Shear Load Transfer in High and Low Stress Tendons

Preprint version of
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Journal of the mechanical behavior of biomedical materials 45 109–120, (2015)

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Abstract

Background: Tendon is an integral part of joint movement and stability, as it functions to transmit load from muscle to bone. It has an anisotropic, fibrous hierarchical structure that is generally loaded in the direction of its fibers/fascicles. Internal load distributions are altered when joint motion rotates an insertion site or when local damage disrupts fibers/fascicles, potentially causing inter-fiber (or inter-fascicular) shear. Tendons with different microstructure (helical versus linear) may redistribute loads differently.

Method of Approach: This study explored how shear redistributes axial loads in rat tail tendon (low stress tendons with linear microstructure) and porcine flexor tendon (high stress with helical microstructure) by creating lacerations on opposite sides of the tendon, ranging from about 20-60% of the tendon width, to create various magnitudes of shear. Differences in fascicular orientation were quantified using polarized light microscopy.

Results and Conclusions: Unexpectedly, both tendon types maintained about 20% of pre-laceration stress values after overlapping cuts of 60% of tendon width (no intact fibers end to end) suggesting that shear stress transfer can contribute more to overall tendon strength and stiffness than previously reported.

All structural parameters for both tendon types decreased linearly with increasing laceration depth. The tail tendon had a more rapid decline in post-laceration elastic stress and modulus parameters as well as a more linear and less tightly packed fascicular structure, suggesting that positional tendons may be less well suited to redistribute loads via a shear mechanism.

Keywords: Tendon, Shear, Structure, Tendon function, Viscoelasticity

1. Introduction

Tendon is a unique, hierarchical structure serving the essential musculoskeletal function of transferring muscle contraction to joint movement or stability. Tendon hierarchy begins on the smallest level with collagen molecules and builds up to microfibrils, fibrils, fibers, and finally fascicles which form tendon as the overall structure. This study will primarily discuss the 2 largest sections of this hierarchy, the tendon fiber and fascicle. Collagen molecules have a triple-helical structure (Ramachandran and Kartha 1955) forming fibrils, however helical structure at larger levels of hierarchy in some tendons has only recently been investigated in terms of structure/function. Fibrils from various tendon types, ranging from equine superficial digital flexor tendon to rat tail tendons, spiral within fibers (Jozsa et al. 1991), which, in turn, are organized in a helix (Vidal 2003; C. T. Thorpe, Klemt, et al. 2013). Fascicles exhibit a helical organization within the whole tendon structure as well (Kalson et al. 2012; Kannus 2000; Khodabakhshi et al. 2013).

Tendons that experience high stress (often termed energy storing tendons which also experience larger strains and help decrease energy loss) and low stress (often called positional tendons, primarily used for intricate movements) are morphologically different in their structural organization and may have different mechanical properties (Birch 2007; Shadwick 1990; Batson et al. 2003; S. L. Woo et al. 1980; S. L.-Y. Woo et al. 1981; Blanton and Biggs 1970; Benedict, Walker, and Harris 1968; C. T. Thorpe, Udeze, et al. 2013; Screen, Toorani, and Shelton 2013). Interestingly, it has been reported that stress relaxation is greater in bovine digital extensor, a largely positional tendon, than the deep digital flexor, a more energy storing tendon (Shepherd et al. 2014), indicating not only a difference in mechanical strength but also in time-dependent adaptation to loading between tissue types. Although there has been little investigation into why these differences are present, it has been shown that inter-fascicular binding is greater in the flexor tendon and that fascicles in the bovine deep digital flexor tendon are less linearly oriented along the length of the tendon than fascicles in the digital extensor tendon (Shepherd et al. 2014). The difference in angulation of fascicles has also been confirmed by demonstrating a greater helical pitch angle in equine energy storing (superficial digital flexor) tendons than positional tendons (common digital extensor) (C. T. Thorpe, Klemt, et al. 2013).

While extensive research has defined the longitudinal behavior of tendons *in vitro* when the load distribution is as uniform as possible (Abrahams 1967; Rigby et al. 1959), shear behavior has been less scrutinized. Understanding the shear behavior during loading is clearly important for redistribution of internal tendon loads 1) as insertion sites rotate during joint motion, 2) during fiber breakage or enzymatic local remodeling, and 3) around damage such as partial tears, lacerations, or other tendinopathies. It is also relevant for tendon lengthening procedures used to treat conditions such as diabetic plantar forefoot ulceration (Mueller et al. 2003) or gastrocsoleus equinus contracture (Hoke 1931; Salamon et al. 2006). In these procedures up to 50% of the tendon width is transected at multiple locations (often 3) on alternating sides of the tendon, necessitating shear transfer to prevent complete tendon rupture (Mueller et al. 2003; Hoke 1931; Salamon et al. 2006). However, studies report that shear force transmission between fascicles, carried by the inter-fascicular connective tissue, is nearly negligible compared to load born by intact fascicles (Haraldsson et al. 2008; Purslow 2009), and that, in equine digital flexor (high stress, energy storing) tendon, sliding between fascicles allows for the large

strain seen by these tendons (C. Thorpe et al. 2012). Fiber sliding is also shown as a dominant mechanism of motion during tendon stretch (Khodabakhshi et al. 2013; Li et al. 2013), particularly in more energy storing, flexor tendons of porcine (Screen, Toorani, and Shelton 2013) and primarily positional, extensor tendons of equine (C. T. Thorpe, Klemt, et al. 2013) where it has been demonstrated that more fiber sliding occurs than their positional or energy storing counterparts, respectively. Taken together, these studies suggest that shear transfer at both hierarchical levels in various tendon types, depending on location and species, may be minor, leaving unexplained the residual strength after tendon lengthening procedures.

Previous studies, including a couple completed in our lab investigating the mechanical properties of partially lacerated flexor tendons (high stress) have shown that mechanical compromise of lacerated tendon is not proportional to the laceration area, indicating that longitudinal loading of fibers and fascicles is not the only load-bearing mechanism within tendon (Kondratko et al. 2012; Pensalfini et al. 2014; Ahmadzadeh et al. 2013; Szczesny and Elliott 2014). This supports the importance of understanding their shear properties during longitudinal loading. Shear transfer between fibers and fascicles would redistribute internal loads around the defect. Therefore, the purpose of the current study is to investigate how shear transfer affects tendon behavior in both high and low stress tendons, describing the elastic and viscoelastic responses after partial laceration. We hypothesize that low and high stress tendons exhibit different shear behavior due to their different tendon structures, with high stress tendons having greater axial strength via internal shear.

2. Materials and Methods

2.1 Specimen Preparation.

Thirty (30) porcine deep digital flexor tendons and 30 rat tail tendons were used in this study. Digital flexor tendons, high stress tendons (often considered as energy storing tendons), were excised from forelimbs of sexually mature 6 month old pigs obtained from a local abattoir. The flexor tendons were isolated from muscle and excess connective tissue, while the distal bone insertion site was left intact. The distal bone was potted in lightweight polyester resin filler in a mold with the internal dimensions of the custom grip used for testing. Rat tail tendons, obtained from sexually mature, 2-3 month old animals with a mass of about 300g were defined in this study as low stress, more positional tendons. The tails were removed at their base from the rat and the tendons were carefully dissected free from tail vertebrae. It is recognized that rat tail tendons differ from other tendons in that they operate in allowing the tail to bend actively. These tendons are nevertheless appropriate for the present research because they have been widely studied by others and there is much literature for comparison.

