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2.1 Introduction

Tendons are soft connective tissues that provide both joint stability and also act to transmit tensile loads between the muscle and bone. Furthermore, tendons may provide a mechanical advantage for force generated by the muscle by acting around a pulley or lengthening a lever arm. Additionally, viscoelastic material properties allow tendons to passively store and release energy during loading cycles. Similarly, tendons dissipate energy and prevent injury by maintaining joint alignment under high loads (O’Brien 1992; Dykxj and Jules 1991). Given the critical function of tendons, it is imperative to understand their structural and mechanical properties in order to optimize their function and healing response. Moreover, tendon injury and degeneration can be highly debilitating, and can result in substantial pain, disability, and health-care costs. Thus, the purpose of this chapter is to provide an overview of tendon biomechanics, including a description of tendon composition and structure, mechanical properties, mechanical testing, and factors that affect mechanics.

2.2 Composition and Structure

The basic composition and structure of tendons are paramount to their mechanical and functional properties. Like most connective tissues, tendons are composed primarily of water and collagen.

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Water comprises 50–60% of tendon weight. Collagen is approximately 75% of the dry weight, with 95% being type I collagen (Woo et al. 2008). Collagen molecule groups form fibrils that are embedded in an extracellular matrix (ECM) that consists of proteoglycans and other components. Proteoglycans consist of a core protein with chains of negatively charged glycosaminoglycans (GAGs). The combination of the structure of collagen fibrils and also the polarized nature of GAGs together contribute to the mechanical properties displayed by tendons.

Tendons are arranged in a hierarchical structure. The structure begins with a triple helix of collagen, then microfibrils, fibrils, fascicles, and ultimately the tendon itself (Fig. 2.1). Tenocytes synthesize the building blocks necessary for tendon structure. Tenocytes are spindle-shaped fibroblast-type cells that synthesize the collagen fibrils and ECM. The overall thickness and quality of the collagen fibrils are dictated by small leucine-rich proteoglycans (SLRPs) such as decorin and biglycan (Kalamajski and Oldberg 2009). The collagen fibrils also have a waveform structure known as crimp, which is present in all tendons. Furthermore, the ultrastructure of tendons shows “crimp” that can be viewed using a microscope (Fig. 2.2). Crimp is important to ten-

don mechanical properties, particularly in the early phase of loading.

2.3 Mechanics

Tendon's longitudinal and fibrillar structure results in anisotropic and nonlinear properties. Anisotropy refers to a material having directionally dependent properties. In the tendon, this results in mechanical properties that are up to 1000 times higher during tensile testing along the longitudinal versus transverse axes (Lake et al. 2010). In addition to anisotropic properties, tendons also display nonlinear characteristics under an applied force, which results in an initial increase in stiffness as more force is applied. The nonlinear characteristics of the tendon result in two distinct regions in a load-elongation curve, termed the toe and linear regions (Fig. 2.3). The toe region of the load-elongation curve describes the behavior of tendons at low deformation, where the collagen fibril crimp is straightened. As deformation increases through the toe region into the linear region, crimp disappears and the collagen fascicle itself stretches (Dale et al. 1972; Diamant et al. 1972; Atkinson et al. 1999). As deformation continues to increase through the

