

Tendon fatigue in response to mechanical loading

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Abstract

Tendinopathies are commonly attributable to accumulation of sub-rupture fatigue damage from repetitive use. Data is limited to late stage disease from patients undergoing surgery, motivating development of animal models, such as ones utilizing treadmill running or repetitive reaching, to investigate the progression of tendinopathies. We developed an *in vivo* model using the rat patellar tendon that allows control of the loading directly applied to the tendon. This manuscript discusses the response of tendons to fatigue loading and applications of our model. Briefly, the fatigue life of the tendon was used to define low, moderate and high levels of fatigue loading. Morphological assessment showed a progression from mild kinks to fiber disruption, for low to high level fatigue loading. Collagen expression, 1 and 3 days post loading, showed more modest changes for low and moderate than high level fatigue loading. Protein and mRNA expression of Interleukin-1 β and MMP-13 were upregulated for moderate but not low level fatigue loading. Moderate level (7200 cycles) and 100 cycles of fatigue loading resulted in a catabolic and anabolic molecular profile respectively, at both 1 and 7 days post loading. Results suggest unique mechanisms for different levels of fatigue loading that are distinct from laceration.

Keywords: Tendinopathy, Tendon Fatigue, Damage Accumulation, Second Harmonic Generation, Animal Model

Introduction

Tendinopathies leading to tendon rupture are common musculoskeletal injuries that are a significant source of disability. Clinical data suggests that the rotator cuff, Achilles and patellar tendons are most prone to tendinopathies¹. Interestingly, these tendons are commonly engaged in repetitive loading or overuse amongst both, the athletic and non-athletic population²⁻⁴, providing evidence that the development of tendinopathy is closely associated with repetitive loading. For instance, the incidence of rotator cuff tears has been shown to increase with increase in age⁵ and is significantly higher in the dominant than non-dominant shoulder of elite overhead young athletes⁶. Similarly, patellar tendinopathy is common in sports that are characterized by repetitive jumping, such as volleyball and

basketball⁷, and in military recruits⁸. Lastly, although Achilles tendon rupture may appear to be a sudden event, degenerative changes are commonly observed in ruptured tendon, supporting a pathology characterized by accumulation of damage⁹.

Despite the frequency of these tendon injuries, clinical management and treatment is limited by the scarcity of data on the progressive nature from early to late stage of tendinopathies. For instance, much of the data regarding tendinopathy stems from tendon biopsies, representing end-stage pathology^{10,11}. However, an understanding of the progressive nature of tendon response to repetitive loading is necessary since the molecular, mechanical and structural response continuously changes as the loading history of the tendon is altered. In addition, while the development of tendinopathy is commonly associated with overuse injuries, some amount of exercise has been shown to result in improved tendon properties¹². However, an understanding of the types and frequency of mechanical loading that can cause tendon damage or alternatively, improve the tendon's ability to withstand loading is unclear.

Several investigators developed animal models to evaluate the response of tendons to overuse or repetitive loading. For instance, overuse injuries in the rotator cuff have been induced by subjecting rats to treadmill running¹³. Similarly, rats trained to perform repetitive reaching and grasping showed diminished motor performance, supporting the use of this model to induce tendon injury¹⁴. However, a limitation to these models is that the amount

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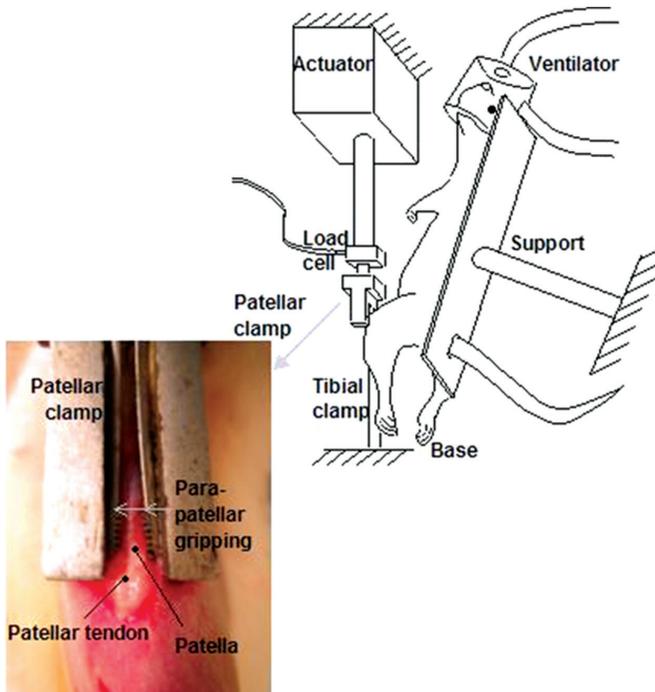


