

Vincent Wang ORCID iD: 0000-0001-5722-9146

Hani Awad ORCID iD: 0000-0003-2197-2610

Stavros Thomopoulos ORCID iD: 0000-0003-1531-4849

Guidelines for *ex vivo* mechanical testing of tendon

Authors:

Spencer P. Lake¹

Jess G. Snedeker²

Vincent M. Wang³

Hani Awad⁴

Hazel RC Screen⁵

Stavros Thomopoulos^{6,*}

¹ Department of Mechanical Engineering & Materials Science, Washington University in St. Louis, St. Louis, MO 63130, USA

² University Hospital Balgrist, ETH Zurich, Zurich, Switzerland

³ Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA 24061, USA

⁴ Department of Orthopaedics, Department of Biomedical Engineering, University of Rochester, Rochester, NY 14642, USA

⁵ School of Engineering & Materials Science, Queen Mary University of London, London, UK

⁶ Department of Orthopaedic Surgery, Department of Biomedical Engineering, Columbia University, New York, NY 10027, USA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jor.25647.

This article is protected by copyright. All rights reserved.

* Corresponding author: Columbia University, Black Building, Room 1408, 650 W 168 ST, New York, NY 10032-3702; sat2@columbia.edu

Running title: Guidelines for tendon mechanical testing

Author Contributions Statement: All authors contributed to drafting and editing the paper. All authors have read and approved the final submitted manuscript.

Abstract

Tendons are critical for the biomechanical function of joints. Tendons connect muscles to bones and allow for the transmission of muscle forces to facilitate joint motion.

Therefore, characterizing the tensile mechanical properties of tendons is important for the assessment of functional tendon health and efficacy of treatments for acute and chronic injuries. In this guidelines paper, we review methodological considerations, testing protocols, and key outcome measures for mechanical testing of tendons. The goal of the paper is to present a simple set of guidelines to the non-expert seeking to perform tendon mechanical tests. The suggested approaches provide rigorous and consistent methodologies for standardized biomechanical characterization of tendon and reporting requirements across laboratories.

Keywords: Tendon, Biomechanics

Introduction

Tendon pathologies, including tendinopathies and tendon ruptures, are common in the active and aging population.¹⁻³ The prevalence of tendon injuries is high, accounting for a substantial portion of physician office visits. Commonly affected tendons include the rotator cuff, Achilles, flexor, and patellar tendons.^{1:4} Several conservative and operative treatments have been attempted, including non-steroidal anti-inflammatory drugs, active and passive rehabilitation protocols, and surgical repair; however, treatment outcomes

This article is protected by copyright. All rights reserved.

remain poor, with limited return to function and high rates of recurrence.⁵⁻⁸ Due to these poor clinical outcomes, many groups are pursuing basic and translational studies to better understand the pathogenesis of tendinopathy and to test new treatment approaches for improved outcomes after surgical repair.

The primary function of tendons is to transmit tensile forces from muscle to bone, facilitating joint motion.⁹⁻¹¹ This function is achieved largely by tensile-bearing type I collagen fibers that are arranged in dense, parallel arrays. This arrangement results in a resilient tissue with a high tensile stiffness in the direction of the predominant fiber orientations. Tensile biomechanical properties are therefore the primary quantitative outcomes and index of tissue health and function for laboratory characterization of acute injury healing and chronic tendinopathy and/or evaluation of treatment efficacy. When determining tendon mechanical properties, a distinction must be made between *material* and *structural* responses. Material properties are inferred from the stress-strain response of the tendon, while the load-deformation response informs the structural properties. For material behavior, load is normalized to tissue cross-sectional area (i.e., engineering stress = force / cross-sectional area) and deformation is normalized to overall length (i.e., linearized strain = change in length / original length). In contrast, determination of the tendon's structural response does not require normalizing the measured forces or elongations to the dimensions of the tissue.

Several mechanical testing protocols can be used to determine the tensile properties of tendon including monotonic ramp to failure, cyclical fatigue loading, and viscoelastic stress relaxation. For testing, tendons are gripped and pulled in tension while simultaneously measuring time, displacement, and load. Perhaps less relevant to the

This article is protected by copyright. All rights reserved.

primary physiologic tensile loading mode of tendon, mechanical testing of tendon can also include shear loading^{12; 13}, compression/indentation¹⁴⁻¹⁷, and planar biaxial loading¹⁸.

There are also non-conventional approaches that can be used to determine tendon mechanical properties, including shear wave elastography¹⁹⁻²¹.

The mechanical behavior of tendon includes features that complicate its interpretation, including material nonlinearity, anisotropy, large strains, and viscoelastic dissipation.^{9; 10;}

^{12; 13} Under uniaxial tension, the typical stress-strain relationship of tendons is initially non-linear (the “toe region”), then enters a linear region, and finally reduces in slope as the tissue yields and fails (Figure 1, Table 1). This shape is representative, but not universal, for all tendon samples; substantial variability is seen, especially in yield and failure modes, depending on tendon type and sample specifics (e.g., *in vivo* treatments).

