Journal of Biomechanics 151 (2023) 111545

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech

Mouse Achilles tendons exhibit collagen disorganization but minimal collagen denaturation during cyclic loading to failure

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ARTICLE INFO

Keywords:
Achilles tendon
Collagen denaturation
Fatigue loading
Mechanical properties
Damage

ABSTRACT

While overuse is a prominent risk factor for tendinopathy, the fatigue-induced structural damage responsible for initiating tendon degeneration remains unclear. Denaturation of collagen molecules and collagen fiber disorganization have been observed within certain tendons in response to fatigue loading. However, no studies have investigated whether these forms of tissue damage occur in Achilles tendons, which commonly exhibit tendinopathy. Therefore, the objective of this study was to determine whether mouse Achilles tendons undergo collagen denaturation and collagen fiber disorganization when cyclically loaded to failure. Consistent with previous testing of other energy-storing tendons, we found that cyclic loading of mouse Achilles tendons produced collagen disorganization but minimal collagen denaturation. To determine whether the lack of collagen denaturation is unique to mouse Achilles tendons, we monotonically loaded the Achilles and other mouse tendons to failure. We found that the patellar tendon was also resistant to collagen denaturation, but the flexor digitorum longus (FDL) tendon and tail tendon fascicles were not. Furthermore, the Achilles and patellar tendons had a lower tensile strength and modulus. While this may be due to differences in tissue structure, it is likely that the lack of collagen denaturation during monotonic loading in both the Achilles and patellar tendons was due to failure near their bony insertions, which were absent in the FDL and tail tendons. These findings suggest that mouse Achilles tendons are resistant to collagen denaturation in situ and that Achilles tendon degeneration may not be initiated by mechanically-induced damage to collagen molecules.

1. Introduction

A major risk factor for tendinopathy is overuse or fatigue loading. Fatigue loading results in damage of tendons in the form of altered tissue structure and organization (Fung et al., 2010; Herod et al., 2016; Zitnay et al., 2017). In addition, the resident cells within tendon initiate a catabolic/degenerative response to fatigue loading characterized by an increase in proteinases, inflammatory markers, and cartilaginous/lipid/bone deposits (Archambault et al., 2007; Devkota and Weinhold, 2010; Spiesz et al., 2015; Thorpe et al., 2015; Zhao et al., 2019). The direct structural damage and resultant degenerative cellular response reduces the mechanical properties of tendon (Arya and Kulig, 2010; Devkota, 2007; Flick et al., 2006; Fung et al., 2009), which increases the probability of a tendon rupture. Since tendon cells are mechanosensitive (Lavagnino et al., 2015), it is possible that the degenerative cellular behavior is a response to the structural damage or altered tissue loading that occurs with overuse. However, it is not clear what specific stimuli in damaged tendons cause the cells to behave in such a manner. Therefore, it is important to understand the mechanisms that are responsible for initiation of tendon damage and degeneration.

Studies have shown that different tendons undergo different kinds of structural damage with mechanical loading. Positional tendons, such as common digit extensor tendons (CDETs), are primarily responsible for fine motor control and exhibit multiple forms of fibrillar damage in response to monotonic loading, including discrete plasticity, fibril kinking, and loss of D-period banding (Chambers et al., 2018; Herod et al., 2016). Energy-storing tendons, such as the Achilles tendon or superficial digit flexor tendon (SDFTs), lack fine control but store and release energy during locomotion resulting in less work done by muscles (Alexander, 1991; Roberts et al., 1997; Roberts and Azizi, 2011). These tendons show more resistance to fibrillar damage during monotonic loading, especially at high strain rates (Chambers et al., 2018). In response to fatigue loading, positional CDETs exhibited fibril kinking, ruptured fibrils and discrete plasticity, while fatigue loading of energy-

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https://doi.org/10.1016/j.jbiomech.2023.111545
Accepted 8 March 2023
Available online 12 March 2023

storing SDFTs did not produce these fibril-level forms of damage (Herod et al., 2016); however, collagen disorganization/kinking at the fiber-level was observed with fatigue loading of rat patellar tendons (Sereysky et al., 2012), which also provides an energy-storing function (Wiesinger et al., 2020). While it is still unclear why energy-storing tendons are resistant to collagen denaturation compared to positional tendons, possible reasons include damage localization within plastic interfascicular or interfibrillar shear (Lee et al., 2017; Thorpe et al., 2012), resistance to intermolecular sliding within fibrils due to an increased density of mature crosslinks (Lin et al., 2022; Quigley et al., 2018; Zitnay et al., 2017), or localized point failure of collagen molecules/fibrils (Chambers et al., 2018).

While the SDFT in large quadrupeds (e.g., horses and cows) is commonly injured and exhibits human-like tendinopathy (Ely et al., 2009; Patterson-Kane and Rich, 2014), it is unclear whether the same damage mechanisms observed in the SDFT occur in the Achilles tendon, which is the primary human energy-storing tendon. Surprisingly, there is no previous work that has looked at the damage mechanisms in Achilles tendons. Therefore, the objective of this study was to investigate whether denaturation of collagen molecules or collagen fiber kinking/disorganization occurs in mouse Achilles tendons during cyclic and monotonic loading to failure. Achilles tendons of mice are of particular interest because they are often studied as a model for tendinopathy (Bell et al., 2013; Wang et al., 2022; Zhang et al., 2020; Zhao et al., 2019) and show similar degeneration patterns to human Achilles tendons. For instance, degenerated Achilles tendons of both mice and humans show deposition of proteoglycans and glycosaminoglycans (Maffulli et al., 2000; Zhang et al., 2020; Cho et al., 2021; Wang et al., 2022; Zhao et al., 2019). Additionally, the availability of transgenic mouse models enables the future investigation of downstream mechanotransduction mechanisms that may drive the etiology of tendinopathy.

Based on the previous literature on energy-storing tendons discussed above, we hypothesized that ruptured mouse Achilles tendons will exhibit collagen fiber disorganization but limited collagen denaturation. A lack of collagen denaturation in mouse Achilles tendons would suggest that the Achilles tendinopathy observed in mouse models (Cho et al., 2021; Wang et al., 2022; Zhang et al., 2020; Zhao et al., 2019) is not initiated by mechanically-induced damage to collagen molecules. We also compared the level of collagen denaturation observed in mouse Achilles tendons after monotonic loading to other mouse tendons to see if this resistance to molecular denaturation is unique to Achilles tendons. Understanding the damage mechanisms in Achilles tendons exposed to fatigue loading will help identify the micromechanical environment that leads to tendon degeneration.