Figure 1. Experimental setup from Fung et al.¹⁵. Rats are anesthetized and fixed onto a support. The patella and the tibia are exposed and clamped to connect the patella to a load cell and fix the tibia to a base allowing tendon loading without direct instrumentation with the tendon.

of load directly applied to the tendon cannot be controlled, and is likely variable between animals as a result of natural variations in gait, anatomical size or strength and temperament.

To address the knowledge gap regarding the progression in tendon response to cyclic loading, we have developed and extensively utilized an *in vivo* model of tendon fatigue damage accumulation using the rat patellar tendon¹⁵. Using this model, we have characterized the progression in fatigue life of the tendon during cyclic loading and found that the tendon has a unique response molecularly, mechanically and structurally that changes with different amounts of applied fatigue loading¹⁵⁻¹⁸. For instance, by comparing the response of a lacerated tendon, moderate level fatigue loaded tendon and a low level fatigue loaded tendon, we found that fatigue loading induces an entirely different biologic response than laceration^{15,17}, and than nominal levels of cyclic loading (representative of exercise)¹⁷. We have utilized a multidisciplinary approach to assess the mechanical, structural, cellular and molecular changes in the tendon, to evaluate the relationship between tendon damage accumulation, and the resulting matrix damage, molecular and mechanical response.

In this manuscript, we will discuss some of our previously published work to review the main findings regarding the tendon's response to fatigue loading. We will also introduce some of the ongoing work utilizing the patellar tendon *in vivo* fatigue loading model to understand damage accumulation and repair.

Patellar tendon model of fatigue damage accumulation

Some of the challenges to developing a model of damage accumulation wherein tendons can be fatigue loaded with control in survival studies, is that the tendon must be easily accessible and must not be damaged from clamping by any instrumentation. The FDL, Achilles, supraspinatus, tail and patellar tendons were all initially evaluated as potential candidates for our *in vivo* fatigue loading model¹⁵. The patellar tendon was chosen as the ideal candidate because it is a superficial tendon that is easily accessible and can be directly loaded without direct instrumentation with the tendon. For instance, by clamping the tibia to a fixed base and clamping the patella to a load cell, the patellar tendon can be directly loaded without direct tendon damage from the clamps. In addition, the patellar tendon commonly exhibits tendinopathy in humans (jumper's knee)^{7,8,19}, further justifying the use of this tendon as the site for development of our *in vivo* fatigue damage model.

After evaluating mice, rats, rabbits and dogs, the rat was chosen for our model because it is a small animal that has previously been extensively used to investigate tendon biomechanics but is sufficiently large for investigation of local, region-specific effects of damage accumulation in future studies¹⁵.

Experimental setup

As previously described by Fung et al., to induce fatigue damage in the tendon rats were anesthetized throughout the experiment with isoflurane (2-3% by volume, 0.4 L/minute) and their left patellar tendons were surgically exposed for fatigue loading¹⁵. The experimental setup is shown in Figure 1. Under aseptic conditions, a clamp was used to fix the tibia at ~30° knee flexion. A custom clamp was then used to grip the patella and connect it to a 50-lb load cell and actuator of a servo-hydraulic loading system, allowing loading of the patellar tendon without direct contact with the tendon¹⁵.

The previously described fatigue loading protocol is shown in Figure 2¹⁵. Briefly, a diagnostic test was applied before fatigue loading (Diag₁) to assess the initial state of the tendon. Fatigue loading was then applied for x cycles that typically ranged from 1N to 40% of the ultimate load of the tendon. Ultimate load was defined from preliminary monotonic load to failure experiments as the highest load reached prior to onset of failure as indicated by inability to reach a higher load level despite further applied loading. The number of fatigue loading cycles (x) was guided by our understanding of the amount of damage induced in the tendon throughout the fatigue life (refer to the sub-section on 'Assessment of mechanical parameters' for details regarding the characterization of the fatigue life of the tendon) and was determined for each experiment by the scientific question being evaluated. A second diagnostic test (Diag₂) was then applied immediately post fatigue loading to assess the initial combined recoverable and non-recoverable effect of fatigue loading. The tendon was allowed to recover for