These features are quantified by the standard structural measures of stiffness (the slope of the linear region of the load-deformation curve), failure load (the maximum load within the load-deformation curve), and work (the area under the load-deformation curve). The corresponding material measures include the modulus (the slope of the linear region of the stress-strain curve), strength (the maximum stress within the stress-strain curve), and energy absorption (the area under the stress-strain curve).^{9; 10} The second feature of the mechanical behavior that merits special attention is viscoelasticity: tendon demonstrates characteristics of both elastic solids and viscous fluids.^{10; 22} These viscous phenomena play important roles for distributing loads among tissue substructures. Therefore, the loading history and the time-varying behavior should be considered to fully describe mechanical properties of tendons. Features of the viscoelastic behavior of tendons include hysteresis (dependence of mechanical behavior on the history of loading), stress

relaxation (reduction in stress over time under constant strain), and creep (increase in strain over time under constant stress). Finally, whilst material properties are generally considered a more appropriate measure to compare samples, the high propensity to imbibe water in, e.g., a hypertonic bath, means cross-sectional area can often be artificially swollen, particularly in slightly damaged samples.²³ Artificially augmented water content will cause a reduction in measured modulus and stress which may not be reflective of a true tissue properties.²⁴

Several phenomenological models have been developed to interpret the mechanical behavior of tendon. For example, the most common test used to determine the viscoelastic properties of tendons is stress relaxation, with time-dependent stress-strain data fit to models such as Y.C. Fung's quasilinear viscoelastic (QLV) theory^{25; 26} to interpret the results. Other modeling approaches consider tendon mechanics at multiple length scales to account for the hierarchical nature of the tissue. Models can incorporate behavior at the molecular, fibril, and tissue levels, e.g., including the effects of helical structure of collagen molecules, crimp pattern of collagen fibers, sliding between fibers and fascicles, and the interaction between extracellular matrix components.^{27; 28}

This "Guidelines" paper will present best practices for standard mechanical testing of tendon in uniaxial tension. The paper will first define the critical methodological and protocol considerations for accurately determining tendon mechanical properties. The paper will then propose the minimum set of mechanical properties necessary to properly describe the tensile mechanical properties of tendon. Standardized reporting criteria will be outlined for publication of tendon mechanical testing results. The goal of this paper is to provide simple guidelines for the non-expert to accurately measure and present a

consensus minimum set of parameters that describe tendon mechanics. This will allow comparisons between studies and enable meta-analyses to obtain reliable evidence on the efficacy of various treatments in preclinical studies. Our recommendations are based on published experiments and practical experience. These recommendations were developed during breakout sessions during the Orthopaedic Research Society (ORS) Tendon Section Satellite Meeting in May 2022.

Methodologic Considerations

The mechanical properties of tendons are affected by numerous biological factors including species, sex, age, disuse, exercise, overuse, drugs, and various acute and chronic injury conditions.^{9; 10} These factors are typically systematically investigated or controlled for in the design of the experiments. In addition, numerous methodologic factors can lead to variability in the measured mechanical properties and can confound the ability to compare results among studies. Particular attention to these variables could enhance rigor and reproducibility in the literature as briefly reviewed herein. We propose the following recommendations:

Tissue storage. While it is preferable to freshly prepare and test tendon samples immediately upon tissue procurement, logistical considerations typically necessitate that tissues are stored, often to ensure that all specimens experience the same preparation and testing protocols. It is recommended that tendon-bone samples or whole joint samples be frozen at ≤ -20 °C. Several studies have evaluated the effects of freezing and thawing on tendons in various species.²⁹⁻³³ In general, one or two freeze-thaw cycles following short term (≤ 3 months) storage at -20 °C or -80 °C do not affect tendon tensile mechanical properties compared to fresh tendon. However, long-term storage and excessive freeze-

thaw cycles should be avoided, as they could potentially alter the structure of the collagen fibers, leading to changes in the tendon's mechanical properties²⁹⁻³³. While the freezing rate is unlikely to have profound effects on tendon mechanical properties, rapid freezing in liquid nitrogen should be avoided³⁴. Based on practical experience, for rodents, it is also recommended to freeze whole limb or joints rather than isolate tissues before freezing, because isolated tissues can dehydrate and tend to degrade faster due to their small size and loss of mechanical tension. With larger animals, the tendon could be dissected immediately after the animal is euthanized, wrapped in phosphate buffered saline (PBS)-soaked gauze, placed in a sealed container, and stored in the freezer.

Tissue hydration and temperature. The hydration state and osmotic pressure of the tendon are important determinants of the tissue's tensile properties. Maintaining the tissue's hydration during preparation and throughout testing should be done by bathing the tissue in isotonic 90% sodium chloride solution or PBS. Increasing the osmotic pressure of the hydration solution leads to loss of water content from the tissue and results in artificial stiffening of the tendon³⁵, especially at higher strain rates³⁶. Although solutions such as PBS can cause a decrease in the apparent modulus, this effect is minor compared to the effects of dehydration. While differences between ambient room and physiologic temperatures are unlikely to have significant effects on tissue properties, efforts should be made to test tendons under consistent temperature and hydration conditions.

Aspect ratio. Material properties, by definition, are intrinsic to the material and thus do not depend on the geometry of the tendon. However, specimen dimensions can influence the determination of tendon material properties. In studies that evaluated samples of different aspect ratios (i.e., ratio of tendon length to width), the reported material

properties, particularly failure strains, varied substantially.^{37; 38} Strain-to-failure significantly decreased with increasing specimen length in both rat and bovine fascicles, while modulus increased.³⁸ The variations in failure strain and modulus with sample length may largely be explained by grip/end effects. One analysis determined that when testing fascicles with a cross sectional area on the order of 250 μm , a sample length of 40 mm would be necessary to eliminate end effects. Such tendon aspect ratios are often unrealistic or impractical, so we recommend maximizing the length of tendon tested relative to the cross-sectional area. Tendon samples with small aspect ratios are expected to demonstrate strains to failure which are artificially large, with an associated lower modulus. Therefore, when testing relatively short tendons, considerable grip/end effects will occur, and this limitation should be acknowledged.