2. Methods

2.1. Mechanical loading

Achilles tendons, flexor digitorum longus (FDL) tendons, tail tendon fascicles, and patellar tendons were harvested from euthanized 15 ± 3 week old male C57BL/6 mice from an approved IACUC study. The Achilles and patellar tendons were harvested with their bone attachments intact whereas the FDL and tail tendons were not. Tendon cross-sectional area was measured using a non-contact laser transducer. For cyclic loading, the bone attachment and myotendinous junction were secured in a custom tensile bioreactor using custom grips (Pedaprolu and Szczesny, 2022). After gripping the tendons, a minimal preload was applied such that there was no visible slack to measure the initial length of the tissue. The Achilles tendons (n = 7) were then cyclically loaded to 5 MPa at 0.5 Hz until rupture. The bioreactor kept the tendons submerged in a PBS bath at 30 °C based on temperature readings of the mouse hindlimb using an infrared thermometer. Based on preliminary testing, the peak cyclic load of 5 MPa was chosen because it was 20% of the ultimate tensile stress (UTS), which is representative of in vivo physiologic loading (West et al., 2004). The peak strain per cycle was recorded.

Additional Achilles tendons (n = 4), FDL tendons (n = 3), patellar tendons (n = 3), and tail tendons (n = 5) were monotonically loaded to failure at a 0.5%/s strain rate in PBS at room temperature with a custom built tensile testing device (Peterson and Szczesny, 2020). The bone attachment of the Achilles tendons was secured in a conical grip during monotonic loading (Supplementary Fig. 1), which minimized stress concentrations by allowing the bone to reset into a natural position during loading. For the patellar tendons, the patella and the tibia were gripped in their anatomical orientations with serrated stainless steel grips (Pedaprolu and Szczesny, 2022). The tendinous ends of the Achilles, FDL, and tail tendons were directly clamped by custom compression grips. A preload of 1 g was applied before monotonic loading to define the zero-displacement position. The UTS and maximum tangent modulus was recorded for all the monotonically loaded tendons. A freshly harvested tendon and a tendon placed under minimal static load (~0.02 MPa) were used as negative controls for monotonic and cyclic loading, respectively. Finally, three Achilles tendons were heated in PBS at 57 °C for 25 mins as a positive control for collagen denaturation. All samples were frozen at –20 °C prior to subsequent analysis for collagen denaturation.

2.2. Assessment of collagen denaturation

All tendons (except tail tendons, which were too thin) were cryosectioned at a thickness of 20 μm. The sections and tail tendons were stained with a fluorescein tagged collagen hybridizing peptide (CHP) at a concentration of 10 μM overnight at 4 °C (3Helix Inc). CHP is a probe that specifically binds to denatured collagen (Li et al., 2013). The samples were washed three times for 10 min each with room temperature distilled water. The sections were cover slipped and the tail tendons were sandwiched between two coverslips and imaged using a confocal microscope (Nikon A1R HD).

Positive signal for CHP staining was identified in the fatigue and monotonically loaded samples by a fluorescence intensity above a baseline threshold value. Baseline threshold values were determined from the negative control sample for each tendon type such that <1% of the total tendon area was positively labeled for CHP staining (Supplementary Fig. 2). This ensured that any background signal (i.e., autofluorescence, denatured collagen in control samples, unwashed CHP probe) unique to each tendon type was not included in the quantification of collagen denaturation in the mechanically loaded samples. The percentage area of collagen denaturation was calculated as the ratio of the area of positive signal to the total area of the tendon sections. For each sample, a total of 2 sections were analyzed, and the percentage area was averaged across the individual tissue sections. The heterogeneity in the CHP staining between tissue sections of each sample was assessed by determining the coefficient of variation for each tendon type.

2.3. Assessment of collagen disorganization

One or two cryosectioned sections of each fatigue loaded Achilles tendon and static samples (n = 4) were imaged via second harmonic generation using a multiphoton microscope (Leica SP8 DIVE) to visualize the collagen fibers. The images were divided into 18 μm × 18 μm subregions, and collagen fiber alignment within those subregions was measured via Fourier transform analysis (Sereysky et al., 2010). Collagen fiber disorganization/kinking was defined as a greater than 30° difference in the fiber angles between the neighboring subregions (Szczesny et al., 2018). The amount of collagen fiber disorganization was determined by calculating the percentage of the total tendon area that contained disorganized regions.

2.4. Statistical analysis

A *t*-test with Welch's correction was used to compare the amount of

collagen denaturation and disorganization between the cyclically loaded and static control Achilles tendons. Differences in the amount of collagen denaturation during monotonic loading and the mechanical properties between the Achilles tendon and all other tendons were determined using a one-way ANOVA with a Dunnett's post hoc correction for multiple comparisons. Statistical significance for all tests was set at $p < 0.05$.

3. Results

3.1. Collagen denaturation and disorganization

Cyclically loaded Achilles tendons exhibited a typical triphasic creep behavior and ruptured at the tissue midsubstance after 109,586 ± 31,330 cycles (Supplementary Fig. 3). Only 3.2 ± 2.7% of the total area of the ruptured samples exhibited positive CHP staining, which was not significantly greater than the residual signal in the static control samples (Fig. 1). In contrast, 17.41 ± 5% of the tendon area exhibited collagen disorganization, which was significantly greater than the amount of disorganization in statically loaded Achilles tendons (7.71 ± 1.64%). Achilles tendons monotonically loaded to rupture also exhibited minimal (5.2 ± 0.4%) positive CHP staining, which was significantly less than FDL tendons (71.6 ± 17.9%) and tail tendons (97.7 ± 2.7%) (Fig. 2). The percentage area of CHP staining in patellar tendons was 11.4 ± 13.3%, which was not significantly different than Achilles tendons ($p = 0.81$). As a positive control, heat denatured Achilles tendons exhibited collagen denaturation across the entire tendon area (97.2 ± 3.6 %). The coefficient of variation of CHP staining between sections within a given sample was on average<0.4, with higher individual sample values due to very small means (Supplementary Fig. 4).

3.2. Tendon mechanical properties

Monotonically loaded Achilles and patellar tendons exhibited soft tissue failure near the bone insertions, while the FDL and tail tendons failed at the tissue midsubstance. Failure at the bone insertions produced a relatively abrupt drop in the stress–strain curves for the Achilles and patellar tendons (Fig. 3). Note that the small jumps in the stress strain curves before failure in some of the samples were due to resetting of the bone attachment in the conical and serrated grips. While the tail tendons exhibited gradual post-yield failure, the FDL tendons exhibited a mixture of abrupt and gradual failure. The UTS and maximum tangent modulus of the patellar tendons was not significantly different than that of the Achilles tendons ($p = 0.12$ and $p = 0.14$, respectively) (Fig. 4). However, the FDL and tail tendons were both significantly stronger and stiffer than the Achilles tendon.