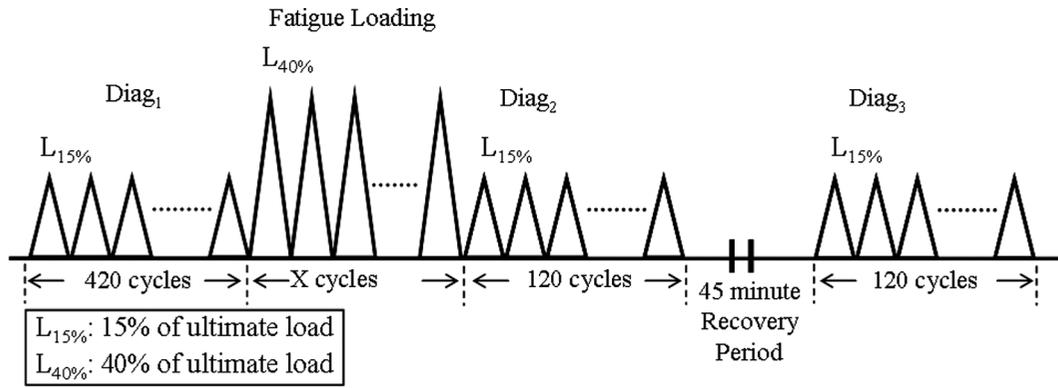


Figure 2. Fatigue loading protocol. Diagnostic tests are applied before (Diag₁), immediately after (Diag₂) and 45 minutes (Diag₃) after fatigue loading.

45 minutes (the longest amount of time possible in conjunction with our longest fatigue loading protocol that satisfied IACUC constraints), and then a final diagnostic test (Diag₃) was applied to deduce the non-recoverable initial damage. Diagnostic tests typically ranged from 1N to 15% of the ultimate load. All data discussed in this manuscript was collected using a loading frequency of 1Hz. The tendon was continuously moistened with phosphate buffered saline throughout the experiment.

Clamps were then removed and skin incisions were sutured with 6-0 prolene. Analgesia (Buprenex) was administered and the animal resumed cage activity.

Multi-disciplinary approach for evaluation of tendon damage and repair

Tendons are complex structures that exhibit a close relationship between structural changes and a molecular response through mechanotransduction. Accumulation of damage is expected to ultimately compromise tendon function and mechanical strength. It is likely that as damage is accumulated, structural damage alters the loading environment of the cells resulting in extracellular matrix changes that lead to changes in tendon function. It is unclear whether increase in amount of damage accumulation induces different levels of one mechanism or induces entirely different mechanisms. A comprehensive assessment that includes molecular, mechanical and structural evaluation of the effect of damage accumulation on the tendon is necessary.

Assessment of molecular changes in response to fatigue loading

Several studies have evaluated the biologic healing response of tendons to laceration^{20,21}. It was expected that the typical healing response that includes overt inflammation may only occur in response to fiber disruption, and therefore was unlikely to occur with sub-rupture damage accumulation until fiber disruption occurred. In addition, tissue biopsies representing late stage tendinopathies provide insight into late stage but not early stage disease leaving little known about the bio-

logic response to early fatigue damage accumulation.

Using our model, we have begun to investigate the progressive nature of the response of the tendon to damage accumulation^{15,17,18}. Since the role of inflammation in chronic tendinopathy is of much debate, studies by Sun et al. evaluated the mRNA and protein expression and of Interleukin-1 β (IL-1 β) and Matrix Metalloproteinase (MMP)-13, 1 and 3 days post low and moderate level fatigue loading¹⁸. Results showed a similar trend for both genes at both timepoints¹⁸. For instance, 1 and 3 days post fatigue loading, low fatigue loaded tendons exhibited suppressed expression of MMP-13 and IL-1 β by 70%¹⁸. Similarly, at both timepoints, moderate fatigue loaded tendons expressed a 5-6 fold upregulation of MMP-13 and IL-1 β ¹⁸. Changes in mRNA expression were always paralleled by changes in protein expression, as confirmed by western blots¹⁸.