Tendon width and thickness were measured on each tendon using a digital caliper prior to tendon preloading. Each dimension was measured in 3 locations along the tendon, one approximately 15mm from either grip and one near the middle of the tendon to ensure the average was representative of the experimental length. The averages were used to determine the cross-sectional area, assuming an elliptical cross-section, similar to Boyer et al. (2001), Duenwald et al. (2009), and LaCroix et al. (2013).

Specimen hydration was maintained throughout setup and testing with physiologic buffered saline (PBS).

2.2 Mechanical Testing.

All specimens were tested in a servohydraulic mechanical testing system (MTS) (Bionix 858; MTS, Minneapolis, Minnesota) with a testing protocol similar to Kondratko et al. (2012). The flexor tendon was setup as described in Kondratko et al. (2012) (Fig. 1). The tail tendon was gripped on both ends by soft tissue grips containing rough, interlocking plates. Paper was glued to the portion of the tendon that was gripped to prevent slipping.

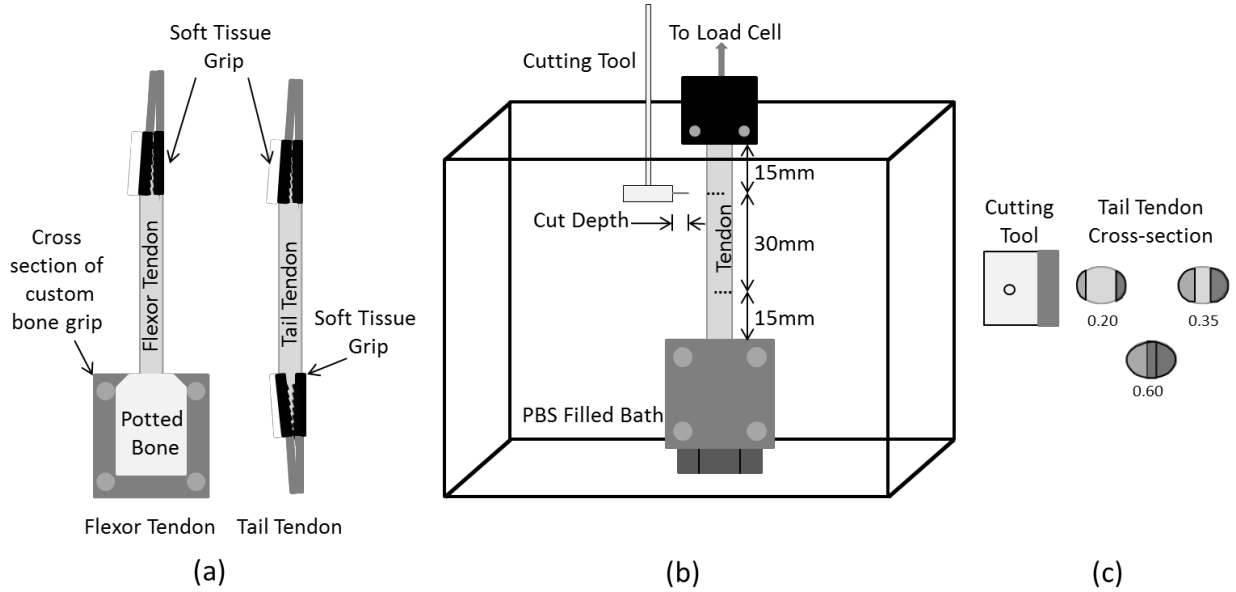

Figure 1. (a) Diagram of flexor tendon gripped in soft tissue and bone grip and tail tendon gripped in 2 soft tissue grips. The soft tissue grips contain two rough surfaces to clamp the non-bone sides of the tendon and the potted bone fits snugly into the custom bone grip. (b) Diagram of the gripped flexor tendon placed in a physiologic buffered saline (PBS) filled bath. The cuts were placed on opposite sides and ends of tendon as shown by the dashed lines. (c) Diagram showing the top view of the cutting tool and cross-sections of the 3 tail tendon laceration depths (numbers refer to the approximate laceration depth/tendon width). The darker sections show the two lacerations.

The tendons were loaded into the system measuring at 60mm, prior to preloading. A preload of 1.0N and 0.1N was slowly placed on the flexor and tail tendons, respectively, and allowed to stabilize before further testing. Preloads for the flexor and tail tendons were chosen based on values reported in Kondratko et al. (2012), Duenwald et al. (2009), Duenwald-Kuehl et al. (2012), and LaCroix et al. (2013), respectively. Load was measured with a 1000lb load cell (Honeywell Model 060-0571-06; Morrison, New Jersey) for the flexor tendon and a 50lb load cell (Lebow Products Model 3397-50; Toronto, Ontario) for the tail tendon. Displacement was controlled and measured by the MTS for all specimens. All time, load, and displacement data were output to a PC with Labtech Notebook software (Laboratory Technology Corporation, Fort Collins, Colorado).

The testing protocol was performed in the same manner as in Kondratko et al. (2012), shown in Fig. 2. Briefly, the initial length, grip-to-grip, was measured while the tendon was under preload. The tendon

was preconditioned with a sinusoidal, cyclic test (10 cycles at 0.5 Hz) to 2% grip-to grip strain. Following a 600s rest period to allow for viscoelastic recovery, a cyclic test of the same frequency and duration was completed to 4% strain. After this test and each subsequent test a 1000s rest period was included with the tendon at gauge length. The tendon then underwent a 100s relaxation test to 4% strain.

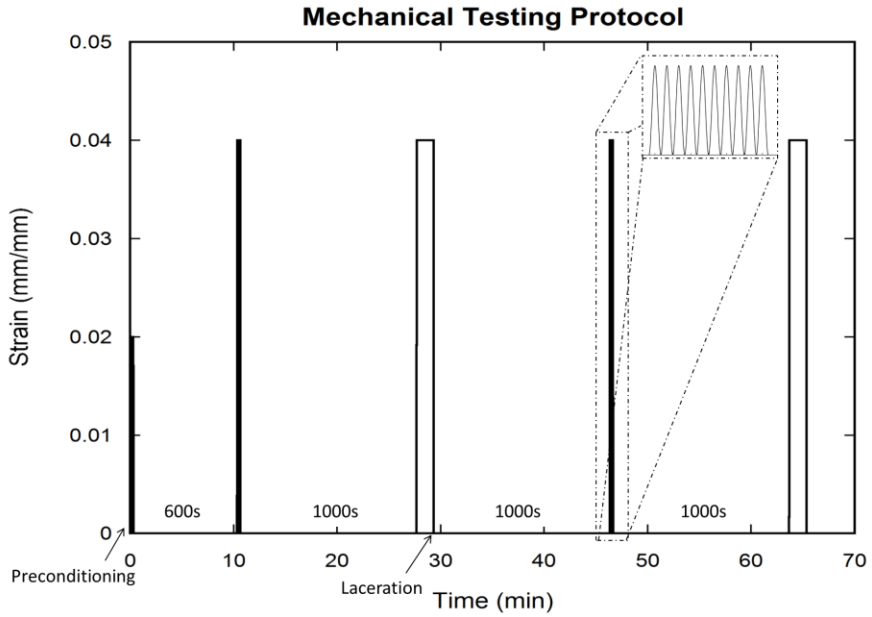

Figure 2. Mechanical testing protocol. Lacerations were created immediately after the first relaxation test and were followed by a 1000s rest period before beginning the second sinusoidal, cyclic test. The inset demonstrates that the bold lines are indicative of a cyclic test with 10 cycles at 0.5 Hz.