4. Discussion

In this study, we found that cyclically loaded mouse Achilles tendons exhibit minimal collagen denaturation but demonstrate collagen fiber disorganization. This is consistent with cyclic loading of the energy-

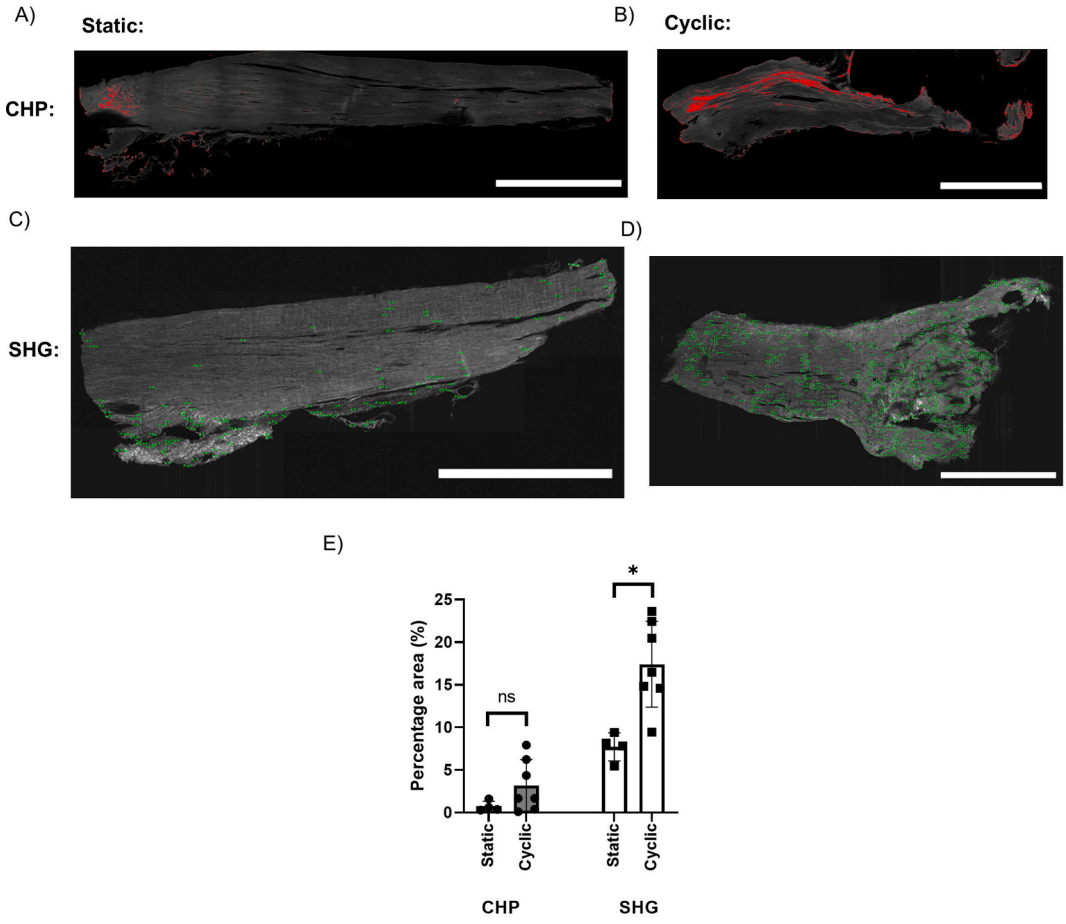

Fig. 1. Cyclically loaded mouse Achilles tendons exhibit collagen fiber disorganization but minimal collagen denaturation. Representative thresholded images of CHP-stained (A) static control and (B) cyclically loaded mouse Achilles tendons (CHP signal is colored red). Representative images of collagen orientation in (C) static control and (D) cyclically loaded mouse Achilles tendons (green signal represents local collagen fiber disorganization). (E) There was a statistically significant difference in collagen disorganization but not collagen denaturation between static control and cyclically loaded mouse Achilles tendons ($p < 0.05$). Scale bar: 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