Fung et al. utilized real time PCR to evaluate early phase (1 and 3 days) mRNA expression of Collagen (Col)-I, Col-III and Col-V of tendons in response to low, moderate and high level fatigue¹⁵. Small but significant changes were observed following low and moderate level fatigue loading but significant large changes were observed following high level fatigue loading at both timepoints¹⁵. More specifically, in comparison to sham controls (animals that experienced identical surgical protocol without fatigue loading), Col-I was slightly downregulated at 1 (1.8 fold) and 3 days (1.5 fold) following low level fatigue loading and was unchanged following moderate level fatigue loading but was highly upregulated at both time points following high level fatigue loading¹⁵. Similarly, 1 day post low and moderate level fatigue loading, Col-III was slightly upregulated (2.8 and 4.0 fold respectively) with no difference observed following high level fatigue loading¹⁵. At 3 days post fatigue loading, no difference was observed following low and a modest decrease (2.9 fold) was observed following moderate level fatigue loading, but a significant increase was observed for high level damage (9.5 fold). Lastly, at 1 day post low and moderate level fatigue loading, Col-V was upregulated by approximately 3.5 fold, and then by 3.2 and 5.8 fold at 3 day post fatigue loading¹⁵. In contrast, a much higher upregulation of 16 and 21 fold was observed for high level fatigue loading at 1 and 3 days respectively¹⁵.

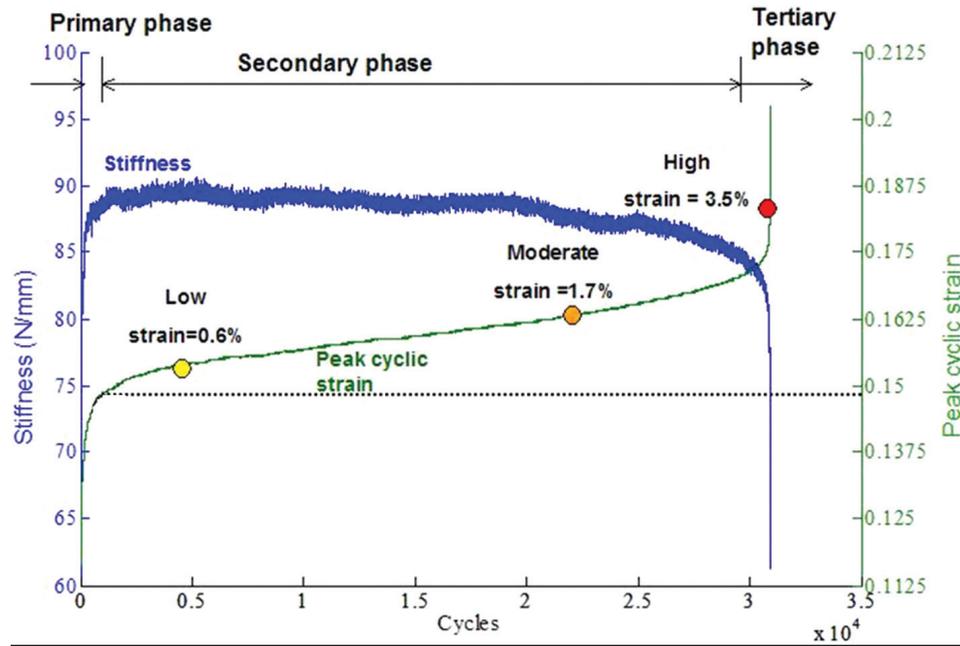


Figure 3. Representative fatigue life of the tendon adapted from Fung et al¹⁵. Stiffness and peak cyclic strain followed 3 phases. Low, moderate and high level fatigue loading were defined by a peak cyclic strain of 0.6% (Low), 1.7% (Moderate) and 3.5% (High) beyond initial measurement determined at cycle 500 (end of the primary phase).