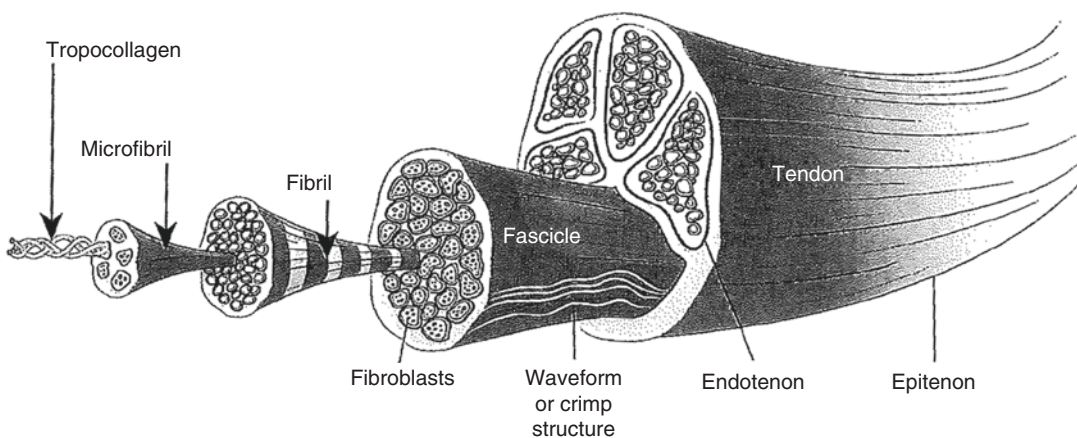


Fig. 2.1 Tendon hierarchical structure. Collagen molecules are assembled into progressively larger bundles, until the level of the tendon itself is reached. Reproduced with permission from Reuther KE, Gray CF, Soslowsky

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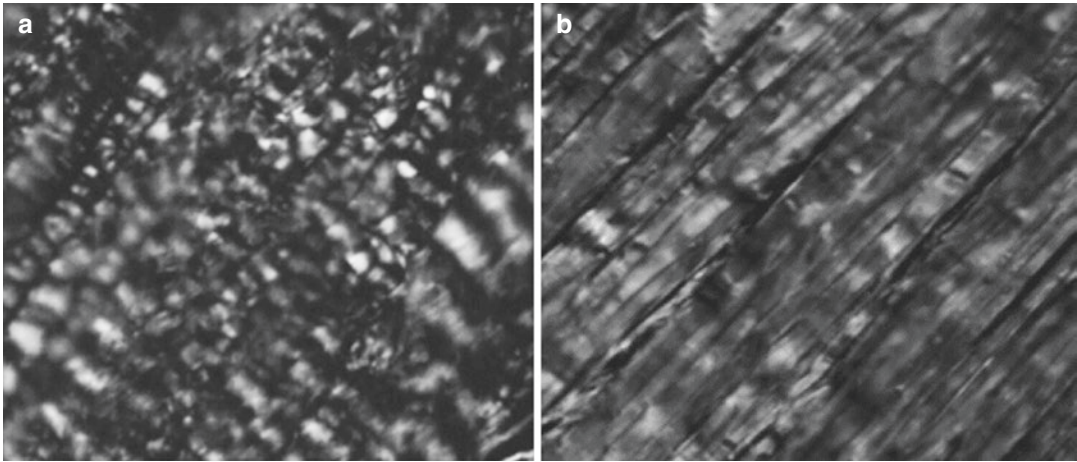


Fig. 2.2 Polarized light microscopy of crimp in a mouse supraspinatus tendon. (a) Collagen fibrils with crimp without an applied load. (b) Collagen fibrils uncrimped while under an applied tensile load. Reproduced with permission from Miller, KS, Connizo, BK, Feeney, F,

Soslowsky, LJ: Characterizing local collagen fiber realignment and crimp behavior throughout mechanical testing in a mature mouse supraspinatus tendon model. *J Biomech* 2012; 45(12)

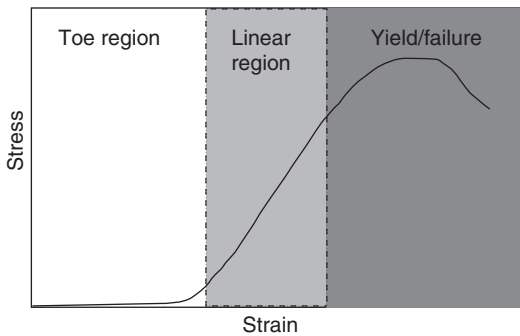


Fig. 2.3 Stress-strain curve demonstrating the toe and linear regions

linear region, the tendon accumulates irreversible damage and eventually ruptures. The anisotropic and nonlinear properties of tendons are further explained by other properties, such as viscoelasticity.

Viscoelasticity is the ability for materials to display both elastic and viscous behavior. One component of a viscoelastic material is viscosity, a measure of resistance to deformation. Although it has been traditionally used to characterize fluids, it can also describe other materials, such as rubber, glass, and biopolymers. Elasticity is the ability of a material to return to its original shape after forces that cause deformation are removed.