Gripping. Gripping tendons for mechanical testing requires careful consideration to minimize artifacts³⁹. When available, it is preferable to grip the bone on one end and the tendon on the other. The bone, which provides a natural attachment to the tendon, can be gripped with form-fitting grips, epoxy, or bone cement and provides a natural attachment to the tendon. The bone can be potted in a container (e.g., a metal or plastic tube) using bone cement or other space filling curing material. Alternatively, recent work has shown effective use of cement-free fixtures through 3D printed inverse molds custom fit for the shape of the bone being gripped⁴⁰. Gripping the soft tissue at the muscle side of the tendon can be challenging, with the primary objective being to prevent tissue slippage or tissue damage that could result in underestimation of the mechanical properties. A variety of tendon gripping techniques can be used; one common approach is to use wedged jaw clamps. Compression clamps can be effective for smaller and thinner tendons (e.g., the

rat supraspinatus). Here, gripping pressure should be carefully considered and maintained to prevent slippage (insufficient force) or tearing at the grip due to stress concentrations at the tendon-grip interface (excess force). However, smooth, flat jaws should be avoided because they do not provide sufficient gripping friction and require large compressive forces to hold the soft tissue, which results in dehydration and stress concentrations at the tendon-grip interface. The recommended approach is to use grips that maximize contact area including sinusoidally-shaped jaws⁴¹ or jaws with serrated or roughened surfaces to increase friction⁴². For larger tendons (e.g., the Achilles), sophisticated freeze clamps become necessary, as it is not possible to apply sufficient force across the tendon end to securely hold the collagen fibers within the core of the tendon^{43; 44}.

Calculation of stress and strain. In order to determine material properties from structural measurements, stress and strain must be calculated from load and deformation, respectively. Calculation of stress requires accurate measurement of tendon cross-sectional area. Historically, various methods including micrometers and calipers have been used to determine cross-sectional area, but these typically involve contact with the specimen that requires reproducibility of the applied contact pressure⁴⁵. Where possible, non-contact methods (e.g., micro computed tomography, magnetic resonance or ultrasound imaging, laser micrometers and scanners, or 3D digital scanners) should be used due to the compliant nature of tendon and its viscoelastic nature which creeps under compression^{46; 47}. Since tendons have irregular cross sections and lengths, averaging several measurements of the tendon should be considered as best practice.

As the strains at the tendon-bone attachment and at the tendon grip are substantially different than strains at the tendon midsubstance, optical strain methods are more

accurate and should be used when available. Optical methods require the application of two or more stain lines in the midsubstance of the tendon for simple calculation of 1D strain or a speckle pattern that can be used for calculation of 2D strain using digital image correlation (DIC) or related image analysis algorithms⁴⁸. Optical strain methods use digital cameras that can track separation of tissue marking lines as the tissue is loaded or using DIC algorithms to compute strain maps⁴⁹. Unless available as a turnkey option with the material testing system, these calculations are generally performed post-testing; therefore, attention must be paid to synchronizing image processing calculations with other measurements directly acquired such as force and grip-to-grip displacement. Data sampling rates should be considered, and high-speed image acquisition can improve accuracy. Care must be taken to ensure that the surface measures are representative of the tendon and not of a superficial connective tissue such as the sheath, which may slide relative to the loaded tendon. If optical strain measures are not available, the initial tendon length can be measured between the grips after the tendon has been mounted and a tare load applied. Calculation of strain can then be made by normalizing grip-to-grip displacement measurements to the tendon's initial length. Alternatively, clip strain gages or contact extensometers can be used with large tendons, but generally should be avoided because their attachment to the tissue may induce local sites of damage.

Mechanical testing protocol recommendations

Tare Load. The rope-like behavior of tendon in the toe-region makes it difficult to establish the “zero” strain (initial length) state. Therefore, it is helpful to apply a small load to the tendon to remove slack and ensure that the tissue is taut prior to initiating the mechanical test. The magnitude of the tare load should be sufficiently small as to not load

the tendon into the toe or linear regions but to provide a consistent starting point for each test. As the size of tested tissues can vary dramatically based on animal, anatomic location, age, etc., a tare stress should be used for consistency across studies. Defining stress as the force divided by the initial tendon cross-sectional area, a load value should be chosen to apply a consistent pre-stress of ~1% of the failure stress to the tendon prior to the start of the testing protocol.

Preconditioning. Tendon is a viscoelastic material and therefore sensitive to strain history and hysteresis dependent responses. “Preconditioning” is the application of loading cycles to provide a consistent strain history to the tendon prior to subsequent mechanical testing. Preconditioning can be strain- or stress-controlled and can vary in terms of waveform, amplitude, number of cycles, and cycling frequency. To date, there are no consistent guidelines regarding specific preconditioning protocols, with wide variation in what has been used in previous studies. The optimal value for each parameter of a preconditioning protocol could vary depending on tissue characteristics. Therefore, a method that will ensure a well-defined strain history and consistent preconditioning should be applied. Using small rodent tendons as the prototypical example, we recommend a common protocol that applies ten cycles of a strain-based triangular waveform from 0% (i.e., at the tare load position) to 2% strain at a rate of 0.5 Hz.^{50; 51} In this protocol, a very low preconditioning strain is recommended to ensure that no damage accumulates in small tendons (e.g., in mouse tendons) prior to testing it to failure. However, there is also a strong theoretical basis for using much higher preconditioning strains, where possible, particularly in larger tendons.⁵² In general, preconditioning levels should be chosen as percentages of failure properties (e.g., 5% of failure stress) to better

account for variability between tendon types. Therefore, we suggest pilot tests consisting of pull-to-failure *without* tare loads or preconditioning to identify the range of reasonable test parameters. The choice of preconditioning parameters should consider: (i) avoidance of damage prior to testing, (ii) keeping within limitations of typical mechanical test systems, and (iii) the overarching goal of applying a consistent strain history that is suitable for each particular tendon.