Finally, mRNA expression was profiled for select Collagens, MMPs and their inhibitor (TIMPs), 1 and 7 days post 100 (representative of exercise) and 7200 cycles (falls within the range of moderate level fatigue loading) fatigue loading¹⁷. The trends observed for Col-I, Col-III and Col-V for 7200 cycles 7 days post fatigue loading were similar to those observed for moderate level fatigue loading as described by Fung et al¹⁵. A hypertrophic response, indicative of tendon adaptation was observed for 100 cycles of fatigue loading, as demonstrated by upregulation of MMP-3 (critical to tendon remodeling), MMP-13 and 14 (necessary for degradation of damaged matrix and remodeling), Col-I, and Col-XII¹⁷. In contrast, at 7200 cycles, a catabolic response was observed that was denoted by upregulation of Col-III and Col-V but downregulation of Col-I¹⁷. The ratio of mRNA expression of Col-III to Col-I increased from 3:10 to 9:10 for 100 to 7200 cycles of fatigue loading¹⁷. A high ratio of Col-III to Col-I is expected in tendinopathy and may be reflective of poor matrix organization²². In addition, no significant change in TIMP activity was observed for 100 cycle fatigue loading, while TIMP-1 and 2 were upregulated, and TIMP-4 was downregulated for 7200 cycle fatigue loading, reflective of altered homeostasis¹⁷.

Assessment of mechanical parameters

To evaluate the immediate effect of fatigue loading on the load bearing capacity of tendons, real time measures were determined from the fatigue loading curves¹⁵. Fung et al. showed that during fatigue loading to failure, peak cyclic strain ($(d_{\max} - d_0)/L_0$ where

d_{\max} : actuator position at maximum load, d_0 : initial actuator position, L_0 : initial tendon length), followed 3 phases (Figure 3)¹⁵. During the primary phase, an increase in peak cyclic strain and stiffness was observed¹⁵. During the secondary phase, the rate of peak cyclic strain increase decreased significantly from that experienced during the primary phase¹⁵. Concurrently, no change in stiffness was induced during the secondary phase. Finally, a steep rate of peak cyclic strain increase and stiffness decrease was induced in the tertiary phase¹⁵.

Baseline hysteresis and tangent stiffness were compared at an initial cycle (cycle 15) and endpoint cycle during fatigue loading to assess the effect of fatigue loading¹⁵. Hysteresis was defined as the area between the loading and unloading curves¹⁵. Interestingly, at the end of the fatigue loading protocol, a significant increase in stiffness ($\sim 18\%$) was observed for low and moderate level fatigue loading but a significant decrease was observed for high level fatigue loading ($\sim 18\%$)¹⁵. Similarly, a significant decrease in hysteresis was observed for low and moderate level fatigue loading ($\sim 30\%$), but an increase was observed for high level fatigue loading ($\sim 25\%$)¹⁵.

In addition to real time measures of induced damage deduced from the fatigue loading portion of the curve, current work has separated the recoverable and non-recoverable changes by first calculating the average of certain mechanical parameters from the last 10 cycles of each diagnostic test and comparing these average values between diagnostics^{23,24}. Parameters that are being evaluated include tendon elongation, hysteresis and stiffness. Comparing the value of any mechan-

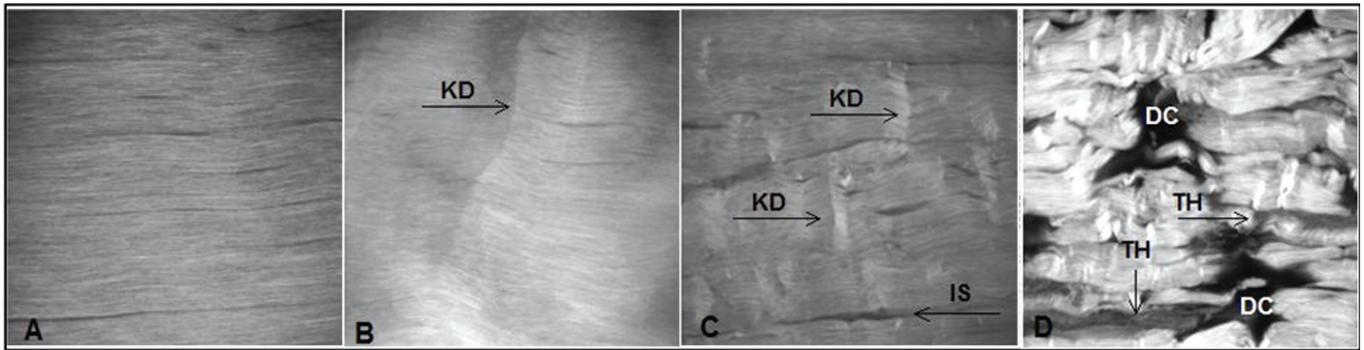


Figure 4. Adapted from Fung et al.¹⁵ fatigue loading to increasing levels of fatigue loading resulted in discernibly progressive changes in tendon structure. (A) Control, non-fatigue loaded tendons, exhibited aligned collagen fibrils. (B) Low level fatigue loaded tendons exhibited kinked fiber deformations (KD). (C) Moderate level fatigue loaded tendons exhibited kinked fiber deformations with widening of the inter fiber space (IS). (D) High level fatigue loaded tendons exhibited severe matrix disruption with fiber thinning (TH) and matrix discontinuities (DC). FOV=400 mm.