Prior to the next rest period, two transverse lacerations were made on opposite sides and ends of the tendon while the tendon was still at the initial gauge length, one 15mm from the proximal grip and one 15mm from the distal grip (Fig. 1b). Lacerations on the flexor tendon were made with a 1.0, 2.5, or 3.05mm (n=10 tendons per group) blade with a stopper as shown in Fig. 1 to ensure correct depth of cut. While carefully stabilizing the tendon, the cutting tool was moved back and forth to allow slicing of the tendon. Laceration depths corresponded to approximately 0.20, 0.55, and 0.60 times the tendon width in the direction of the cut, respectively. The lacerations for the tail tendon were made to a depth of 0.75, 1.0, and 2.5mm (n=10 tendons for each group), which corresponded to approximately 0.20, 0.35, 0.60 times the tendon width (Fig. 1c). Laceration depths were chosen, based on preliminary calculations using approximate tendon dimensions, to fall within the laceration depth/tendon width range of 0.20 to 0.60. However due to variation in tendon width, the ratios of the 2 tendon types were not the same. After the lacerations were created the 1000s rest period began. The lacerated tendon was then submitted to another cyclic and relaxation test as completed prior to laceration.

2.3 Specimen Imaging.

Upon completion of mechanical testing, 6 porcine flexor tendons and 6 rat tail tendons were randomly selected for specimen imaging to visualize fascicular structure. Whole tendons were visualized under a stereomicroscope (Stemi SV11; Carl Zeiss Microscopy, Jena, Germany) using a microscope camera (AxioCam; Carl Zeiss Microscopy, Jena, Germany). The microscope was equipped

with 2 polarizing filters, one fixed to the microscope lens and another to an external light source, to allow better visualization of tendon fascicles. Images were collected with the AxioVision Rel. 4.7 software (Carl Zeiss Microscopy, Jena Germany, 2008).

2.4 Mechanical Parameter Calculation.

To normalize for tendon width, each laceration depth was reported as a ratio of laceration size to average tendon width in the direction of the laceration. Percent overlap area was also considered to ensure differences in tendon thickness, perpendicular to the laceration, did not affect results. This was determined to be the percent of the tendon cross-sectional area in which the lacerations were overlapping; a negative percentage indicates the percent area of the tendon that remains intact between the lacerations (for lacerations less than 50% of the tendon width). This "overlap area" is represented as the middle portion of the tendon cross-sections shown in Fig. 1c.

Nominal stress values were calculated by dividing the load by the initial cross-sectional area of the tendon. For cyclic and relaxation tests, both elastic and viscoelastic parameters were considered. The elastic parameter for both tests was reported as the maximum stress observed during the test, $\sigma_{c\text{-}max}$ for cyclic tests and $\sigma_{r\text{-}max}$ for relaxation tests (Fig. 3). The cyclic maximum occurred at the peak of the initial cycle, and was determined by fitting a third-order polynomial curve to the initial stretch and subtracting the initial value (t=0s) from the peak value determined from the curve fit. The relaxation maximum always correlated to the initial stress, recorded at $t=2.5t_r$ (t_r=40ms=rise time), with time zero defined half way through the rise time, as recommended by Lakes (Lakes 2009). Viscoelastic parameters were reported as $\sigma_{c\text{-}decay}$ and $\sigma_{r\text{-}decay}$ for the cyclic and relaxation tests, respectively (Fig. 3). $\sigma_{c\text{-}decay}$ corresponded to the decrease in peak values between the initial (cycle 1) and final (cycle 10) cycles, the final cycle peak was determined in the same fashion as the initial cycle peak described above. Similarly, $\sigma_{r\text{-}decay}$ is defined as the decrease in the stress from the beginning of the relaxation test to the end of the relaxation test. This was calculated by fitting a logarithmic curve to the data and subtracting the value determined from the curve fit at t=100s by the maximum stress (t=0). Curve fitting for both cyclic and relaxation data was used to prevent errors in analysis due to noise in the system. Relaxation tests after the largest lacerations of the rat tail tendon produced data with an average signal-to-noise ratio of 4.2 and R^2 value of 0.74. Curve fitting was used to aid in the analyses of these data and was then used in all relaxation groups for consistency.

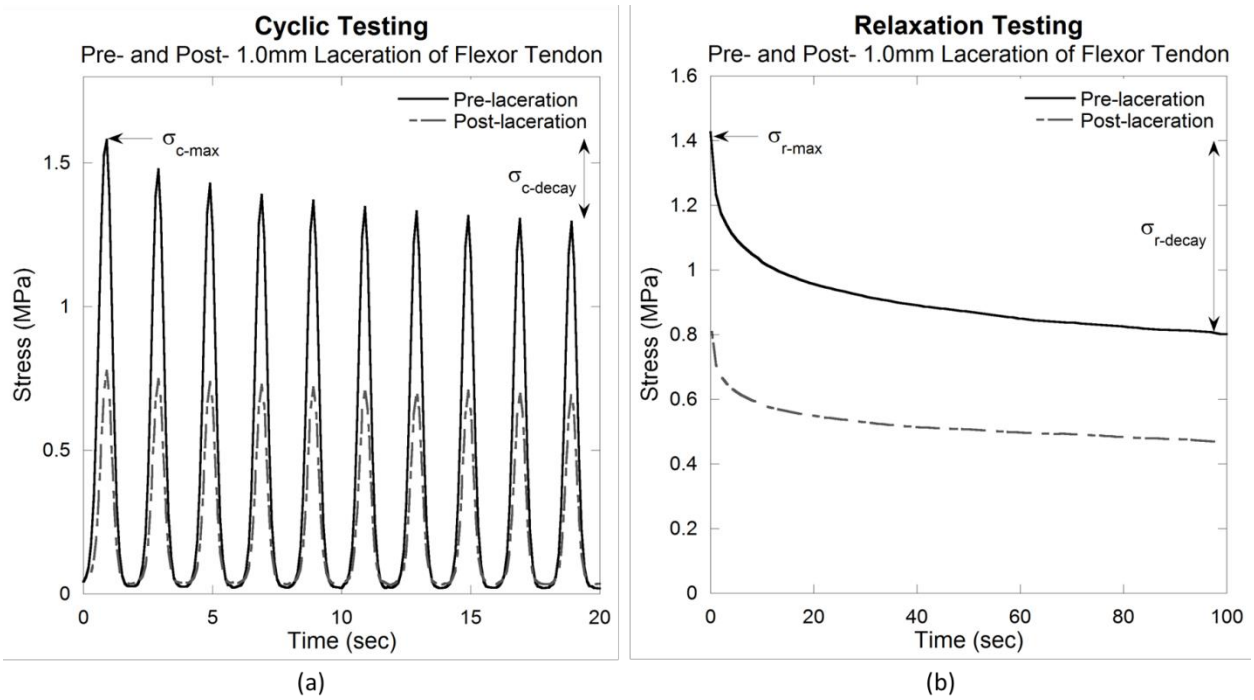

Figure 3. Representative stress data for the (a) cyclic and (b) relaxation tests of a flexor tendon pre- and post-shear laceration to a depth of 1.0mm (20% of the tendon width). Cyclic ($\sigma_{c\text{-}max}$ and $\sigma_{c\text{-}decay}$) and relaxation ($\sigma_{r\text{-}max}$ and $\sigma_{r\text{-}decay}$) testing parameters are shown. Stress decreased after laceration for both tests.

