Injured Achilles Tendon Exhibits Inferior Mechanical and Structural Response During Fatigue Loading

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Poster No: 0615
2013 Annual Meeting
Orthopaedic Research Society
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Introduction

Achilles tendon ruptures occur frequently and result in significant pain and disability with extensive recovery time.[1] Since the Achilles tendon experiences repetitive loads at or near failure during normal activity,[2] knowledge of tendon fatigue properties is critical for determining when patients may resume normal activity. Several studies have suggested the benefits of fatigue testing over traditional failure tests, such as the ability to detect subfailure damage accumulation and changes in structure with repetitive loading.[3] Specifically, during fatigue loading, tissue stiffness initially increases and then gradually decreases as sub-rupture damage accumulates, followed by a dramatic increase in peak deformation prior to failure.[4] While it is generally believed that injury affects the mechanical and structural response of tendon during fatigue loading, this relation has not yet been fully established. Therefore, the objective of this study was to examine the mechanical and structural properties of mouse Achilles tendon during fatigue loading following an acute injury. We hypothesized that an acute injury would dramatically decrease tendon mechanical and structural fatigue properties.

Methods

Twenty four tendons from twelve C57BL/6 mice at 120 days of age were used (IACUC approved). Six P120 mice were immediately euthanized and received bilateral excisional injuries in the mid-substance of the Achilles tendon using a 0.5 mm diameter biopsy punch (~50% of the width). The remaining six P120 mice were used as controls. Following tissue harvest, surrounding musculature was removed and the bone-tendon unit was prepared for mechanical testing. Cross-sectional area was measured using a laser device.[5] Tendons were fatigue tested and imaged with an integrated polarized light system which consisted of a backlight, 90° offset rotating polarizer sheets on both sides of the test sample, and a digital camera.[6] Specimens were fatigue loaded between 1 and 3.8N at 1Hz using a sinusoidal waveform, with the maximum load corresponding to 75% of the monotonic failure strength.[7] During loading, force and displacement data were acquired at 100 Hz. Sets of alignment images were captured at 0.5 and 1.0 N after preconditioning, 10 cycles of fatigue loading, and on intervals of 100 fatigue loading cycles until failure.

Peak cyclic strain, tangent stiffness, hysteresis, and cycles to failure were computed from mechanical data. To quantify collagen alignment, the birefringent signal phase and magnitude were determined from alignment image series and used to determine the circular standard deviation[6] (CSD) and the signal's peak-to-mean and peak-to-peak intensity. All parameters were analyzed at time points proportional to the fatigue life of the specimen (5%, 50%, 95% of fatigue life) as well as for comparisons in the first 200 cycles of loading (1, 10, 100, and 200 cycles). T-tests compared parameters between injured and control tendons.

Results

In all tests, peak cyclic strain followed the three phase pattern typically reported in fatigue testing literature.[3,4] As hypothesized, injury caused a dramatic change in both mechanical and structural fatigue properties. Specifically, following injury, the number of cycles to failure decreased dramatically (CTRL: 3759±2578 cycles; INJ: 301±360 cycles (12.5-fold), $p<0.001$), stiffness decreased (at 5%, 50% and 95% of fatigue life, Figure 1A, Table1), and hysteresis increased (at 5%, and 50% of fatigue life). Peak strain was not different at 5%, 50% or 95% of fatigue life. In terms of tissue birefringence, injured tendon had increased CSD at both loads (initially and at 5% ($p=0.05$), with trends at 50% ($p=0.06$) and 95% ($p=0.07$) of fatigue life), and decreased peak-to-peak intensity (at 5% ($p=0.03$), 50% ($p=0.04$) and 95% ($p=0.003$) of fatigue life). Results through the first 200 cycles were similar, indicating that differences between groups can be detected early with fatigue loading (Figure 1B).