Monotonic load to failure. The most common test for determining tendon mechanical properties is a monotonic uniaxial tensile ramp to failure. This test should be performed following tare load application and preconditioning. It can also be performed after application of non-damaging tests such as stress relaxation or cyclic loading (see below).

Load-to-failure tests should be “quasi-static”, meaning the strain rate during loading should be relatively slow to minimize viscoelastic effects.⁵³⁻⁵⁵ Increasing the strain rate by 100% increases the tendon’s elastic modulus and maximum stress by only ~15-30%, suggesting that tendon mechanical properties are only moderately strain rate sensitive^{56; 57}. Nevertheless, “quasi-static” tests are recommended to avoid inertial and viscoelastic effects; this setup minimizes the effects of viscous behaviors and allows for a reasonable simplification to determine elastic properties such as modulus. We recommend a quasi-static ramp to failure at a strain rate of 0.5% per second^{50; 53; 55}, with extension applied until the measured force drops by at least 75% of the maximum force to ensure full tissue failure prior to the completion of the protocol. As failure can occur at the clamp edges due to stress concentrations and/or gripping effects, failure properties (i.e., maximum load or stress, strain at failure, etc.) should only be reported for samples that are visually observed to fail in the midsubstance of the tendon. As best practice, we also recommend

that the mode and definition of failure should be clearly described (e.g., grip-to-grip strain at maximum load). For testing of surgically repaired tendons, additional failure mechanisms such as repair-site gap formation and suture pullout from tendon can provide further insight into the mechanical behavior.⁵⁸

Stress Relaxation and Creep. Static stress-relaxation is the most common mode used to evaluate the viscoelastic properties of tendon^{9; 10}. This protocol involves the application of a linear ramp increase in strain followed by a hold period at a subfailure strain wherein the load is measured to quantify time-dependent relaxation. While typical theoretical frameworks for viscoelastic testing assume that a strain is applied in an instantaneous step, physical limitations require an application of strain across a finite ramp. We recommend using the maximum practical rate of translation allowable with the test equipment; analysis methods have been developed to account for finite ramp rates and mechanical overshoot.^{59; 60} For tendon testing, the recommended protocol is a rapid ramp to a fixed strain in the middle of the linear region (e.g., typically ~5% for small rodent tendons) and a subsequent hold period of at least 10 minutes to allow for adequate relaxation and achievement of equilibrium stress.^{61; 62} Although not commonly used for tendon analysis, a creep test can also be performed to quantify viscoelastic properties. Like the guidelines for stress-relaxation, the protocol should consist of a rapid ramp to a fixed stress value. Based on practical experience, one suggested protocol is a ramp to 50% of the tendon's failure stress followed by a hold period of 30 minutes or longer to allow for adequate creep and achievement of equilibrium strain. For long-duration experiments of multiple hours, tissues must be tested in a bath of isotonic saline and protease inhibitors should be added to the testing bath to prevent degradation of the

tendon. We note, however, that the details of these parameters may vary according to tendon and species, and a wide range of strain rates and levels have been reported in the literature.

Cyclic Loading. During typical activities of daily living, tendons are loaded repetitively for many cycles. To reproduce this physiologic pattern, examine fatigue, and/or determine viscoelastic behavior, application of sinusoidal oscillations about an equilibrium strain or stress value can be performed. For viscoelastic characterization, the dynamic modulus and phase angle, which describe the sample's dynamic stiffness and energy dissipation, respectively, can be calculated by applying a set of sinusoidal waves across a range of frequencies and comparing the relative amplitudes and phases of the stress-time and strain-time data.^{63; 64} In addition, the analysis of cyclic loading data enables the calculation of tendon hysteresis behavior, which relates to the difference in the mechanical response during the loading versus unloading portion during a given cycle of testing. For full oscillatory evaluation, cycling should be performed through an appropriate range of the stress-strain curve. Scientific and clinical questions typically guide the specific focus of biomechanical evaluation (e.g., toe region, linear region, or post yield region). A typical protocol may consist of sinusoidal cycles at frequencies that span several orders of magnitude (a “frequency sweep”, e.g., 0.01 - 10 Hz) with small amplitude loading about equilibrium stress level(s) within the toe region.⁶⁵⁻⁶⁷

Primary outcome measures and interpretation of mechanical responses

The mechanical behavior of tendon depends on the multi-scale organization of collagen structures within the tissue (Table 2).⁶⁸⁻⁷⁰ Factors such as collagen fiber organization, collagen fiber packing, collagen molecular crosslinking, and the complexity of

hierarchical collagen organization all affect the mechanical behavior of tendon. Therefore, structure-function relationships should be considered in the interpretation of tendon mechanical testing results. For monotonic uniaxial tensile tests to failure, the primary structural properties are determined from the load-deformation curve: failure load (maximum load), stiffness (slope of the linear portion of the curve), and work to failure (area under the curve through the maximum load). Additionally, the primary material properties are determined from the stress-strain curve: strength (maximum stress), modulus (slope of the linear portion of the curve), and energy absorption (area under the curve through the maximum stress) (Table 3). For viscoelastic characterization, stress relaxation is the most common test performed. Simple outcome measures include peak load or stress, equilibrium load or stress, ratio of peak to equilibrium load or stress, and time to 50% relaxation. However, proper interpretation of viscoelastic responses should include fitting the data to standard models such as the QLV theory, which will produce parameters describing the nonlinear elastic behavior (A, B) during the loading phase and the viscoelastic parameters (τ_1 , τ_2 , C) during the stress relaxation phase.^{25; 26} When deciding on mechanical testing protocols and analyses, one must carefully consider the question(s) being posed (Table 4). For instance, ‘what constitutes an appropriate mechanical assessment of a tissue engineered construct grown *in vitro*’ will differ considerably from that of an ‘explanted animal tendon from an exercise intervention study’. In many cases (e.g., investigating the efficacy of an intervention), relatively simple mechanical tests are sufficient to answer a study’s question, while in other cases (e.g., quantifying the effect of a genetic or compositional perturbation), complex viscoelastic modeling may be necessary.