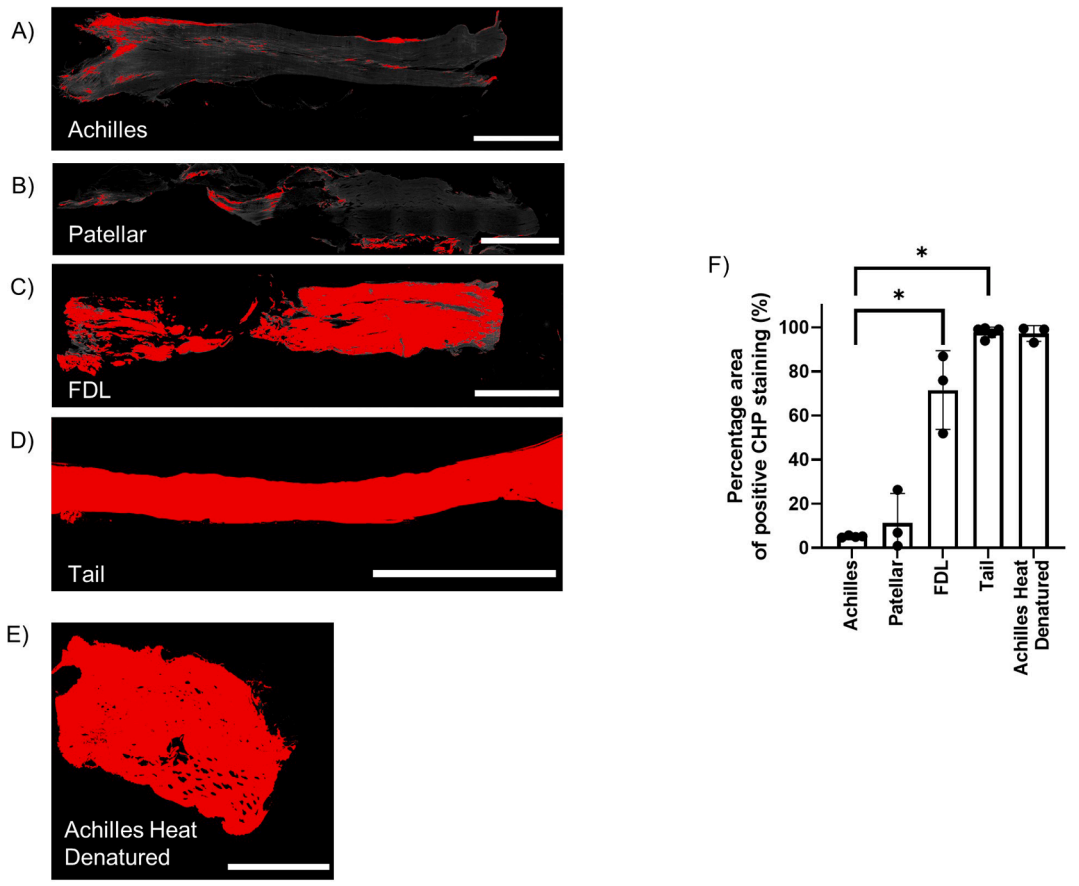

Fig. 2. Levels of collagen denaturation in various mouse tendons monotonically loaded to failure. Representative thresholded images of ruptured mouse (A) Achilles tendons, (B) patellar tendons, (C) FDL tendons, and (D) tail tendon fascicles. (E) Heat denatured Achilles tendon acted as positive control for collagen denaturation. (F) Monotonically ruptured Achilles tendons showed significantly less collagen denaturation than FDL tendons and tail tendon fascicles ($p < 0.05$). Scale bar: 1 mm.

storing bovine SDFT (Herod et al., 2016) and rat patellar tendon (Sereysky et al., 2012), which has also been suggested to provide an energy-storing function (Wiesinger et al., 2020). We also observed little collagen denaturation in monotonically loaded mouse Achilles tendons and patellar tendons. However, this is in contrast to monotonic loading of bovine SDFTs at strains rates similar to those used in this study, which do exhibit collagen molecule denaturation (Chambers et al., 2018). Additionally, the resistance to molecular damage in mouse Achilles and patellar tendons was also unique compared to mouse FDL tendons, which may also serve an energy-storing purpose (Lin et al., 2022, 2020).

It is tempting to conclude from these data that Achilles tendons are highly adapted to their particular in vivo function and that tissue damage mechanisms differ between energy-storing tendons. In comparison to positional tendons, energy-storing tendons exhibit greater interfascicular sliding under load (Thorpe et al., 2012) and have a higher density of mature crosslinks (Svensson et al., 2018). This reduces the amount of strain transmitted to tendon fascicles for a given applied tissue-level strain and reduces the amount of intermolecular sliding, both of which protects energy-storing tendons from collagen denaturation (Herod et al., 2016; Quigley et al., 2018; Zitnay et al., 2017). However, these structural properties also lead to a lower tissue modulus/strength as well as local brittle failure (Chambers et al., 2018; Thorpe et al., 2012). Interestingly, we found that the Achilles and patellar tendons had a lower modulus and tensile strength than the FDL and tail tendons (Fig. 4). Since FDL tendons experience lower in vivo forces and provide less energy storage than Achilles tendons (Biewener and Baudinette, 1995), it is possible that the rodent FDL may serve a dual positioning and energy-storing function, with structural characteristics, mechanical properties, and damage mechanisms that are a blend of "pure" energy-storing and positional tendons. This would explain the moderate susceptibility of FDL tendons to collagen denaturation that is intermediate between the Achilles and tail tendon fascicles (Fig. 2) and would be in agreement with a potential tradeoff between cyclic loading capacity and stiffness/strength (Herod et al., 2016).

However, it is unclear what structural differences (if any) exist between the FDL and Achilles tendons. Mouse Achilles tendons do not contain separate fascicles (Eekhoff et al., 2022; Lee and Elliott, 2018), so increased interfascicular sliding cannot explain the difference in collagen denaturation and mechanical behavior between the Achilles and FDL tendons. Additionally, rat FDL and Achilles tendons have a similar resistance to acid solubility (Lin et al., 2022; Svensson et al., 2018), suggesting that they also have comparable amounts of mature crosslinks. Finally, greater interfibrillar sliding could potentially protect collagen molecules in Achilles tendons from denaturation and also produce a lower tensile modulus. However, rat tail tendon fascicles exhibit substantial interfibrillar sliding as well as collagen denaturation (Szczesny and Elliott, 2014), suggesting that increased interfibrillar sliding alone is not enough to prevent damage to collagen molecules. Therefore, it is unlikely that a difference in tissue structure can explain the difference in collagen denaturation between the Achilles and FDL tendons.

An alternative explanation is that the minimal collagen denaturation observed in mouse Achilles tendons within this study was due to point failure at the enthesis. Both the Achilles and patellar tendons in our study were gripped at the bone attachments, and they both failed at the

