tendon damage. Interestingly, in some lateral regions, the patterns of crimp frequency mirrored increases in cyclic peak strain with fatigue loading. This suggests that localized regions of tissue may be experiencing a failure response that may not have been detected with the current method. Thus, future work will investigate crimp parameters at several regions throughout the tendon width to elucidate the specific locations of failure. Such changes may also be incorporated into structural fit fiber recruitment models to study cases of tendon damage.[9]

Significance

Knowledge of tendon structural and mechanical properties throughout fatigue loading remains critical in elucidating the mechanics of subrupture damage accumulation and ultimate failure. Such information 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 and recovery.

Acknowledgments

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References

1. **Sereysky JB, Andarawis-Puri N, Ros SJ, et al.** Automated image analysis method for quantifying damage accumulation in tendon. *J. Biomech.* 43, 2641–2644 (2010).

2. Veres SP, Harrison JM, Lee JM. Repeated subrupture overload causes progression of nanoscaled discrete plasticity damage in tendon collagen fibrils. *J. Orthop. Res.* 31, 731–737 (2013).

3. Buckley MR, Sarver JJ, Freedman BR. The dynamics of collagen uncrimping and lateral contraction in tendon and the effect of ionic concentration. *J. Biomech.* 46, 2242–2249 (2013).

4. Favata M. Scarless healing in the fetus: Implications and strategies for postnatal tendon repair. *Diss. Available ProQuest* 1–218 (2006).

5. Miller KS, Connizzo BK, Feeney E. Characterizing local collagen fiber re-alignment and crimp behavior throughout mechanical testing in a mature mouse supraspinatus tendon model. *J. Biomech.* 45, 2061–2065 (2012).

6. Provenzano PP, Heisey D, Hayashi K. Subfailure damage in ligament: a structural and cellular evaluation. *J. Appl. Physiol.* 92, 362–371 (2002).

7. Fung DT, et al. Subrupture tendon fatigue damage. *J. Orthop. Res.* 27, 264–273 (2009).

8. Veres SP, Lee JM. Designed to fail: a novel mode of collagen fibril disruption and its relevance to tissue toughness. *Biophys. J.* 102, 2876–2884 (2012).

9. Peltz CD, 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.* 28, 841–845 (2010).