This article is protected by copyright. All rights reserved.

Conclusions

Currently, there is wide variation in the tendon field regarding mechanical testing methods, analyses, and choice of reported outcomes. This paper provides guidelines to the tendon research community towards standardizing methodology and reporting for tendon mechanical properties. A minimum set of parameters is proposed for describing tendon mechanical behavior (Table 5). Application of these guidelines and consistent reporting of suggested outcome measures will enhance the rigor of tendon mechanical testing methods in the field and allow for reproducibility and more appropriate comparisons across laboratories and research studies.

Acknowledgements

The guidelines paper was supported by the National Institutes of Health (R01 AR077793, R01 AR056696, and P30 AR069655) and National Science Foundation (2037125).

References

1. Thomopoulos S, Parks WC, Rifkin DB, et al. 2015. Mechanisms of tendon injury and repair. *J Orthop Res* 33:832-839.
2. Voleti PB, Buckley MR, Soslowky LJ. 2012. Tendon healing: repair and regeneration. *Annu Rev Biomed Eng* 14:47-71.
3. Woo SL, Debski RE, Zeminski J, et al. 2000. Injury and repair of ligaments and tendons. *Annual Review of Biomedical Engineering* 2:83-118.
4. Yang G, Rothrauff BB, Tuan RS. 2013. Tendon and ligament regeneration and repair: clinical relevance and developmental paradigm. *Birth Defects Res C Embryo Today* 99:203-222.

5. Galatz LM, Ball CM, Teefey SA, et al. 2004. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am* 86-A:219-224.
6. Lipman K, Wang C, Ting K, et al. 2018. Tendinopathy: injury, repair, and current exploration. *Drug Des Devel Ther* 12:591-603.
7. Snedeker JG, Foolen J. 2017. Tendon injury and repair - A perspective on the basic mechanisms of tendon disease and future clinical therapy. *Acta Biomater* 63:18-36.
8. Li ZJ, Yang QQ, Zhou YL. 2021. Basic Research on Tendon Repair: Strategies, Evaluation, and Development. *Front Med (Lausanne)* 8:664909.
9. Woo SL, An K, Frank CB, et al. 2000. Anatomy, biology, and biomechanics of tendon and ligament. *Orthopaedic Basic Science*, 2 ed. Rosemont, IL: AAOS; pp. 581-616.
10. Thomopoulos S, Genin GM. 2013. Tendon and ligament biomechanics. In: Winklestein BA editor. *Orthopaedic Biomechanics*. Boca Raton, FL: CRC Press; pp. 49-74.
11. Zajac FE. 1989. Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Crit Rev Biomed Eng* 17:359-411.
12. Fang F, Lake SP. 2017. Multiscale Mechanical Evaluation of Human Supraspinatus Tendon Under Shear Loading After Glycosaminoglycan Reduction. *J Biomech Eng* 139:0710131-0710138.
13. Fang F, Lake SP. 2016. Multiscale mechanical integrity of human supraspinatus tendon in shear after elastin depletion. *J Mech Behav Biomed Mater* 63:443-455.

14. Marturano JE, Arena JD, Schiller ZA, et al. 2013. Characterization of mechanical and biochemical properties of developing embryonic tendon. *Proc Natl Acad Sci U S A* 110:6370-6375.
15. Connizzo BK, Grodzinsky AJ. 2018. Multiscale Poroviscoelastic Compressive Properties of Mouse Supraspinatus Tendons Are Altered in Young and Aged Mice. *J Biomech Eng* 140:0510021-0510028.
16. Fang F, Lake SP. 2015. Multiscale strain analysis of tendon subjected to shear and compression demonstrates strain attenuation, fiber sliding, and reorganization. *J Orthop Res* 33:1704-1712.
17. Lee SB, Nakajima T, Luo ZP, et al. 2000. The bursal and articular sides of the supraspinatus tendon have a different compressive stiffness. *Clin Biomech (Bristol, Avon)* 15:241-247.
18. Szczesny SE, Peloquin JM, Cortes DH, et al. 2012. Biaxial tensile testing and constitutive modeling of human supraspinatus tendon. *J Biomech Eng* 134:021004.
19. Gotschi T, Widmer J, Cornaz F, et al. 2021. Region- and degeneration dependent stiffness distribution in intervertebral discs derived by shear wave elastography. *J Biomech* 121:110395.
20. Martin JA, Biedrzycki AH, Lee KS, et al. 2015. In Vivo Measures of Shear Wave Speed as a Predictor of Tendon Elasticity and Strength. *Ultrasound Med Biol* 41:2722-2730.
21. Gotschi T, Scharer Y, Gennisson JL, et al. 2023. Investigation of the relationship between tensile viscoelasticity and unloaded ultrasound shear wave measurements in ex vivo tendon. *J Biomech* 146:111411.