Discussion

This study provides evidence for inferior mechanical and structural fatigue properties following injury in the Achilles tendon. Although injured tissues were ~70% of the normal tendon stiffness, specimen failure occurred at ~8% of the

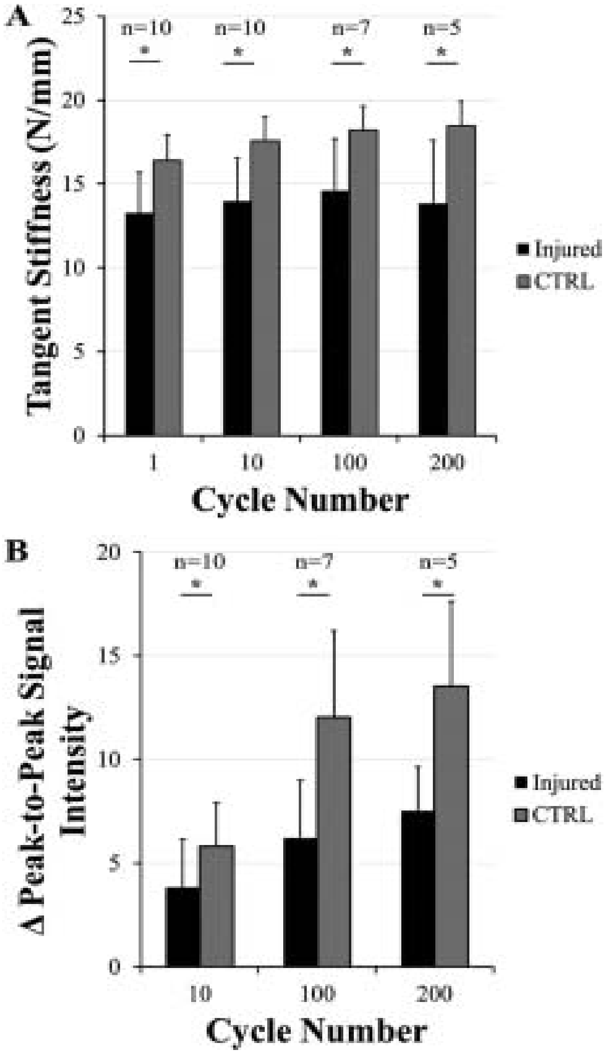

Figure 1. (A) Tangent stiffness was significantly lower in injured tissues compared to control ($p<0.01$). **(B)** Peak-to-peak intensity signal for collagen alignment was not different between groups prior to fatigue loading, but increased relative to the first map at 0.5N, by cycles 10 ($p=0.05$) 100 ($p=0.006$) and 200 ($p=0.008$) of fatigue loading. Similar results existed for alignment maps at 1.0N (not shown). These results, together with CSD, indicate that an injured tissue has inferior fatigue properties and alignment initially, and that these changes progress rapidly with induction of fatigue loading. *Indicates $p<0.05$. "n" in figure indicates the number of Achilles tendons in each injured group at each cycle number and n=6 for the control group (8 tendons total lost during preparation).

normal fatigue life, suggesting the importance of measuring fatigue properties to assess the mechanical integrity of tissues that experience cyclical loading near their failure strength. Altered structural changes were consistent with studies using other imaging methods,[8,9] but the tissue response near failure had not previously been reported. Current work is investigating the role of healing at 1, 3, and 6 weeks post injury and the effect of different genotypes on the mechanical and structural response of tendon to fatigue loading. Future work to investigate peak-to-peak intensity and CSD regionally may provide evidence of local changes in tissue structure due to injury and in response to loading.

Significance

Injury to the Achilles tendon resulted in a dramatic decrease in the number of cycles to failure. Significant changes in mechanical and structural properties of the Achilles tendon became larger in response to fatigue loading. Knowledge of these changes in response to loading is critical to best determine when it may be safe for patients recovering from an Achilles tendon rupture to return to normal activity.

Acknowledgements

We thank A Dunkman, M Hast, C Yuan, C Riggin, E Feeney, and S Thomas for assistance.

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Table 1. Mechanical parameters throughout fatigue life. Although the mechanical properties for control and injured specimens vary throughout fatigue life, their respective values differ significantly for tangent stiffness and hysteresis.

		% Fatigue Life		
Parameter	**Group**	**5%**	**50%**	**95%**
Peak Strain	INJ CTRL	0.20±0.06	0.22±0.07	0.24±0.08
(mm/mm)	p-value	0.11±0.04	0.21±0.05	0.23±0.04
		0.4	0.5	0.4
Tangent Stiffness	INJ CTRL	13.1±3.2	13.7±2.9	12.3±2.7
(N/mm)	p-value	18.3±1.4	18.3±1.4	16.7±1.6
		*0.001	*0.001	*0.002
Hysgteresis	INJ CTRL	1.01±0.65	0.80±0.37	1.65±1.81
(N*mm/mm) × 100%	p-value	0.44±0.06	0.41±0.05	0.74±0.64
		*0.03	*0.007	0.1

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