The elasticity of a material can be described in the context of structural or material properties. Stiffness is a structural property that defines the extent to which a material resists deformation from an applied force. Stiffness is derived from the slope of the load-elongation curve (Fig. 2.4a). In contrast with structural properties, material properties are normalized properties, taking into account tissue size or shape. Material properties are calculated using stress and strain. Stress is the intensity of the load or force normalized by the cross-sectional area. Strain is a relative measure of deformation, a change in length divided by the original length. These parameters are used to calculate Young's modulus, a material property that describes elasticity and is defined as the slope of the stress-strain curve (Fig. 2.4b). Both load-elongation and stress-strain curves are generated through mechanical testing. Mechanical testing protocols can be adapted to measure different types of properties, including viscoelastic properties.

Dynamic mechanical analysis is one methodology used to assess tendon viscoelastic properties. This analysis is performed by applying an oscillatory stress and measuring the strain response. A phase lag is a delay between the applied stress and resulting strain response.

Phase lags may be measured to describe the viscous or elastic behavior of a material. In purely elastic materials, there is no phase lag; the strain response occurs simultaneously with the applied stress (Fig. 2.5a). In purely viscous materials, strain lags stress by 90° or one quarter cycle behind the stress applied (Fig. 2.5b). The phase lag for any material will always be between 0° and 90° , making purely elastic and purely viscous materials both the upper- and lower-bounds for phase lag, respectively. Additionally, viscoelasticity of tendons can also be characterized via hysteresis. Hysteresis represents the amount of energy dissipated as a result of internal friction during mechanical loading and unloading. Like all materials, tendon dissipates energy throughout loading and unloading cycles. Thus, hysteresis can be derived from the area between loading and unloading load-elongation curves during mechanical testing (Fig. 2.6).

The viscoelastic properties of tendon also lead to phenomena known as creep and stress relaxation. Creep is an increase in deformation of a material under a constantly applied load. Initially, elongation under a constant load occurs quickly, however, this response slows with time (Fig. 2.4c). Cyclic creep occurs during cyclic dynamic testing, where each consecutive load cycle generally causes an increase in the amount of deformation. In contrast with creep, stress relaxation holds strain constant. Stress relaxation in tendon is demonstrated when a measured load in a tendon decreases over time with a constant strain (Fig. 2.4d). This load initially decreases under a constant strain quickly, but the rate of change decreases over time as it approaches equilibrium. Cyclic stress relaxation also generally occurs when a tendon is exposed to cycling dynamic testing, requiring a decreased force to reach a constant strain over time.

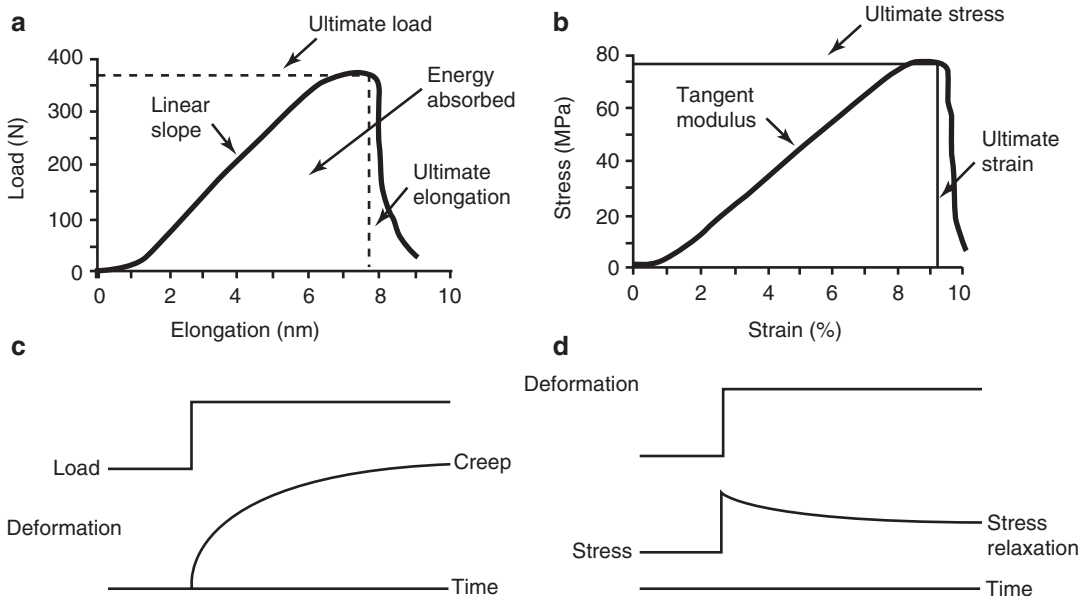


Fig. 2.4 (a) Tendon load-elongation curve. Various material properties can be derived from this curve. (b) Tendon stress-strain curve. This curve normalizes properties to each individual tendon's parameters, including length and area. Reproduced with permission from Woo SL, Debski RE, Withrow JD, Janushek MA: Biomechanics of knee ligaments. *Am J Sports Med* 1999; 27(4):533–543. (c)