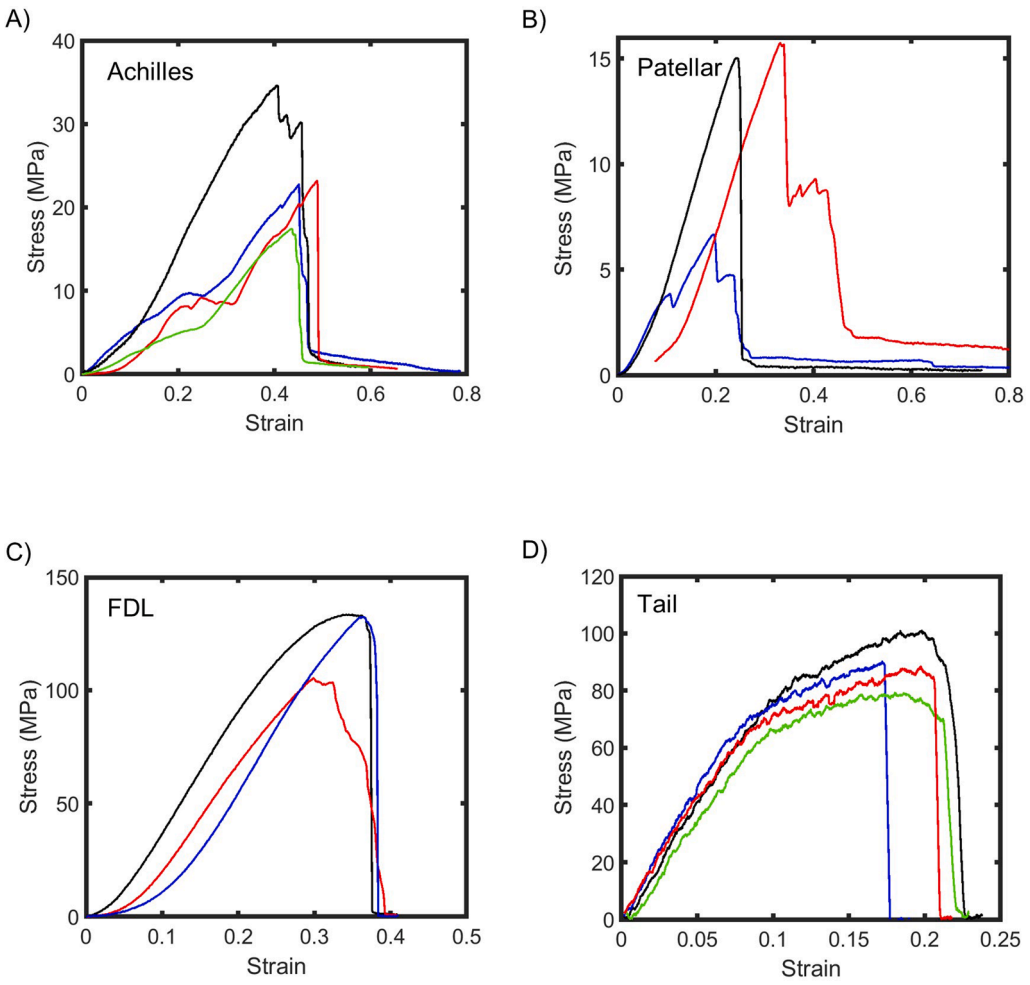

Fig. 3. Stress strain curves of monotonically loaded mouse tendons. Individual stress strain curves of (A) Achilles tendons, (B) patellar tendons, (C) FDL tendons, and (D) tail tendon fascicles.

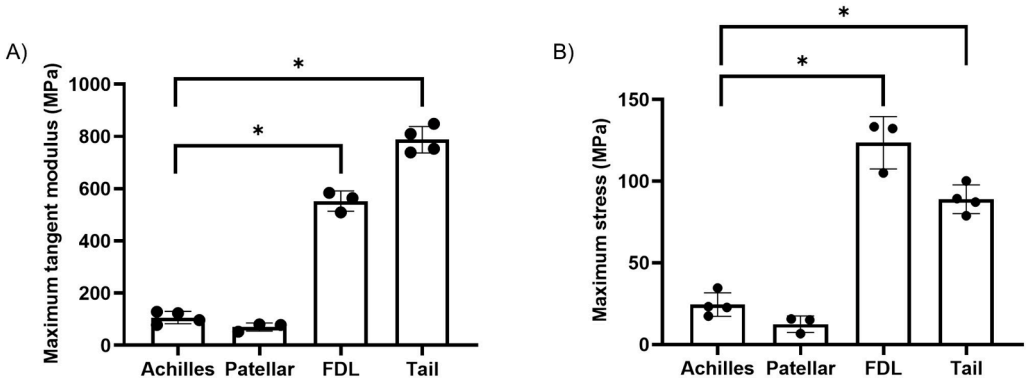

Fig. 4. Mechanical properties of mouse tendons. (A) Maximum tangent modulus and (B) maximum stress during monotonic loading of Achilles tendons are lower than both FDL tendons and tail tendon fascicles ($p < 0.05$).

enthesis under monotonic loading. In contrast, the FDL and tail tendons failed at the tissue midsubstance, and these tendons were gripped directly since the bony attachments could not be harvested intact. We found that the modulus and UTS for the Achilles and patellar tendons were substantially lower than that for the FDL and tail tendons, which is consistent with prior literature suggesting that the enthesis is a weak point in tendons (Liu et al., 2012; Miller et al., 2012). Additionally, the failure point in the stress–strain curves for both the Achilles and patellar tendons is relatively abrupt compared to the FDL tendons and especially the tail tendons (Fig. 3). This suggests that the enthesis failed prior to significant yielding of the tendon midsubstance for both the Achilles and patellar tendons. Previous studies have demonstrated that collagen denatures only after tendons and fibrils are stretched beyond their yield point (Lin et al., 2022, 2020), so this would explain the low amount of CHP staining in the Achilles and patellar tendons during monotonic loading. Interestingly, the fatigue-loaded Achilles tendons also failed at the tissue midsubstance yet still exhibited little collagen denaturation. This suggests that under cyclic physiologic loading the enthesis remains

intact and that mouse Achilles tendons rupture via brittle point failure within the tissue midsubstance, which is consistent with the failure mechanisms in other energy-storing tendons (Chambers et al., 2018; Herod et al., 2016). This is in contrast to fatigue loading of rat tail tendon fascicles (i.e., positional tendons), which did exhibit collagen denaturation in response to fatigue loading (Zitnay et al., 2017), albeit at higher loading levels (40% UTS). Therefore, while failure at the enthesis may explain the limited collagen denaturation observed during monotonic loading, the lack of collagen denaturation observed in the mouse Achilles tendon during fatigue loading is likely due to the structural characteristics (e.g., increased mature crosslinks) of the tendon itself (Herod et al., 2016).

Another important implication of our data is that the initiation of tendon degeneration observed in Achilles tendons may not be due to mechanically-induced collagen denaturation. It should be noted that the tissues in this study were not viable, so it is possible that collagen denaturation/molecular damage does occur within tendinopathic tissue in vivo as a result of secreted proteinases and cell-mediated tissue remodeling (Chen et al., 2020; Steplewski et al., 2019), which could in turn aggravate the degenerative cascade. Still, the presence of collagen disorganization suggests that altered tissue topography may stimulate a degenerative cellular response in fatigue-loaded Achilles tendons. Previous in vitro studies have shown that nonaligned substrates cause tendon cells to lose their native phenotype and increase their osteogenic potential (Yin et al., 2010; Zhu et al., 2010). Additional studies have also shown that the response of cells to mechanical loading is sensitive to angular variations in fibrillar matrices (e.g., crimping, kinking), which may be due to altered strain transmission and mechanosensing (Chao et al., 2014; Szczesny et al., 2016). Therefore, it is possible that collagen fiber disorganization is an early driver of the degenerative cellular behavior observed in tendinopathic Achilles tendons.