Modulus was calculated by determining the slope of the linear region of the stress-strain curve for the initial pull of the each cyclic test. The linear region varied slightly between tests; however the slope was generally taken between 1.5 and 3.5% strain.

Post-laceration values for each parameter were reported as a ratio of pre-laceration values to allow direct comparisons between tendons.

2.5 Image Analysis.

Fascicle angles were measured using ImageJ (NIH; Bethesda, Maryland). Fascicles were traced and their angle relative to the longitudinal direction of the tendon was measured. The fascicle angles on both the medial and lateral sides of the porcine flexor tendon were determined (Fig. 4a), as they are separate fascicles that become interwoven and plunge into the interior of the tendon at the midline. The absolute value of each angle was taken and the average was calculated for each tendon type.

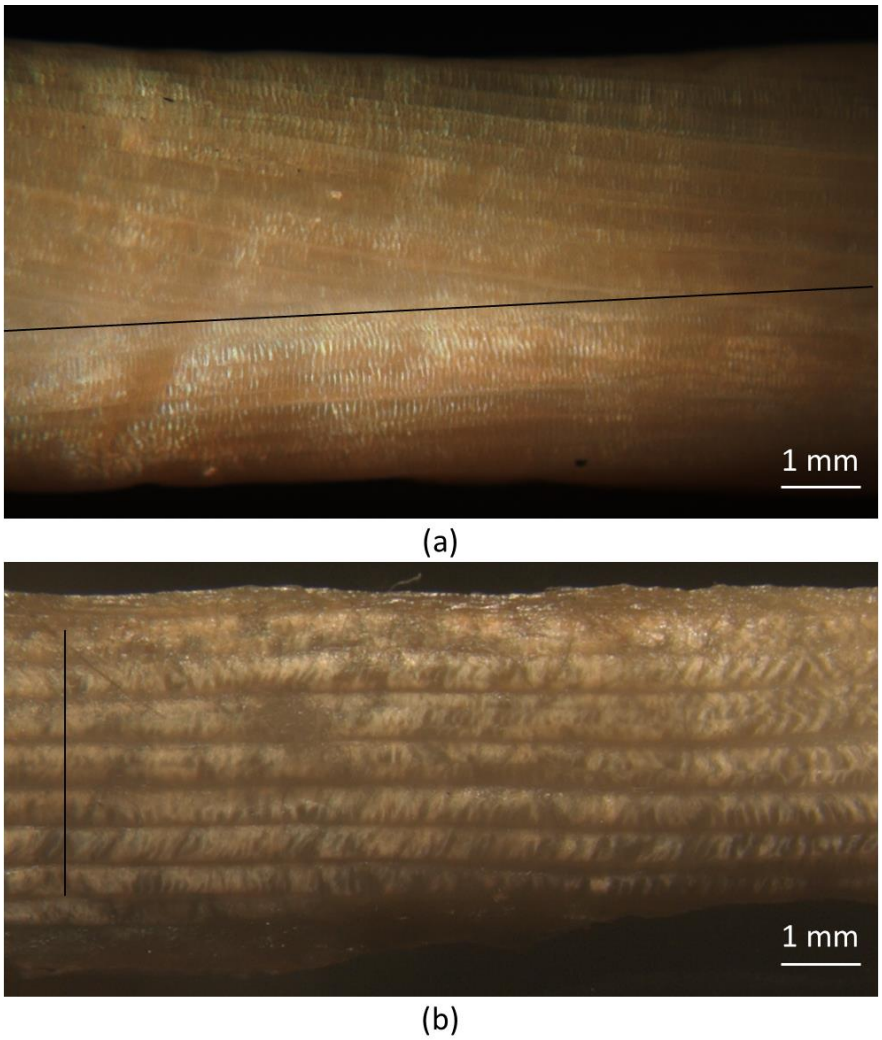

Figure 4. Image of porcine flexor tendon (a) and rat tail tendon (b) using polarized light microscopy. For analysis, angles for fascicles on both sides of their convergence, as shown with the black line in (a), for the flexor tendon were measured. The absolute value of the angles was taken before averages were taken. The black line in (b) demonstrates an example of a line drawn perpendicular to the fascicular direction. Fascicular packing was determined by dividing the number of fascicles crossed by each line (here 7) by the length of the line and averaging.

Fascicular packing was reported as the number of fascicles per unit length in the transverse direction. Lines containing only whole fascicles were drawn in five different locations perpendicular to the longitudinal direction of the tendon (Fig. 4b). The number of fascicles crossed by each line was counted and an average was taken for each specimen.

2.6 Statistics.

Post-laceration/pre-laceration ratios for each parameter were statistically compared by completing a one-way ANOVA. Within each parameter, a Fisher's least significant difference (LSD) post-hoc test was completed to specify differences between laceration sizes. Significance between pre- and post-laceration values for each laceration depth within each parameter was then calculated using a separate paired Student's t-test. Additionally, an unpaired Student's t-test was completed to compare relaxation and cyclic post-laceration ratios within each tendon type and laceration depth.

After plotting post-laceration/pre-laceration ratio versus laceration depth/tendon width ratio, an ANCOVA was completed to determine differences in slope and y-intercept between tendon types. Data from each specimen were used to calculate the ANCOVA.

A Student's t-test was also used to determine if a difference was present between specimens for fascicle orientation and packing. Significance was defined as $p \leq 0.05$, and trends were defined as $p \leq 0.1$.

3. Results

3.1 Mechanical Analysis.

As expected, when comparing the laceration depth/tendon width ratio to the percent overlap area of the lacerations, there is a linear relationship between the two geometric parameters with a similar slope for both tendon types (Fig. 5). This indicates that either parameter may be used for analysis with little effect on the results. It also demonstrates that for both tendon types, the width to thickness ratio is similar for all specimens in the group.

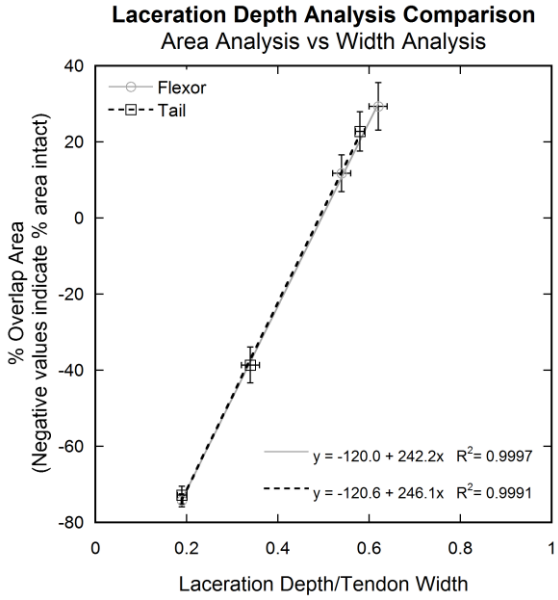

Figure 5. Comparison of laceration depth/tendon width ratio and percent overlap area. In both specimen types, the relationship between the parameters is linear. The error bars indicate standard error.

All parameters decrease following shear lacerations (Fig. 3 and Fig. 6). The ANOVA for each parameter in Fig. 6, in addition to the laceration depth/tendon width ratio, demonstrated a significant difference between the three groups based on laceration depth within the tendon type ($p<0.01$ for all parameters). The average post-laceration/pre-laceration ratio decreases with increasing laceration depth for all parameters and both tendon types, with most having significant differences based on the Fisher's LSD post-hoc analysis (Fig. 6).