Creep test where load is held constant and amount of tendon deformation is measured. (d) Stress relaxation test where deformation is held constant and stress is measured. Both the creep test and stress relaxation test demonstrate viscoelastic behavior of tendon in response to load

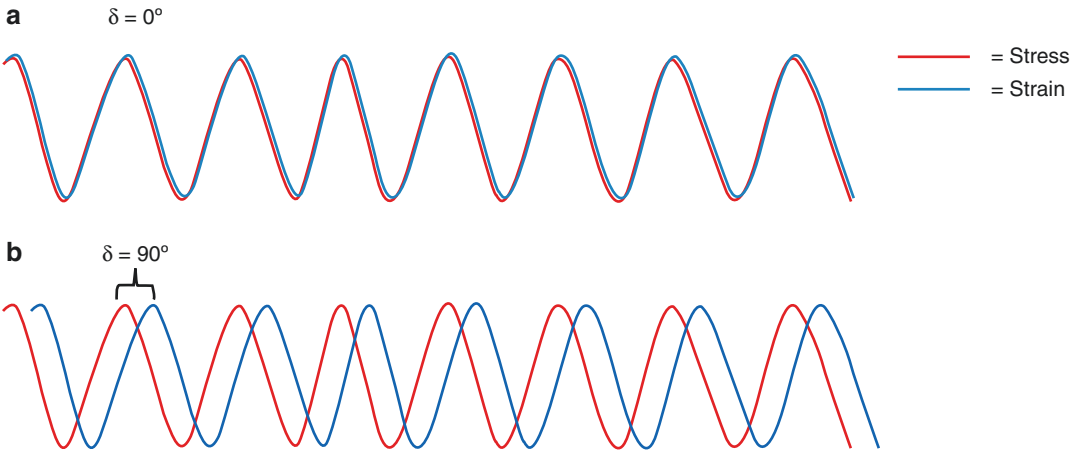


Fig. 2.5 Stress and strain response curves that represent a perfectly elastic material (a), with 0° phase lag between stress and strain, and a perfectly viscous material (b), with a 90° phase lag between stress and strain

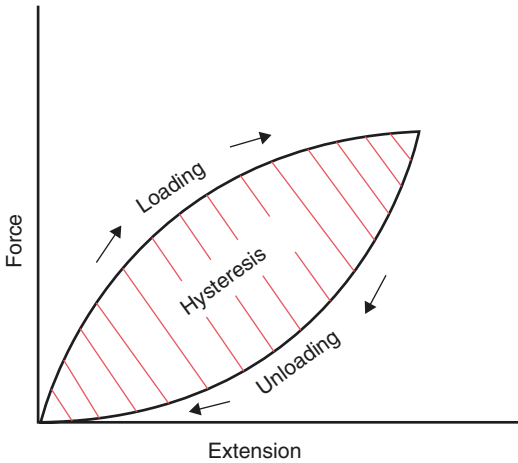


Fig. 2.6 A graph showing the loading and unloading curves of an idealized rubber band, with the area between the curves, highlighted by red lines, representing hysteresis