We acknowledge that there are limitations to this study. One is that our findings in mice may not be applicable to human Achilles tendons. Indeed, some question whether rodent Achilles tendons are suitable models of the human equivalent given the different physiological demands between humans and rodents as well as the difference in size and structure (Patterson-Kane and Rich, 2014). As mentioned above, rodent Achilles tendons lack an interfascicular matrix (Eekhoff et al., 2022; Lee and Elliott, 2019), which is an important determinant of the mechanical differences between energy-storing and positional tendons in larger animals (O'Brien et al., 2021). Nevertheless, mouse Achilles tendons do exhibit tissue degeneration in response to overuse (Zhang et al., 2020; Zhao et al., 2019), similar to human Achilles tendons and SDFTs in the horse (Maffulli et al., 2000; O'Brien et al., 2021; Patterson-Kane and Rich, 2014). Additionally, mouse Achilles tendons exhibit a similar resistance to collagen denaturation as bovine SDFTs (Chambers et al., 2018; Herod et al., 2016; Quigley et al., 2018), suggesting that our results are consistent with larger animal models. A second technical limitation is that we used optical microscopy and image analysis of individual tissue sections to quantify the percent of the tendon area that contains denatured collagen, which should not be confused with the total amount of denatured collagen within the tissue that is measured using biochemical techniques (Lin et al., 2019). For example, prior testing of rat tail tendon fascicles demonstrated that when 100% of the tendon area contains denatured collagen, only 2.5% of the total collagen is actually denatured (Zitnay et al., 2017). However, this also suggests that microscopic imaging of CHP staining is a highly sensitive technique for identifying the presence of denatured collagen molecules in tendon. Additionally, the areal fluorescence measurements used in this study are correlated with biochemical microplate and trypsin digestions assays (Lin et al., 2019). The technical variation in CHP staining within our data is also small compared to the biological variation between samples and tendon types (Supplementary Fig. 4). Therefore, our data is likely an accurate proxy for the relative total collagen denaturation between tissues. Finally, we were unable to directly test whether the lack of collagen denaturation during monotonic loading of mouse Achilles and patellar tendons was indeed due to failure at the tendon enthesis. The short length of both tendons precluded the ability to remove the bone attachments and directly grip the tendon midsubstance. Conversely, the separation of the distal FDL tendon into multiple digits made it difficult to test the proximal tissue with an intact bone attachment. Nevertheless, the Achilles tendon entheses are obviously intact in situ; therefore, our data suggest that, even for supraphysiologic loading (e.g., ramp loading to failure/extreme traumatic event), mouse Achilles tendons do not experience significant collagen denaturation in vivo.

In summary, our data demonstrate that fatigue loading creates collagen fiber disorganization in mouse Achilles tendons but minimal denaturation of collagen molecules. Previous studies of SDFTs (Chambers et al., 2018; Herod et al., 2016; Quigley et al., 2018) and our monotonic loading data of patellar tendons suggest that the resistance to collagen denaturation with fatigue loading is not unique to Achilles tendons. Still, the finding that fatigue loading does not induce collagen denaturation in mouse Achilles tendons, which exhibit tendinopathic changes with overuse (Cho et al., 2021; Zhang et al., 2020; Zhao et al., 2019; Wang et al., 2022), suggests that mechanically-induced collagen denaturation may not be responsible for the initiation of tendon degeneration and tendinopathy.

CRediT authorship contribution statement

Krishna Pedaprolu: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Spencer E. Szczesny:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Dr. Benjamin Freedman for his suggestion to test mouse patellar tendons as a comparison. This study was supported by the Alliance for Regenerative Rehabilitation and Training, the National Institutes of Health (R21 AR079095), and the National Science Foundation (2142627).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiomech.2023.111545.

References

Alexander, R.M., 1991. Energy-saving mechanisms in walking and running. J Exp Biol 160, 55–69. https://doi.org/10.1242/jeb.160.1.55.

Archambault, J.M., Jelinsky, S.A., Lake, S.P., Hill, A.A., Glaser, D.L., Soslowsky, L.J., 2007. Rat supraspinatus tendon expresses cartilage markers with overuse. J. Orthop. Res. 25, 617–624. https://doi.org/10.1002/jor.20347.

Arya, S., Kulig, K., 2010. Tendinopathy alters mechanical and material properties of the Achilles tendon. J. Appl. Physiol. 108, 670–675. https://doi.org/10.1152/japplphysiol.00259.2009.

Bell, R., Li, J., Gorski, D.J., Bartels, A.K., Shewman, E.F., Wysocki, R.W., Cole, B.J., Bach, B.R., Mikecz, K., Sandy, J.D., Plaas, A.H., Wang, V.M., 2013. Controlled treadmill exercise eliminates chondroid deposits and restores tensile properties in a new murine tendinopathy model. J. Biomech. 46, 498–505. https://doi.org/10.1016/j.jbiomech.2012.10.020.

Biewener, A., Baudinette, R., 1995. In vivo muscle force and elastic energy storage during steady-speed hopping of tammar wallabies (Macropus eugenii). J. Exp. Biol. 198, 1829–1841. https://doi.org/10.1242/jeb.198.9.1829.