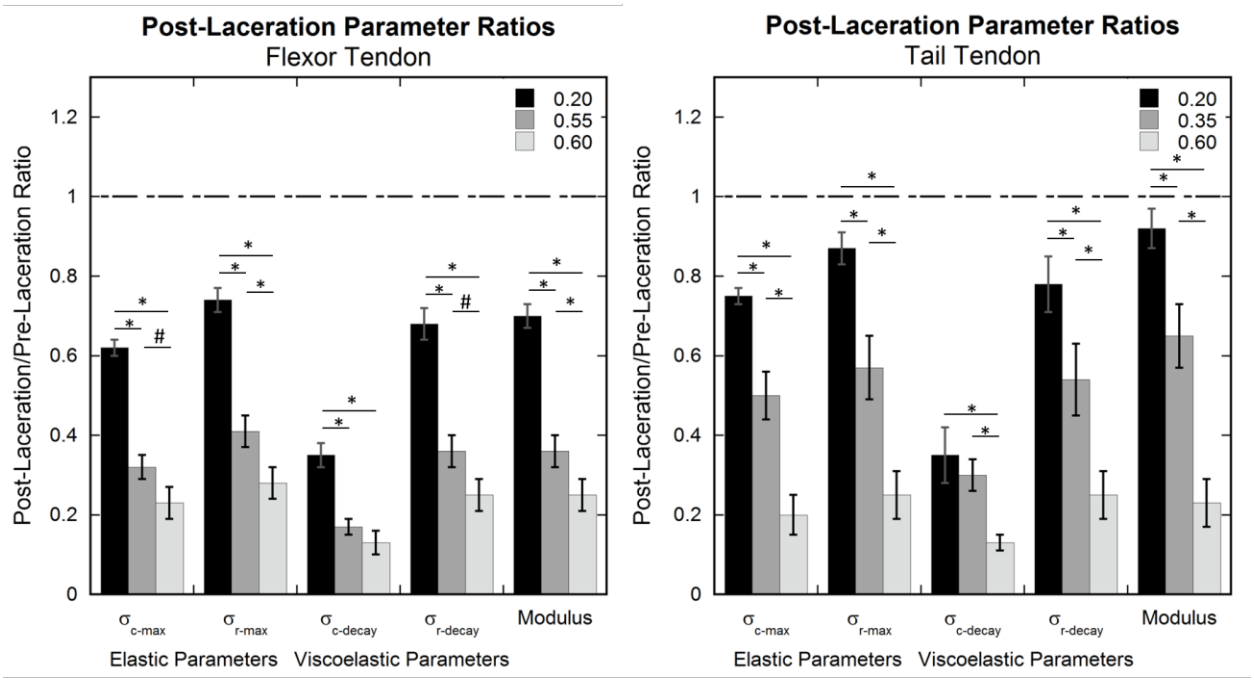

Figure 6. Post-laceration parameters plotted as a ratio of the pre-laceration value for (a) porcine flexor tendon and (b) rat tail tendon. The stress and modulus ratio averages decrease for all parameters with increasing laceration depth. The values in the legend represent the laceration depth/tendon width ratio. The dotted line represents a ratio of 1, where the post-laceration value would equal the pre-laceration value. Error bars represent standard error (n=10) and significant differences ($p \leq 0.05$) between laceration groups within each parameter determined by the Fisher's least significant difference post-hoc analysis are shown with an asterisk (*) and trends ($p \leq 0.1$) are shown with a pound sign (#).

The flexor tendon and tail tendon parameter ratios are plotted in Fig. 6. Each flexor tendon parameter demonstrated a significant difference between pre- and post-laceration values for each laceration depth ($p<0.006$ for all groups). With the exception of the 0.75mm (0.2 laceration depth/tendon width ratio) laceration group for the modulus parameter ($p=0.174$), all tail tendon parameters also displayed differences between pre- and post-laceration values for all laceration depths ($p<0.03$ for all groups). The intact tendon values for each parameter and cross-sectional area for both tendon types are listed in Table 1.

Parameter Values Prior to Laceration			
		Flexor Tendon	Tail Tendon
Cyclic Parameters	$\sigma_{c\text{-}max}$	1.42 ± 0.16 MPa	1.65 ± 0.20 MPa
	$\sigma_{c\text{-}decay}$	0.28 ± 0.03 MPa	0.33 ± 0.04 MPa
Relaxation Parameters	$\sigma_{r\text{-}max}$	1.28 ± 0.14 MPa	1.51 ± 0.19 MPa
	$\sigma_{r\text{-}decay}$	0.61 ± 0.06 MPa	0.61 ± 0.07 MPa
Stiffness Parameter	Modulus	61.4 ± 6.83 MPa	65.9 ± 7.69 MPa
Cross-sectional Area		27.8 ± 0.74 mm^2	4.88 ± 0.26 mm^2

Table 1. Parameter values prior to laceration. Values reported are mean percentage ± standard error.

Greater differences were found between testing methods in viscoelastic parameters than elastic parameters (Fig. 6). The cyclic post-laceration ratios of stress decay were significantly lower than the relaxation counterpart for both tendon types at each laceration depth ($p<0.045$ for all groups). Conversely, the elastic parameter, maximum stress, only demonstrated differences between cyclic and relaxation tests for the small laceration depth for both tendon types ($p<0.015$ for both groups).

A strong linear correlation was observed when plotting the parameter averages against the average laceration depth/tendon width ratio, separated by tendon type, as seen in Figs. 7-9 ($R^2>0.97$ for all linear regressions). The ANCOVA demonstrated a difference in slopes between tendon types for both elastic parameters, $\sigma_{c\text{-}max}$ ($p=0.0045$) and $\sigma_{r\text{-}max}$ ($p=0.0108$) (Fig. 7). The viscoelastic parameter $\sigma_{c\text{-}decay}$ did not demonstrate a significant difference between slopes of the post-laceration/pre-laceration ratio versus laceration depth/tendon width ratio plots for tail and flexor tendon, ($p=0.7923$), while $\sigma_{r\text{-}decay}$ demonstrated a trend towards significance ($p=0.0506$) between tendon types (Fig. 8). The elastic modulus also resulted in a significant difference between slopes of the flexor and tail tendon ($p=0.0023$) (Fig. 9).