22. Woo SL, Johnson GA, Smith BA. 1993. Mathematical modeling of ligaments and tendons. *J Biomech Eng* 115:468-473.
23. Bloom ET, Lee AH, Elliott DM. 2021. Tendon Multiscale Structure, Mechanics, and Damage Are Affected by Osmolarity of Bath Solution. *Ann Biomed Eng* 49:1058-1068.
24. Screen HR, Chhaya VH, Greenwald SE, et al. 2006. The influence of swelling and matrix degradation on the microstructural integrity of tendon. *Acta Biomater* 2:505-513.
25. Fung YC. 1993. *Biomechanics: Mechanical Properties of Living Tissues*. New York: Springer;
26. Fung YC. 1973. Biorheology of soft tissues. *Biorheology* 10:139-155.
27. Fang F, Lake SP. 2016. Modelling approaches for evaluating multiscale tendon mechanics. *Interface Focus* 6:20150044.
28. Akintunde AR, Miller KS. 2018. Evaluation of microstructurally motivated constitutive models to describe age-dependent tendon healing. *Biomech Model Mechanobiol* 17:793-814.
29. Arnout N, Myncke J, Vanlauwe J, et al. 2013. The influence of freezing on the tensile strength of tendon grafts: a biomechanical study. *Acta Orthop Belg* 79:435-443.
30. Jung HJ, Vangipuram G, Fisher MB, et al. 2011. The effects of multiple freeze-thaw cycles on the biomechanical properties of the human bone-patellar tendon-bone allograft. *J Orthop Res* 29:1193-1198.

31. Lee AH, Elliott DM. 2017. Freezing does not alter multiscale tendon mechanics and damage mechanisms in tension. *Ann N Y Acad Sci* 1409:85-94.
32. Quirk NP, Lopez De Padilla C, De La Vega RE, et al. 2018. Effects of freeze-thaw on the biomechanical and structural properties of the rat Achilles tendon. *J Biomech* 81:52-57.
33. Giannini S, Buda R, Di Caprio F, et al. 2008. Effects of freezing on the biomechanical and structural properties of human posterior tibial tendons. *Int Orthop* 32:145-151.
34. Oswald I, Rickert M, Brüggemann G-P, et al. 2017. The influence of cryopreservation and quick-freezing on the mechanical properties of tendons. *Journal of Biomechanics* 64:226-230.
35. Lozano PF, Scholze M, Babian C, et al. 2019. Water-content related alterations in macro and micro scale tendon biomechanics. *Scientific Reports* 9:7887.
36. Haut TL, Haut RC. 1997. The state of tissue hydration determines the strain-rate-sensitive stiffness of human patellar tendon. *Journal of Biomechanics* 30:79-81.
37. Anssari-Benam A, Legerlotz K, Bader DL, et al. 2023. On the specimen length dependency of tensile mechanical properties in soft tissues: Gripping effects and the characteristic decay length. *Journal of Biomechanics* 45:2481-2482.
38. Legerlotz K, Riley GP, Screen HR. 2010. Specimen dimensions influence the measurement of material properties in tendon fascicles. *J Biomech* 43:2274-2280.
39. Wale ME, Nesbitt DQ, Henderson BS, et al. 2021. Applying ASTM Standards to Tensile Tests of Musculoskeletal Soft Tissue: Methods to Reduce Grip Failures and Promote Reproducibility. *J Biomech Eng* 143.

40. Kurtaliaj I, Golman M, Abraham AC, et al. 2019. Biomechanical Testing of Murine Tendons. *J Vis Exp*.
41. Butler DL, Grood ES, Noyes FR, et al. 1984. Effects of structure and strain measurement technique on the material properties of young human tendons and fascia. *J Biomech* 17:579-596.
42. Ng BH, Chou SM, Krishna V. 2005. The influence of gripping techniques on the tensile properties of tendons. *Proc Inst Mech Eng H* 219:349-354.
43. Matthews GL, Keegan KG, Graham HL. 1996. Effects of tendon grip technique (frozen versus unfrozen) on in vitro surface strain measurements of the equine deep digital flexor tendon. *Am J Vet Res* 57:111-115.
44. Wang T, Yu H. 2022. Templated freezing: a simple method may increase gripping force of the clamp on the tendon. *Journal of Orthopaedic Surgery and Research* 17:317.
45. Ellis DG. 1969. Cross-sectional area measurements for tendon specimens: a comparison of several methods. *J Biomech* 2:175-186.
46. Ge XJ, Zhang L, Xiang G, et al. 2020. Cross-Sectional Area Measurement Techniques of Soft Tissue: A Literature Review. *Orthop Surg* 12:1547-1566.
47. Hayes A, Easton K, Devanaboyina PT, et al. 2019. A review of methods to measure tendon dimensions. *Journal of Orthopaedic Surgery and Research* 14:18.
48. Boyle JJ, Kume M, Wyczalkowski MA, et al. 2014. Simple and accurate methods for quantifying deformation, disruption, and development in biological tissues. *Journal of the Royal Society, Interface / the Royal Society* 11:20140685.

49. Luyckx T, Verstraete M, De Roo K, et al. 2014. Digital image correlation as a tool for three-dimensional strain analysis in human tendon tissue. *Journal of Experimental Orthopaedics* 1:7.
50. LaCroix AS, Duenwald-Kuehl SE, Brickson S, et al. 2013. Effect of age and exercise on the viscoelastic properties of rat tail tendon. *Ann Biomed Eng* 41:1120-1128.
51. Johnson GA, Tramaglini DM, Levine RE, et al. 1994. Tensile and viscoelastic properties of human patellar tendon. *J Orthop Res* 12:796-803.
52. Sverdlik A, Lanir Y. 2002. Time-dependent mechanical behavior of sheep digital tendons, including the effects of preconditioning. *Journal of Biomechanical Engineering* 124:78-84.
53. Deymier AC, Schwartz AG, Cai Z, et al. 2019. The multiscale structural and mechanical effects of mouse supraspinatus muscle unloading on the mature enthesis. *Acta Biomater* 83:302-313.
54. Newton JB, Fryhofer GW, Rodriguez AB, et al. 2021. Mechanical properties of the different rotator cuff tendons in the rat are similarly and adversely affected by age. *J Biomech* 117:110249.
55. Fessel G, Cadby J, Wunderli S, et al. 2014. Dose- and time-dependent effects of genipin crosslinking on cell viability and tissue mechanics - toward clinical application for tendon repair. *Acta Biomater* 10:1897-1906.
56. Ng BH, Chou SM, Lim BH, et al. 2004. Strain rate effect on the failure properties of tendons. *Proc Inst Mech Eng H* 218:203-206.