Chambers, N.C., Herod, T.W., Veres, S.P., 2018. Ultrastructure of tendon rupture depends on strain rate and tendon type. J. Orthop. Res. 36, 2842–2850. https://doi.org/10.1002/jor.24067.
Chao, P.G., Hsu, H.-Y., Tseng, H.-Y., 2014. Electrospun microcrimped fibers with nonlinear mechanical properties enhance ligament fibroblast phenotype. Biofabrication 6, 035008. https://doi.org/10.1088/1758-5082/6/3/035008.
Chen, Y., Shen, W., Tang, C., Huang, J., Fan, C., Yin, Z., Hu, Y., Chen, W., Ouyang, H., Zhou, Y., Mao, Z., Chen, X., 2020. Targeted pathological collagen delivery of sustained-release rapamycin to prevent heterotopic ossification. Sci. Adv. 6, eaay9526. https://doi.org/10.1126/sciadv.aay9526.
Cho, Y., Kim, H.-S., Kang, D., Kim, H., Lee, N., Yun, J., Kim, Y.-J., Lee, K.M., Kim, J.-H., Kim, H.-R., Hwang, Y., Jo, C.H., Kim, J.-H., 2021. CTRP3 exacerbates tendinopathy by dysregulating tendon stem cell differentiation and altering extracellular matrix composition. Sci. Adv. 7, eabg6069. https://doi.org/10.1126/sciadv.abg6069.
Devkota, A.C., 2007. Distributing a Fixed Amount of Cyclic Loading to Tendon Explants over Longer Periods Induces Greater Cellular and Mechanical Responses. J. Orthop. Res. 11, 1609–1612. https://doi.org/10.1002/jor.
Devkota, A.C., Weinhold, P.S., 2010. Prostaglandin E2, collagenase, and cell death responses depend on cyclical load magnitude in an explant model of tendinopathy. Connect. Tissue Res. 51, 306–313. https://doi.org/10.3109/03008200903318261.
Eekhoff, J.D., Abraham, J.A., Schott, H.R., Solon, L.F., Ulloa, G.E., Zellers, J.A., Cannon, P.C., Lake, S.P., 2022. Fascicular elastin within tendon contributes to the magnitude and modulus gradient of the elastic stress response across tendon type and species. Acta Biomater. https://doi.org/10.1016/j.actbio.2022.03.025.
Ely, E.R., Avella, C.S., Price, J.S., Smith, R.K.W., Wood, J.L.N., Verheyen, K.L.P., 2009. Descriptive epidemiology of fracture, tendon and suspensory ligament injuries in National Hunt racehorses in training. Equine Vet. J. 41, 372–378. https://doi.org/10.2746/042516409X371224.
Flick, J., Devkota, A., Tsuzaki, M., Almekinders, L., Weinhold, P., 2006. Cyclic loading alters biomechanical properties and secretion of PGE 2 and NO from tendon explants. Clin. Biomech. 21, 99–106. https://doi.org/10.1016/j.clinbiomech.2005.08.008.
Fung, D.T., Wang, V.M., Laudier, D.M., Shine, J.H., Basta-Pljakic, J., Jepsen, K.J., Schaffler, M.B., Flatow, E.L., 2009. Subrupture tendon fatigue damage. J. Orthop. Res. 27, 264–273. https://doi.org/10.1002/jor.20722.
Fung, D.T., Wang, V.M., Andarawis-Puri, N., Basta-Pljakic, J., Li, Y., Laudier, D.M., Sun, H.B., Jepsen, K.J., Schaffler, M.B., Flatow, E.L., 2010. Early response to tendon fatigue damage accumulation in a novel in vivo model. J. Biomech. 43, 274–279. https://doi.org/10.1016/j.jbiomech.2009.08.039.
Herod, T.W., Chambers, N.C., Veres, S.P., 2016. Collagen fibrils in functionally distinct tendons have differing structural responses to tendon rupture and fatigue loading. Acta Biomater. 42, 296–307. https://doi.org/10.1016/j.actbio.2016.06.017.
Lavagnino, M., Wall, M.E., Little, D., Banes, A.J., Guilak, F., Arnoczky, S.P., 2015. Tendon mechanobiology: current knowledge and future research opportunities. J. Orthop. Res. 33, 813–822. https://doi.org/10.1002/jor.22871.
Lee, A.H., Elliott, D.M., 2018. Comparative multi-scale hierarchical structure of the tail, Plantaris, and Achilles Tendons in the Rat. bioRxiv, 396309. https://doi.org/10.1101/396309.
Lee, A.H., Elliott, D.M., 2019. Comparative multi-scale hierarchical structure of the tail, plantaris, and Achilles tendons in the rat. J. Anat. 234, 252–262. https://doi.org/10.1111/joa.12913.
Lee, A.H., Szczesny, S.E., Santare, M.H., Elliott, D.M., 2017. Investigating mechanisms of tendon damage by measuring multi-scale recovery following tensile loading. Acta Biomater. 57, 363–372. https://doi.org/10.1016/j.actbio.2017.04.011.
Li, Y., Ho, D., Meng, H., Chan, T.R., An, B., Yu, H., Brodsky, B., Jun, A.S., Michael, Y., 2013. Direct detection of collagenous proteins by fluorescently labeled collagen mimetic peptides. Bioconjug. Chem. 24, 9–16. https://doi.org/10.1021/bc3005842.
Lin, A.H., Zitnay, J.L., Li, Y., Yu, S.M., Weiss, J.A., 2019. Microplate Assay for Denatured Collagen using Collagen Hybridizing Peptides. J. Orthop. Res. 37, 431–438. https://doi.org/10.1002/jor.24185.
Lin, A.H., Allan, A.N., Zitnay, J.L., Kessler, J.L., Yu, S.M., Weiss, J.A., 2020. Collagen denaturation is initiated upon tissue yield in both positional and energy-storing tendons. Acta Biomater. 118, 153–160. https://doi.org/10.1016/j.actbio.2020.09.056.
Lin, A.H., Slater, C.A., Martinez, C.-J., Eppell, S.J., Yu, S.M., Weiss, J.A., 2022. Collagen fibrils from both positional and energy-storing tendons exhibit increased amounts of denatured collagen when stretched beyond the yield point. Acta Biomater. https://doi.org/10.1016/j.actbio.2022.11.018.
Liu, Y.X., Thomopoulos, S., Birman, V., Li, J.-S., Genin, G.M., 2012. Bi-material attachment through a compliant interfacial system at the tendon-to-bone insertion site. Mech. Mater. Microstruct. Anisotropies 44, 83–92. https://doi.org/10.1016/j.mechmat.2011.08.005.
Maffulli, N., Barrass, V., Ewen, S.W.B., 2000. Light microscopic histology of achilles tendon ruptures: a comparison with unruptured tendons. Am. J. Sports Med. 28, 857–863. https://doi.org/10.1177/03635465000280061401.
Miller, K.S., Connizzo, B.K., Feeney, E., Soslowsky, L.J., 2012. Characterizing local collagen fiber re-alignment and crimp behavior throughout mechanical testing in a mature mouse supraspinatus tendon model. J. Biomech. 45, 2061–2065. https://doi.org/10.1016/j.jbiomech.2012.06.006.
O'Brien, C., Marr, N., Thorpe, C., 2021. Microdamage in the equine superficial digital flexor tendon. Equine Vet. J. 53, 417–430. https://doi.org/10.1111/evj.13331.