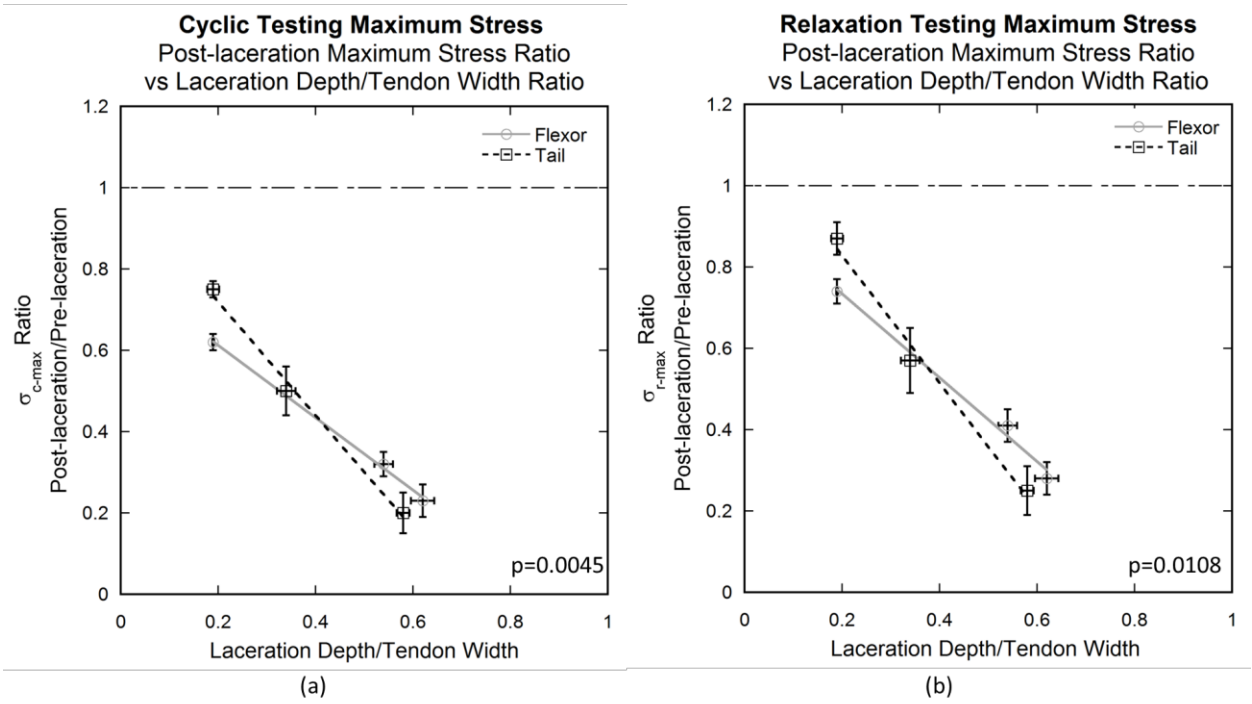

Figure 7. Plots of average post-laceration maximum stress values, presented as a fraction of the pre-laceration value, versus laceration depth/tendon width for (a) cyclic testing, $\sigma_{c\text{-}max}$ and (b) relaxation testing $\sigma_{r\text{-}max}$. Linear regression lines are plotted for both tendon types for both parameters ($R^2>0.97$ for all regression lines). The horizontal dashed line at 1.0 demonstrates an equivalent pre- and post-laceration value. The reported p-value is the ANCOVA result for the differences between slopes of the linear regression lines determined using data from all specimens. Error bars represent standard error.

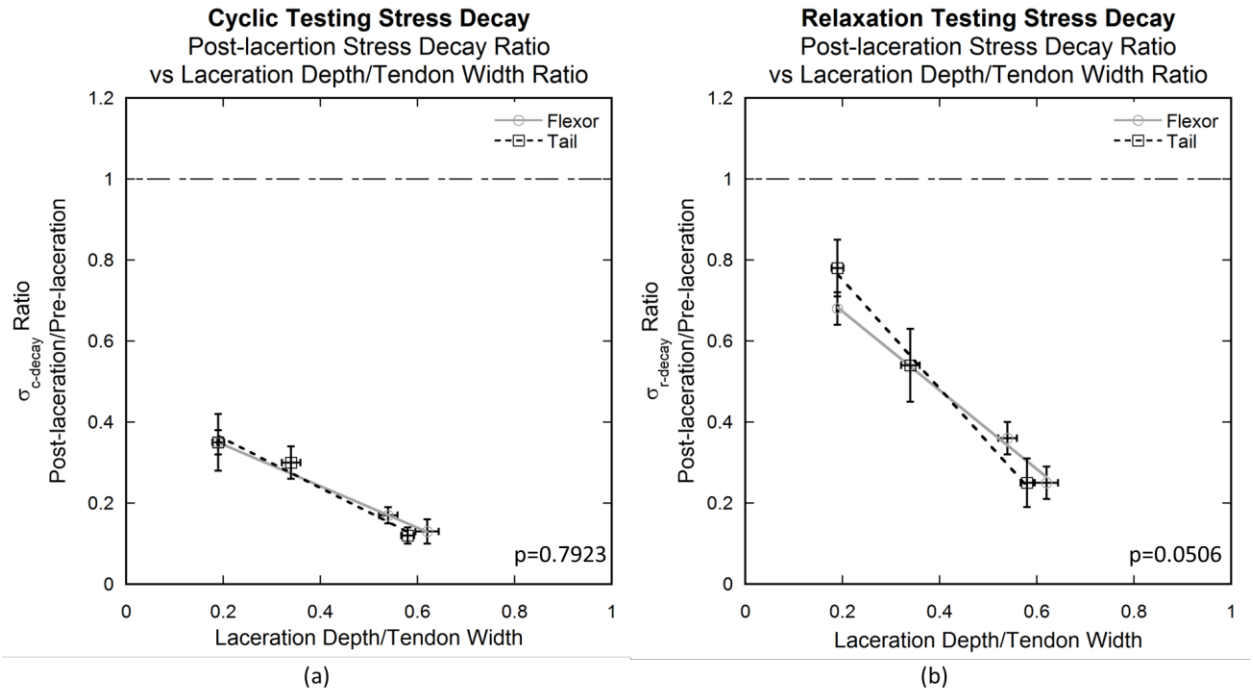

Figure 8. Plots of average post-laceration stress decay values, presented as a ratio of the pre-laceration value, versus laceration depth/tendon width for (a) cyclic testing, $\sigma_{c\text{-}decay}$ and (b) relaxation testing, $\sigma_{r\text{-}decay}$. Linear regression lines are plotted for both tendon types for both parameters (R^2>0.97 for all regression lines). The horizontal dashed line at 1.0 demonstrates an equivalent pre- and post-laceration value. The reported p-value is the ANCOVA result for the differences between slopes of the linear regression lines determined using data from all specimens. Error bars represent standard error.

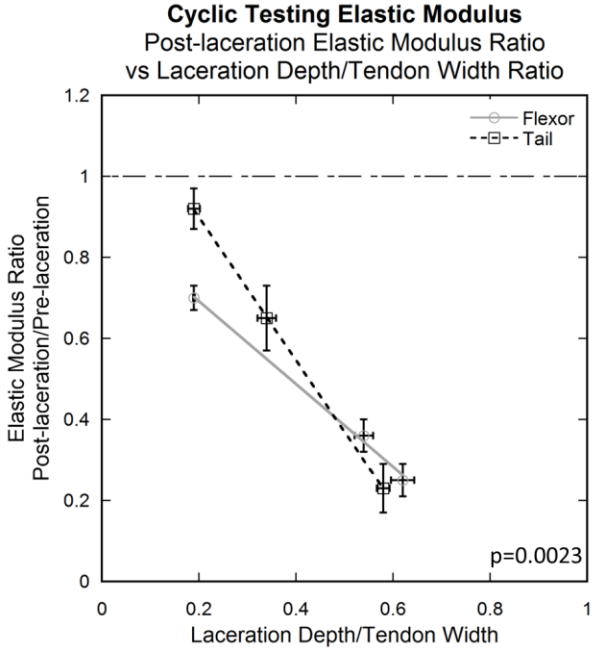

Figure 9. Plot of average post-laceration elastic modulus values, presented as a ratio of the pre-laceration value, versus laceration depth/tendon width. Linear regression lines are plotted for both tendon types (R^2>0.97 for both regression lines). The horizontal dashed line at 1.0 demonstrates an equivalent pre- and post-laceration value. The reported p-value is the ANCOVA result for the differences between slopes of the linear regression lines determined using data from all specimens. Error bars represent standard error.