57. Rosario MV, Roberts TJ. 2020. Loading Rate Has Little Influence on Tendon Fascicle Mechanics. *Front Physiol* 11:255.
58. Cole BJ, ElAttrache NS, Anbari A. 2007. Arthroscopic rotator cuff repairs: an anatomic and biomechanical rationale for different suture-anchor repair configurations. *Arthroscopy* 23:662-669.
59. Gimbel JA, Sarver JJ, Soslowky LJ. 2004. The effect of overshooting the target strain on estimating viscoelastic properties from stress relaxation experiments. *J Biomech Eng* 126:844-848.
60. Abramowitch SD, Woo SL. 2004. An improved method to analyze the stress relaxation of ligaments following a finite ramp time based on the quasi-linear viscoelastic theory. *J Biomech Eng* 126:92-97.
61. Vafek EC, Plate JF, Friedman E, et al. 2017. The effect of strain and age on the mechanical properties of rat Achilles tendons. *Muscles Ligaments Tendons J* 7:548-553.
62. Safa BN, Meadows KD, Szczesny SE, et al. 2017. Exposure to buffer solution alters tendon hydration and mechanics. *J Biomech* 61:18-25.
63. Fessel G, Snedeker JG. 2009. Evidence against proteoglycan mediated collagen fibril load transmission and dynamic viscoelasticity in tendon. *Matrix Biol* 28:503-510.
64. Ikoma K, Kido M, Nagae M, et al. 2013. Effects of stress-shielding on the dynamic viscoelasticity and ordering of the collagen fibers in rabbit Achilles tendon. *J Orthop Res* 31:1708-1712.

65. Dourte LM, Pathmanathan L, Jawad AF, et al. 2012. Influence of decorin on the mechanical, compositional, and structural properties of the mouse patellar tendon. *J Biomech Eng* 134:031005.
66. Zuskov A, Freedman BR, Gordon JA, et al. 2020. Tendon Biomechanics and Crimp Properties Following Fatigue Loading Are Influenced by Tendon Type and Age in Mice. *J Orthop Res* 38:36-42.
67. Bonilla KA, Pardes AM, Freedman BR, et al. 2019. Supraspinatus Tendons Have Different Mechanical Properties Across Sex. *J Biomech Eng* 141:0110021-0110028.
68. Fang F, Lake SP. 2017. Experimental evaluation of multiscale tendon mechanics. *J Orthop Res* 35:1353-1365.
69. Freedman BR, Knecht RS, Tinguely Y, et al. 2022. Aging and matrix viscoelasticity affect multiscale tendon properties and tendon derived cell behavior. *Acta Biomater* 143:63-71.
70. Connizzo BK, Adams SM, Adams TH, et al. 2016. Multiscale regression modeling in mouse supraspinatus tendons reveals that dynamic processes act as mediators in structure-function relationships. *J Biomech* 49:1649-1657.
71. Thorpe CT, Udeze CP, Birch HL, et al. 2012. Specialization of tendon mechanical properties results from interfascicular differences. *J R Soc Interface* 9:3108-3117.
72. Gautieri A, Passini FS, Silvan U, et al. 2017. Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biol* 59:95-108.