Patterson-Kane, J.C., Rich, T., 2014. Achilles tendon injuries in elite athletes: lessons in pathophysiology from their equine counterparts. ILAR J. 55, 86–99. https://doi.org/10.1093/ilar/ilu004.
Pedaprolu, K., Szczesny, S.E., 2022. A novel, open-source, low-cost bioreactor for load-controlled cyclic loading of tendon explants. J. Biomech. Eng. 144 https://doi.org/10.1115/1.4053795.
Peterson, B.E., Szczesny, S.E., 2020. Dependence of tendon multiscale mechanics on sample gauge length is consistent with discontinuous collagen fibrils. Acta Biomater. 117, 302–309. https://doi.org/10.1016/j.actbio.2020.09.046.
Quigley, A.S., Bancelin, S., Deska-Gauthier, D., Légaré, F., Kreplak, L., Veres, S.P., 2018. In tendons, differing physiological requirements lead to functionally distinct nanostructures. Sci. Rep. 8, 1–14. https://doi.org/10.1038/s41598-018-22741-8.
Roberts, T.J., Azizi, E., 2011. Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. J. Exp. Biol. 214, 353–361. https://doi.org/10.1242/jeb.038588.
Roberts, T.J., Marsh, R.L., Weyand, P.G., Taylor, C.R., 1997. Muscular force in running Turkeys: The economy of minimizing work. Science 275, 1113–1115. https://doi.org/10.1126/science.275.5303.1113.
Sereysky, J.B., Andarawis-Puri, N., Ros, S.J., Jepsen, K.J., Flatow, E.L., 2010. Automated image analysis method for quantifying damage accumulation in tendon. J. Biomech. 43, 2641–2644. https://doi.org/10.1016/j.jbiomech.2010.04.043.
Sereysky, J.B., Andarawis-Puri, N., Jepsen, K.J., Flatow, E.L., 2012. Structural and mechanical effects of in vivo fatigue damage induction on murine tendon. J. Orthop. Res. 30, 965–972. https://doi.org/10.1002/jor.22012.
Spiesz, E.M., Thorpe, C.T., Chaudhry, S., Riley, G.P., Birch, H.L., Clegg, P.D., Screen, H.R.C., 2015. Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise. J. Orthop. Res. 33, 889–897. https://doi.org/10.1002/jor.22879.
Steplewski, A., Fertala, J., Tomlinson, R., Hoxha, K., Han, L., Thakar, O., Klein, J., Abboud, J., Fertala, A., 2019. The impact of cholesterol deposits on the fibrillar architecture of the Achilles tendon in a rabbit model of hypercholesterolemia. J. Orthop. Surg. Res. 14, 172. https://doi.org/10.1186/s13018-019-1217-7.
Svensson, R.B., Smith, S.T., Moyer, P.J., Magnusson, S.P., 2018. Effects of maturation and advanced glycation on tensile mechanics of collagen fibrils from rat tail and Achilles tendons. Acta Biomater. 70, 270–280. https://doi.org/10.1016/j.actbio.2018.02.005.
Szczesny, S.E., Elliott, D.M., 2014. Incorporating plasticity of the interfibrillar matrix in shear lag models is necessary to replicate the multiscale mechanics of tendon fascicles. J. Mech. Behav. Biomed. Mater. 40, 325–338. https://doi.org/10.1016/j.jmbbm.2014.09.005.
Szczesny, S.E., Driscoll, T.P., Tseng, H.-Y., Liu, P.-C., Heo, S.-J., Mauck, R.L., Chao, P.-H.-G., 2016. Crimped Nanofibrous Biomaterials Mimic Microstructure and Mechanics of Native Tissue and Alter Strain Transfer to Cells [WWW Document]. ACS Publications. https://doi.org/10.1021/acsbiomaterials.6b00646.
Szczesny, S.E., Aeppli, C., David, A., Mauck, R.L., 2018. Fatigue loading of tendon results in collagen kinking and denaturation but does not change local tissue mechanics. J. Biomech. 71, 251–256. https://doi.org/10.1016/j.jbiomech.2018.02.014.
Thorpe, C.T., Udeze, C.P., Birch, H.L., Clegg, P.D., Screen, H.R.C., 2012. Specialization of tendon mechanical properties results from interfascicular differences. J. R. Soc. Interface 9, 3108–3117. https://doi.org/10.1098/rsif.2012.0362.
Thorpe, C.T., Chaudhry, S., Lei, I.I., Varone, A., Riley, G.P., Birch, H.L., Clegg, P.D., Screen, H.R.C., 2015. Tendon overload results in alterations in cell shape and increased markers of inflammation and matrix degradation. Scand. J. Medicine Sci. Sports 25, e381–e391. https://doi.org/10.1111/sms.12333.
Wang, X., Liu, S., Yu, T., An, S., Deng, R., Tan, X., Crane, J., Zhang, W., Pan, D., Wan, M., Carr, A., Cao, X., 2022. Inhibition of Integrin αvβ6 Activation of TGF-β Attenuates Tendinopathy. Adv. Sci. (Weinh) e2104469. https://doi.org/10.1002/advs.202104469.
West, J.R., Juncosa, N., Galloway, M.T., Boivin, G.P., Butler, D.L., 2004. Characterization of in vivo Achilles tendon forces in rabbits during treadmill locomotion at varying speeds and inclinations. J. Biomech. 37, 1647–1653. https://doi.org/10.1016/j.jbiomech.2004.02.019.
Wiesinger, H.P., Seynnes, O.R., Kösters, A., Müller, E., Rieder, F., 2020. Mechanical and material tendon properties in patients with proximal patellar tendinopathy. Front. Physiol. 11, 1–11. https://doi.org/10.3389/fphys.2020.00704.
Yin, Z., Chen, X., Chen, J.L., Shen, W.L., Hieu Nguyen, T.M., Gao, L., Ouyang, H.W., 2010. The regulation of tendon stem cell differentiation by the alignment of nanofibers. Biomaterials 31, 2163–2175. https://doi.org/10.1016/j.biomaterials.2009.11.083.
Zhang, J., Li, F., Nie, D., Onishi, K., Hogan, M.V., Wang, J.-H.-C., 2020. Effect of metformin on development of tendinopathy due to mechanical overloading in an animal model. Foot Ankle Int. 41, 1455–1465. https://doi.org/10.1177/1071100720966318.
Zhao, G., Zhang, J., Nie, D., Zhou, Y., Li, F., Onishi, K., Billiar, T., Wang, J.-H.-C., 2019. HMGB1 mediates the development of tendinopathy due to mechanical overloading. PLoS One 14, e0222369. https://doi.org/10.1371/journal.pone.0222369.
Zhu, J., Li, J., Wang, B., Zhang, W.J., Zhou, G., Cao, Y., Liu, W., 2010. The regulation of phenotype of cultured tenocytes by microgrooved surface structure. Biomaterials 31, 6952–6958. https://doi.org/10.1016/j.biomaterials.2010.05.058.
Zitnay, J.L., Li, Y., Qin, Z., San, B.H., Depalle, B., Reese, S.P., Buehler, M.J., Yu, S.M., Weiss, J.A., 2017. Molecular level detection and localization of mechanical damage in collagen enabled by collagen hybridizing peptides. Nat. Commun. 8, 1–12. https://doi.org/10.1038/ncomms14913.