3.2 Image Analysis.

As anticipated, porcine flexor tendon and rat tail tendon exhibited different fascicle orientation. Unlike the rat tail tendon, whose fascicles are nearly parallel to the long axis of the tendon (Fig. 4b), the porcine flexor tendon fascicles are positioned at slight angles off the long axis (Fig. 4a). Rat tail tendon fascicles are oriented at an average of 0.41 ± 0.43° and the porcine flexor tendon fascicles are on average 8.44 ± 3.05° off the longitudinal direction of the tendon (Fig. 10a). These angles are significantly different from each other ($p<0.0001$).

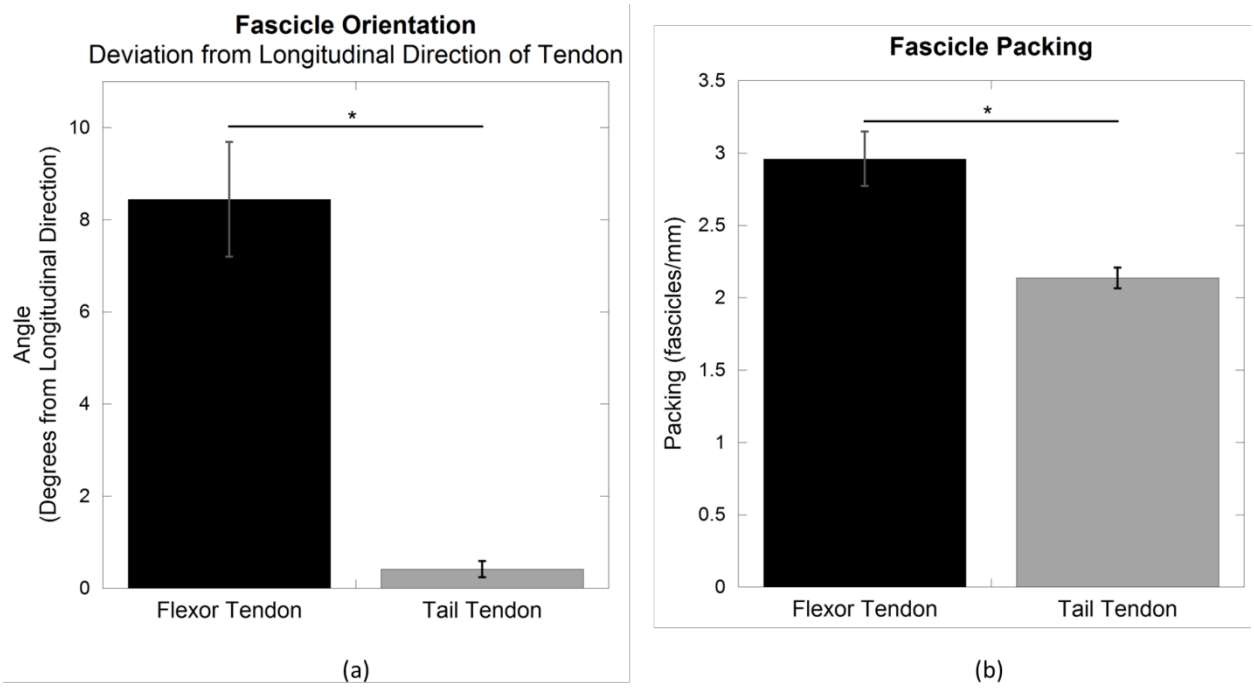

Figure 10. Fascicle analysis completed with images obtained using polarized light microscopy. (a) Fascicle orientation of the porcine flexor tendon and rat tail tendon presented as the angle from the longitudinal direction of the tendon. The angles were measured and their absolute values were taken before an average was calculated. (b) Fascicle packing of the flexor tendon and tail tendon determined by counting the number of fascicles in the transverse direction per unit length. An asterisk (*) indicates statistical significance ($p<0.003$).

The number of fascicles per transverse unit length within the tendons was also different between tendon types. The flexor tendon exhibited more fascicles per transverse unit length, 2.96 ± 0.46 fascicles/mm, while the tail tendon had 2.14 ± 0.18 fascicles/mm ($p=0.002$) (Fig. 10b). Observationally, the porcine flexor tendon fascicles also appeared to have tighter fascicle packing (fascicles closer together) than rat tail tendons (Fig. 4).

4. Discussion

Tendon hierarchical structure is complex, and the way its structure affects function requires continued investigation. The intricate, helical structure (Vidal 2003; C. T. Thorpe, Klemt, et al. 2013; Kalson et al. 2012; Kannus 2000; Khodabakhshi et al. 2013; Jozsa et al. 1991) results in complex mechanical properties, which are unexpected and hard to predict when tendons are loaded in a non-uniform

manner, such as near rotated insertion sites or around broken fibers or fascicles or torn or lacerated tendon. Therefore the goal of this study was to investigate how shear behavior redistributes axial loads in whole tendon via elastic and viscoelastic properties and their differences in functionally different tendons.

In this study, shear was created by lacerating the tendon on opposite sides, a fixed axial distance apart. With increasing laceration depth, shear load transfer becomes more responsible for the overall tendon strength. Using the basic assumption that tendons consist of linearly parallel fascicles (Fallon et al. 2002), lacerations greater than 50% of the tendon width would result in a tendon with no fascicles running its full length and therefore load would only be supported along the length of the tendon by transfer between fascicles, carried by inter-fascicular matrix. Although several studies suggest that there is little load carried between fascicles in shear to contribute to overall tendon strength in certain tendon types (Haraldsson et al. 2008; Purslow 2009; Berenson et al. 1996; C. T. Thorpe, Udeze, et al. 2013), others, including a study in our lab (Kondratko et al. 2012) and a study completed by Thorpe et al. (2012), both on digital flexor tendons (though from different species) suggest that shear is an important component of tendon function. Kondratko et al. (2012) demonstrated that compromised tendon strength after partial laceration is not linearly proportional to the percent area lacerated, indicating that fascicles are not independent, load-bearing structures but rather that they interact laterally at a functional level. In further support of this, the inclusion of shear-lag was necessary to model the mechanical results reported in the partial laceration study (Kondratko et al. 2012; Pensalfini et al. 2014) and has been used in other studies to model the mechanical response of whole tendon (Ahmadzadeh et al. 2013) and tendon fascicles (Szczesny and Elliott 2014).

The present study supports the hypothesis that fascicles do not act independently in tendon. Although there was increasing compromise with increasing laceration depth for both elastic and viscoelastic parameters, significant load-bearing occurred for lacerations greater than 50% of the tendon width, reinforcing the idea that shear lag is an important mechanism to redistribute non-uniform loads. The relationship between tendon compromise and laceration depth was linear in the range tested (approximately 20 to 60% of the tendon width), even after all continuous structures were disrupted. The linear relationship was not proportional to the laceration depth ratio such that when approximately 60% of the tendon width was lacerated on both proximal and distal ends (resulting in a cut overlap of approximately 25% of the tendon area, leaving no part of the cross-sectional footprint intact) the tendon parameters remained at approximately 20% of the intact value for both tendon types. With no full-length fascicles left intact (assuming near-parallel fascicles), the inter-fascicular matrix load transfer was responsible for the residual 20% of the tendon's original strength. Shear load transfer through the matrix was also suggested, though on a different hierarchical level (fibril), by Ahmadzadeh and colleagues (2013) and Szczesny and Elliott (2014) to play a role in overall tendon and fascicle mechanics.