Figure Legends

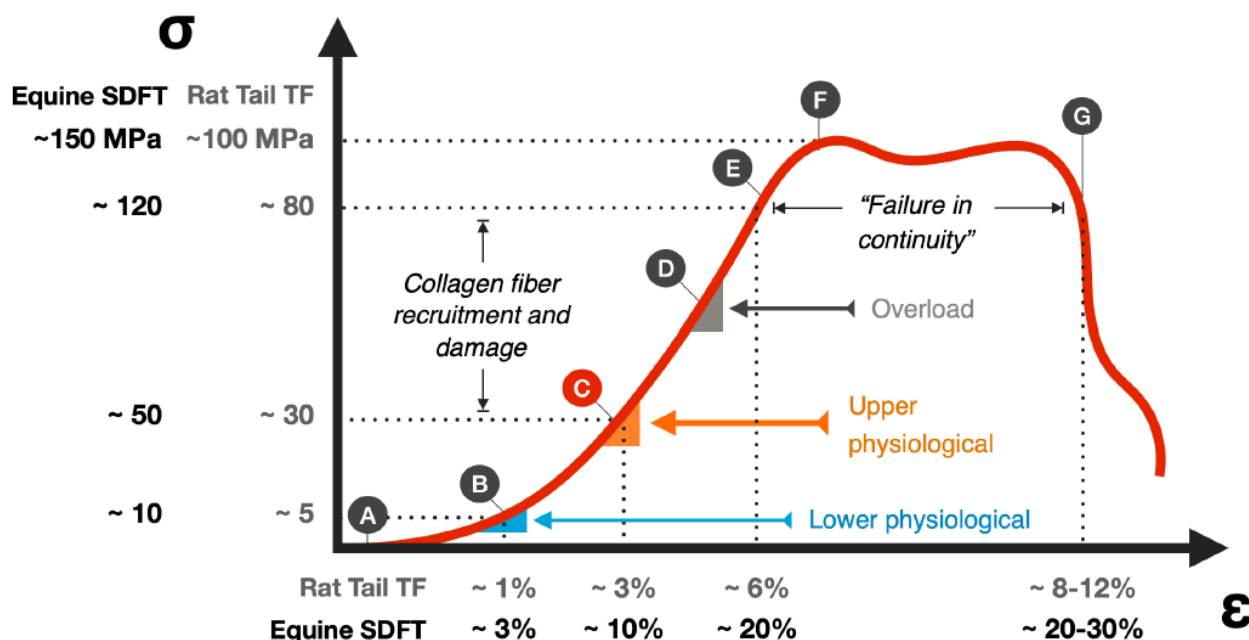


Figure 1: Representative mechanical behavior of two commonly used animal model tendons under uniaxial tension (superficial digital flexor tendon SDFT⁷¹, tail fascicle TF⁷²). To describe the material properties, stress (σ) is plotted against strain (ϵ), after normalization according to tissue dimensions. The curve is annotated to show important behaviors that are interpreted in Table 1. A-B represent the toe region and B-E represent the elastic region of the loading curve. The stress at E represents the yield point, or the onset of “failure in continuity”. The maximum stress at F represents the ultimate strength or failure stress. G represent the point at which the tissue loses its continuity and load bearing capacity. The modulus is determined as the slope of σ - ϵ curve in the elastic region within the physiological strain range. The area under the curve between point A-F represents the energy to failure, which can be interpreted as the toughness of the tissue. Note that this curve will differently scale depending on the material properties of the load-bearing collagen matrix and multiscale collagen structure of the particular tendon being tested.

Table 1: Tendon mechanical behavior under uniaxial tension. Representative values for stress and strain range from those typical of rat tail tendon fascicles (RTTF) to those of equine superficial digital flexor tendons (SDFT).

Stage	Stress ^{29,30}	Strain ^{29,30}	Curve Feature	Functional State
A	~ 1 MPa	~ 0 %	Slack	Unloaded
B	~ 5-10 MPa	~ 1-10 %	Toe	Crimp disappearance
C	~ 30-50 MPa	~ 3-10 %	Linear onset	Full collagen fiber recruitment; sub-damage
D	~ 30-120 MPa	~ 3-20 %	Linear region	Full collagen recruitment; sub-damage accumulation
E	~ 80-120 MPa	~ 6-20 %	Yield onset	High damage
F	~100-200 Mpa	~ 8-30 %	Failure onset	High damage, extensive tendon elongation or abrupt disruption
G	~100-200 MPa	~ 8-30 %	Failure	Complete disruption

Table 2: Relationships between collagen structure and mechanical function.

Collagen Structure	Mechanical Tissue Function
Increased collagen organization, increased collagen packing (higher collagen area fraction) ^{73,74}	Higher tissue modulus
	Lower tissue diffusivity / convective transport
Increased enzymatic collagen crosslinking ⁷⁵⁻⁷⁸	Higher tissue strength and modulus
	Lower molecular / tissue creep
Increased complexity of meso- / macro-scale hierarchy (e.g., interfascicular matrix, paratenon) ⁷⁹⁻⁸²	Lower rate of muscle force development (decreased maximum muscle power)
	Increased injury resistance (increased work to failure)
	Increased diversity of muscle function (multi-axis; multi-position joint function)

Table 3: Standard outcome measures determined from monotonic uniaxial tensile test to failure.

Outcome measure	Method
Failure load	Maximum recorded force
Stiffness	Slope of linear portion of load-deformation curve, calculated using linear regression of appropriate range
Work to failure	Integral of load-deformation curve through maximum load
Failure stress	Failure load divided by cross sectional area
Modulus	Slope of linear portion of stress-strain curve, calculated using linear regression of appropriate range
Energy absorption	Integral of stress-strain curve through maximum stress

Table 4: Guidelines for reporting and interpretation of data.

Functional question	What to measure & report	How to interpret
Is the tendon mechanically functional? <i>(joint torque and power)</i>	length	A too short (or overly stiff) tendon can restrict joint motion and predispose reinjury (short tendon-muscle units can yield abnormally high forces for a given joint position). A too long (or overly compliant) tendon can place the muscle outside its optimal force-elongation curve, potentially leading to reduced muscle strength and/or diminished rate of muscle force development.
	toe-linear transition	
	stiffness of the linear region	
Will the tendon allow (return to) activity? <i>(injury resistance)</i>	maximal load / stress	If the maximal load (and maximal material stress) and work to failure are all higher than would be expected during anticipated daily activities, the tendon can be assumed as able to support these activities.
	work to failure	
	stiffness / modulus of the linear region	An overly stiff tendon (or tendon subregion) can predispose tissue to tearing (local high stiffness regions can lead to stress concentrations, high stiffness may come at the expense of lower toughness)
Do tissue mechanics suggest normal tissue viscoelasticity? <i>(viscous dissipations, injury protective mechanisms)</i>	creep behavior	Abnormal viscoelastic behavior suggests abnormal tissue structure, with abnormal collagen kinematics and solid-fluid interactions, potentially across size scales. Dissipative viscoelastic mechanisms may also be essential for injury resistance.
	stress relaxation	
	hysteresis	
	elastic moduli	Low elastic moduli (toe, linear) suggest non-optimal collagen structure; this may reflect both ultrastructural and meso-structural derangement, with mechanobiological implications.

Table 5: Minimum reporting guidelines for tendon mechanical testing. All factors listed must be reported in order to properly interpret mechanical testing data.

Category	Variable(s) reported
Environmental	Storage conditions
Testing methodology	Hydration
	Temperature
	Cross-sectional area method
	Strain measurement method
	Zero strain determination (e.g., tare load)
Outcome measures	Anatomic: cross-sectional area, length
	Structural: failure load, stiffness, work to failure
	Material: strength, modulus, energy absorption