ical parameter between $Diag_1$ (pre-fatigue) and $Diag_3$ (post-recovery) reflects the non-recoverable changes in the tendon. Similarly, comparing the value of any mechanical parameter between $Diag_2$ (post-fatigue) and $Diag_3$ (post-recovery) reflects the recoverable damage in the tendon. Whether these parameters can serve as indices of damage and can predict the molecular and mechanical response of the tendon over time is being evaluated in current work. These measures, evaluated over a time course, can be used to determine whether tendons recover, further degenerate or are slow to experience any change over time.

Structural analysis of tendon damage

Tendons primarily function in tension and their tensile properties are predominantly attributable to their high content of Col-I. Several investigators have shown that Col-I content is directly affected by tendinopathy¹⁰ and exercise²⁵ emphasizing the importance of inclusion of collagen analysis in assessment of tendon damage. In addition, since damage is not expected to accumulate uniformly in the tendon^{26,27}, it is expected that damaged and undamaged regions will exhibit a different cellular response. Therefore, an ability to visualize tendon structural damage, with the long term goal of correlating it with protein expression and mechanical function is integral to understanding the response of tendons to fatigue loading.

Conventional histological techniques utilizing thin paraffin or plastic sections with polarized light, bright-field, scanning electron and confocal microscopy provide insight into collagen structure. To better retain tendon morphology and minimize thin section cutting artifacts, Laudier et al. developed a novel procedure for tendon histology that utilized Methyl Methacrylate (MMA) instead of the typically used paraffin²⁸. Further advancement was then made in visualization of collagen structure damage by extending the application of second harmonic generation (SHG) imaging for use with tendon embedded in thick plastic (MMA) sections¹⁶. SHG imaging allows visuali-

zation of collagen microstructure by invoking the second-order optical property of collagen using a laser tuned to the near-infrared range. The ability of this imaging modality to penetrate into the depth of the tissue allows evaluation of tendon structure through thick plastic sections (~40 thick), minimizing the effect of sectioning artifact¹⁶. In addition, current work has expanded utilization of SHG imaging for tendon structure visualization on non-fixed mouse patellar tendons and rat tail tendons, extending the potential application of this technique to *in vivo* studies²⁹.

Analysis techniques to visualize tendon structural changes have been developed to analyze SHG images^{16,30}. As described previously, using Fast Fourier Transform based analysis (FFT), a method indicative of the disorganization of the entire tendon was first developed¹⁶. Briefly, FFT was performed on the entire image, to convert it from spatial to frequency domain. It was expected that fiber disorganization that results from fatigue damage would be reflected in changes in the frequency components¹⁶. A graphical representation of the distribution of the frequency components was obtained from a plot of the power spectrum (amplitude of the transform equation)¹⁶. The field of view (FOV) was divided into 30 x 30 pixel windows, and a power spectrum was generated for each window¹⁶. The distribution of angular deviations from the principal axis of the tendon was graphed and served as an indicator of overall image anisotropy¹⁶. As expected, results showed greater anisotropy with each level of increased damage¹⁶. Building on this work, quantification of structural damage was further advanced through the development of a method to quantify the damage area fraction (DAF, the fractional area of a 2D SHG image of tendon determined to be damaged)³⁰. First, manual analysis was conducted by a trained blinded user and confirmed that DAF increases with increased level of fatigue loading³⁰. Since manual image analysis is time intensive and subject to user variability, development of an automated method became clearly valuable. In this method developed by Sereysky et al., Fourier transform was calculated, its power spectrum (ps) was

plotted, and an ellipse was fitted to the filtered power spectrum³⁰. The orientation of the collagen fibers within each image subsection was determined from the short axis of the fitted ellipse³⁰. The two user-defined input variables, image subsection size and fiber orientation angle difference necessary for two adjacent subsections to be considered damaged were then adjusted to optimize the correlation between manual and automated analysis. Results showed that damage measured manually and automatically colocalized $78 \pm 13\%$ of the time, supporting the use of this automated method³⁰.