While it is not commonly reported, shear viscoelasticity, here reported as relaxation (stress decay), is an interesting component of tendon mechanics. Unlike longitudinal relaxation, which results from both fascicle stretch and sliding of fascicles relative to each other, shear relaxation is primarily describing fascicle sliding in the case of large laceration depths, helping to separate the two mechanical components. Therefore, as the pre-laceration value is reporting longitudinal relaxation and the post-

laceration value is primarily reporting shear relaxation (in the overlapping groups and if the fascicles are not helical to the extent that they bypass both lacerations), the decrease in relaxation after laceration describes the relationship of fascicular sliding to stretching taking place within the tendon. Fascicular sliding is an important mechanism for protecting the fascicles from mechanical damage during tendon overload (C. Thorpe et al. 2012).

The larger decrease seen here in the viscoelastic parameter ratio in cyclic tests compared to relaxation tests after lacerations suggests that shear load transfer within tendon can better maintain load during static strain application than during repetitive strain (prior to allowing time for viscoelastic recovery). This observed difference was not a consequence of experimental protocol order, as the greater decrease was seen in cyclic testing which was completed prior to relaxation. A similar observation was reported in Kondratko et al. (2012), where all cyclic parameters decreased more after laceration than relaxation parameters. In both studies it is possible that strain rate differences are causing the larger decrease during cyclic testing. Due to slower strain application during cyclic testing, reorganization and separation of entangled collagen fibrils (Bozec, van der Heijden, and Horton 2007) and other hierarchical extracellular components may be more significant at low strain levels. The additional cycles of tendon displacement occurring in the cyclic testing protocol may also result in further reorganization during each subsequent cycle, potentially releasing the mechanisms causing shear transfer to take place.

As reported in the literature, fascicles in the high stress tendon analyzed here have a greater angle relative to the longitudinal direction of the tendon, suggesting a greater helical pitch angle, and also exhibit tighter fascicle packing (*i.e.* more fascicles per transverse unit length) compared to fascicles in the low stress tendon (C. T. Thorpe, Klemt, et al. 2013; Shepherd et al. 2014). Although the differences seen in the fascicles per transverse unit length may be a result of either fascicle size or packing, qualitative analysis of the images supported a difference in fascicle packing (Fig. 4). This tighter packing of the flexor tendon fascicles may enhance their ability to transfer shear load and explain why the low stress, tail tendon exhibited a more rapid decline in post-laceration parameters as the laceration depths increased. While the low stress tendon displays a more rapid decline in post-laceration parameters as laceration depth increases, it conversely shows a smaller decrease in post-laceration values after the small laceration. This may also be due to the flexor tendon's helical structure causing small lacerations to have an effect on more of the overall cross-section since the fascicles do not remain in the same longitudinal plane, although more testing is required.

Helical pitch angle of collagen fascicles may contribute to differences in apparent Poisson's ratios between tendon types (Reese, Maas, and Weiss 2010). The Poisson's ratios reported in the literature vary between and within different tendon types (Cheng and Screen 2007; Lynch et al. 2003; Vergari et al. 2011; Chernak and Thelen 2012). However, one study reports the Poisson's ratio for rat tail tendon fascicle to be 0.8 (Cheng and Screen 2007) while another reports a higher Poisson's ratio for whole porcine flexor tendon, between 0.8 and 1.64 (Chernak and Thelen 2012). The greater helical pitch at the fascicle level in the flexor tendon in this study therefore supports a higher apparent Poisson's ratio (Reese, Maas, and Weiss 2010), which may contribute to shear differences observed.

Limitations of this study include: specimens tested were obtained from different species, porcine and rat. While their origin and size are different, the basic function of the two tendon types are as desired, a high stress (typically energy storing) and a low stress (typically positional) tendon. Additionally, although the porcine deep digital flexor tendon is not an extremely high stress tendon, the comparison to the rat tail tendon provides an interesting contrast because the structure of the rat tail tendon is unique in its extremely low fascicular binding. This tendon structure provides us with a tendon that should have minimal shear load transfer. Furthermore, normalization of the data was used to provide comparative information on the differences between the tendon types. Another limitation in this study is that an elliptical cross-sectional area is assumed to calculate the stress and percent overlap area. However, since this assumption was made for each specimen, errors will be consistent across the study. Moreover, the preloads for the tendon types were selected based on previous experience published in the literature, but do not necessarily correlate to the tendon strength. Additionally, differences in fascicular orientation were only measured at the tendon surface and not the interior of the tendon. However, 3D imaging in other studies indicate that fascicular structure is similar throughout the tendon (Kalson et al. 2012; Jozsa et al. 1991; Khodabakhshi et al. 2013). Finally, further studies would benefit from probing for detailed shearing mechanisms and determining specific contributions to shear at the various hierarchical levels as this study did not investigate these aspects of the shear loading.

Energy storing (generally high stress) and positional (generally low stress) tendons have been shown to display different mechanical properties (Birch 2007; Shadwick 1990; Batson et al. 2003; S. L. Woo et al. 1980; S. L.-Y. Woo et al. 1981; Blanton and Biggs 1970; Benedict, Walker, and Harris 1968; Shepherd et al. 2014). This study indicates that differences in mechanical behavior between high and low stress tendons are also present when lacerations increase internal shear. Our results show that high stress tendons (flexor tendon) and low stress tendons (tail tendon) function differently in shear by differing slopes of post-laceration ratio versus laceration depth plots. Overall, results indicate that high stress, energy storing tendons may better withstand shear loading, supporting our hypothesis. Alternatively, low stress tendons decrease stiffness more rapidly as laceration depths increase, indicating that they have less capability to transfer loads in shear. A possible explanation is the more linearly oriented fascicles and lower fascicle density observed in this study.

4.1 Conclusions.

This *in vitro* study describes elastic and viscoelastic mechanical responses of porcine flexor tendons (which function as high stress, energy storing tendons) and rat tail tendons (which function as low stress, positional tendons) to varying amounts of laceration-induced shear loading. All mechanical parameters are increasingly compromised as larger laceration depths require increased shear load-bearing. Both tendon types exhibit a linear decrease in post-laceration/pre-laceration ratios (elastic and viscoelastic stress and elastic modulus) relative to the laceration size in the range tested. However, the tail tendon displays a more rapid decline in the post-laceration ratios with increasing laceration depth (larger negative slope). This, coupled with more linearly parallel and less densely packed fascicles, suggests a lesser ability for shear load transfer between fascicles in rat tail tendons than porcine digital flexor tendons. Nevertheless, approximately 20% of the pre-laceration values for all parameters were maintained in both tendon types after overlapping lacerations of about 60% of the tendon width (*i.e.* no

continuous elements end to end). This indicates that even tendons reported to have low fascicular binding can create significant shear load transfer during tendon lengthening procedures, although further studies should investigate site-specific mechanics and mechanisms. Additionally, the unexpected strength of tendons tested in shear suggests that shear transfer between tendon fascicles contributes more to normal (but non-uniformly distributed) tendon loadings than often anticipated. This behavior likely plays an important role near insertion sites and around tendon pathologies and injuries and perhaps during reorientation of insertion sites during movement. The significant levels of shear transfer shown here suggest that tendons should neither be considered nor modeled exclusively as independent load-bearing fibers or fascicles for any of the above scenarios.

5. Acknowledgments

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Disease of the National Institutes of Health (Award EB008548) are gratefully acknowledged. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors also thank Ron McCabe for his technical assistance.

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