Qualitative assessment of SHG images of the tendon mid-substance showed discernible differences in images representative of different levels of damage (Figure 4)¹⁵. More specifically, control tendons exhibited aligned collagen fibers without matrix disruption whereas fatigue loaded tendons exhibited progressive structural damage. Tendons that were loaded to low level fatigue exhibited isolated fiber kinks that were more abundant for moderate level fatigue loaded tendons¹⁵. Moderate level fatigue loaded tendons also exhibited widening of interfiber space¹⁵. High level fatigue loaded tendons exhibited severe matrix disruption¹⁵. The qualitatively observed manifestation of structural damage was reflected in our automated methods^{16,30}.

Discussion

Despite the prevalence of tendinopathy, its clinical management is limited by the scarcity of information about progression from the early, more clinically manageable stage, to the late, less manageable stage. An *in vivo* model of tendon fatigue loading that allows for accurate control over the input experimental parameters without damage to the tendon from the experimental setup has tremendous potential applications for the study of progression of tendon damage. In this manuscript, we reviewed early applications using this model. Studies were conducted to characterize the fatigue life of the tendon. In agreement with findings from *ex vivo* evaluation, during fatigue loading to failure, creep elongation and secant stiffness exhibited 3 distinct phases^{15,31}. The steep increase in peak elongation during the primary phase is likely attributable to a complex combination of fibers uncrimping, and others being elastically loaded while water is being exuded. We expect that in the secondary phase, most fibers are elastically responding, accounting for the slow increase in peak elongation. Finally, in the third phase, overt fiber rupture (decrease in number of load bearing fibers) results in steep increases in creep elongation. Evaluation of low, moderate and high level fatigue loading supports a progressive nature to damage accumulation, evidenced by morphological and molecular changes.

It is unclear how early in the fatigue life the tendon begins to accumulate damage. Although there is an established association between prolonged, strenuous exercise and the development of tendinopathies³², exercise has also been shown to improve the ability of the tendon to withstand loads. For instance, eccentric calf muscle training has been shown to increase Achilles tendon stiffness³³, a mechanical property that

typically decreases with the development of tendinopathy³⁴. The increase in tendon stiffness observed with exercise likely results in decreased strain in response to loading, reducing the risk of tendon injury³⁵. In contrast, increased strains have been measured in tendons of patients suffering from tendinopathy³⁶, suggesting that they may be at increased risk of further injury. Other studies have shown that exercise increases vascularization, upregulates vascular endothelial growth factor (VEGF) and increases cellular proliferation³⁷⁻⁴⁰. Comparison of concentric and eccentric running showed upregulation of angiogenic factors for both groups, with earlier downregulation in eccentric loading, which is likely more clinically favorable to avoid scar tissue build-up³⁹. Using our *in vivo* model of fatigue damage accumulation, we compared the molecular profile of 100 cycles (representative of exercise) and moderate level fatigue loading (7200 cycles), and found an anabolic response to 100 cycles, and a catabolic response to 7200 cycles¹⁷. These data suggest that there is a threshold of loading frequency and magnitude that once overcome, changes the response of the tendon to loading from beneficial to degenerative. In addition, current work is evaluating whether exercise (treadmill running) can protect a tendon from accumulating damage or improve the functional ability of fatigue damaged tendons. Preliminary data showed a distinctly different response to exercise for fatigue damaged tendons than healthy tendons, suggesting that a short bout of exercise can induce an adaptive response and improve overall function of fatigue damaged tendons⁴¹. An understanding of the types and frequency of mechanical loading that results in tendon damage or improve the tendon's functional ability is unclear. In addition, while it is expected that the tendon's attempt to repair may be more successful with lower than higher amounts of accumulated damage, data to establish these relationships is scarce and is the focus of our ongoing work.

The unexpected increase in stiffness and decrease in hysteresis that was observed for low and moderate levels of fatigue loading is likely attributable to redistribution of loads from non-load bearing damaged fibers to undamaged fibers¹⁵. It is not until high level fatigue loading where the majority of load bearing fibers are damaged that the expected stiffness loss and increase in hysteresis are observed. In our previous studies, we have evaluated the bulk tissue molecular response and concluded that molecular response for low and moderate level fatigue loading significantly differed from that of 100 cycle (representative of exercise), high level fatigue loading, and laceration. It remains unclear how the bulk tissue response relates to the local tissue response. For instance, cells in damaged regions of the matrix may all express the same genes with the difference in observed molecular profiles associated with different levels of fatigue loading being attributed to the difference in number of cells in damaged regions. Alternatively, the difference in profile observed between different levels of damage may be attributable to a different cellular response associated with different severities of matrix changes. The relationship between matrix damage and cellular response in the context of damage accumulation and repair is being further evaluated in current work.

Inflammation is known to be a key component of the tendon healing response to laceration. However, the role of inflammation in tendinopathy is far from established and is continuously debated. The commonly reported absence of inflammatory cells in tendons from patients suffering from late stage tendinopathy⁴² has led many investigators to discount inflammation as a component of chronic tendinopathy. Millar et al. found inflammatory cells in intact subscapularis tendons exhibiting early to moderate tendinopathy in patients with supraspinatus tendon tears.

Interestingly, in these patients, the severe degenerative changes associated with the torn supraspinatus tendon were associated with a much lower presence of inflammation than was observed in the intact subscapularis⁴³. While inflammatory cell infiltration may not be associated with late stage tendinopathy, the presence of molecular mediators of inflammation in early tendinopathy may implicate its role in the progression of the disease. We found that inducing moderate level fatigue resulted in a 5-6 fold upregulation of IL-1 β , a pro-inflammatory cytokine, that is paralleled by a similar increase in MMP-13¹⁸. Archambault et al. found an increase in MMP activity to result from introducing exogenous IL-1 β or stretch, with a greater effect due to the combination of both, IL-1 β and stretch⁴⁴. These findings suggest that upregulation of IL-1² that results in response to moderate level fatigue loading may initiate a cascade of molecular inflammation that results in stimulation of MMPs leading to matrix degeneration. Interestingly, a suppressed expression of MMP-13 and IL-1 β was observed for low fatigue loaded tendons¹⁸. Results suggest that a certain amount of accumulated damage is necessary to induce molecular inflammation, which once induced, may play a role in altering the response of the tendon to further loading.

Our model of fatigue damage accumulation allows us to induce injury from fatigue loading that results in degenerative changes, as is commonly seen in tendinopathy. Our model is specifically conducive for investigation of early stage tendinopathy; the area of tendinopathy research exhibiting the greatest knowledge gap. While our current applications of the model have focused on fatigue loading for 1 bout, the model can be used to apply multiple bouts of fatigue loading provided that the animal is given sufficient recovery time from surgery between bouts. Since skin incisions are made each time fatigue loading is applied, a finite number of separate bouts will ultimately be ethically possible. The loading frequencies and loading force chosen can be adjusted in our model to mirror different clinical scenarios that are expected to lead to development of tendinopathy. For instance, applying 1 bout of moderate level fatigue loading simulates a very important and common clinical scenario such as the effect of 1 episode of intense military training, or 1 session of very intense exercise. Per our goals, our model allows us to investigate the development of early stage tendinopathy and exhibits some limitations in its application for long term chronic tendinopathy.

Future studies will investigate the underlying cellular mechanisms associated with the distinctly different responses to different amounts of accumulated fatigue loading. The studies described in this manuscript focused on the early *in vivo*

response to different levels of fatigue loading by evaluating the molecular response of the tendon 1, 3, and 7 days post fatigue loading. Current studies are evaluating the molecular, mechanical and structural response of the tendon up to 6 weeks post fatigue loading to determine if fatigue loaded tendons repair or further degenerate overtime. In addition, to investigating the mechanisms of development and progression of tendon sub-rupture damage accumulation, future studies will investigate the ability of surgically repaired fatigue loaded tendons to heal, mimicking the confounding degeneration observed in conjunction with a clinical tendon tear. Finally, we have recently developed a mouse model of *in vivo* fatigue damage accumulation that parallels our model in the rat to investigate the effect of variation in genetic background on the tendon's response to fatigue loading⁴⁵. By comparing inbred genetic strains or mice with specific genes knocked out, insight will be gained into the underlying molecular mechanisms associated with the biological response to damage accumulation.

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