2.4 Mechanical Testing Methods

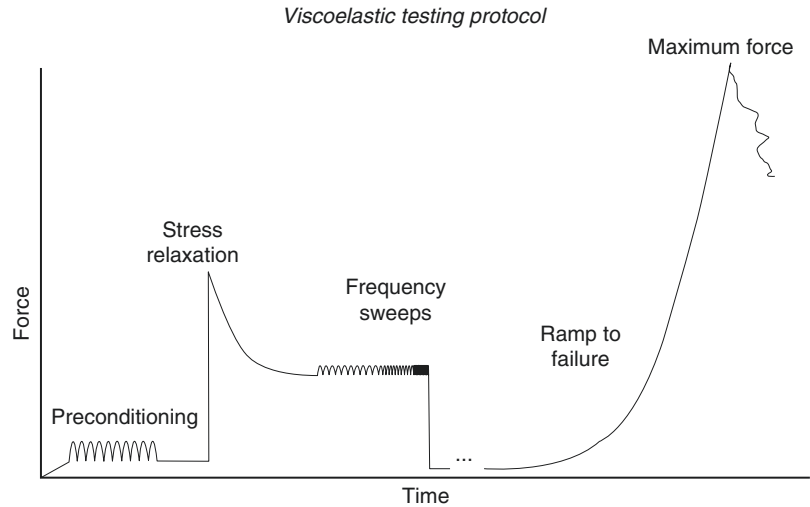
There are many parameters that affect the results of mechanical testing. In order to standardize measurements for comparison, cyclic preconditioning is commonly performed before mechanical testing. Preconditioning is performed at low loads to stretch the tendon without causing irreversible damage. After applying a preconditioning protocol, a steady state is reached where no further changes occur unless the protocol is

altered (Fung 1993; Miller et al. 2012). After preconditioning, many mechanical testing methods such as ramp to failure, dynamic cyclic, and fatigue testing can be used to assess tendon mechanical properties.

Ramp to failure testing is the most common form of mechanical testing used to assess tendons. One purpose of this method is to find the maximum force and displacement a sample can endure before failure. It is typically performed by applying a constantly increasing displacement on the sample until failure. In addition, this test is also used to determine the stiffness and modulus of both the toe and linear regions and the transition point between these regions. It is not uncommon for ramp to failure testing to be performed in series with dynamic testing. This is because the low strains used in dynamic testing to measure creep and stress relaxation do not alter the tendon's integrity, making it possible to combine these tests together (Fig. 2.7).

Many different parameters set during dynamic cyclic testing affect tendon response due to the viscoelastic nature of the tissue. The dynamic modulus derived from cyclic dynamic testing is defined as the stress amplitude divided by the strain amplitude. Thus, the dynamic modulus measurement is affected by testing parameters, based on the strains and frequencies that are selected. These moduli describe how tendons

Fig. 2.7 An example of a tendon viscoelastic testing protocol. The test begins with preconditioning, followed by a stress relaxation and a series of frequency sweeps. Multiple stress relaxations can be performed at higher strains, followed by more frequency sweeps. The test ends with a ramp to failure test



behave depending on rate of loading and loading history. As previously discussed, creep and stress relaxation can also be used to characterize the viscoelastic behavior of tendons. Creep and stress relaxation are affected by the magnitude of force or strain, respectively. A more comprehensive method of evaluating creep and stress relaxation is to perform dynamic testing using various magnitudes of force. Examining these properties across a range of values provides a deeper insight into the viscoelastic characteristics of the sample.

Fatigue testing is another type of cyclic testing which consists of cycling within a defined range of force or displacement, and recording the number of cycles until failure. During this testing, tendons have three phases marked by changes in stiffness (Freedman et al. 2014). Initially, the tendon increases in stiffness, reaches a maximum, and finally decreases in stiffness, demonstrating a triphasic behavior pattern. This decrease in stiffness is attributed to an accumulation of sub-rupture damage, which results in increases in deformation and decreases in stiffness prior to failure. This testing is useful for characterization of tendons that undergo repetitive loads, such as the Achilles tendon and other tendons that function in locomotion (Fung et al. 2009, 2010; Wren et al. 2003).

2.5 Experimental Factors Influencing Tendon Mechanics

Several technical aspects must be considered when preparing for testing of tendon mechanical properties. The overall process typically includes sample isolation, storage, preparation, and testing. Tendons are prepared by isolating them for independent testing by removing bony or muscular attachments that may confound results. This process may be labor intensive, thus, it is not uncommon to store samples in a freezer before further preparation and testing. However, when storing these samples prior to testing, freeze-thaw cycles must be taken into consideration. While studies have reported no changes in mechanics with less than five freeze-thaw cycles (Suto et al. 2012), other studies have noted a decline in mechanics with each cycle and to use caution when exposing tendons to more than five cycles (Chen et al. 2011; Huang et al. 2011). The environment in which the tendon is tested also requires careful consideration. Specifically, hydration and temperature of the tendon must be controlled, as changed in either of these parameters will have profound effects on tendon behavior. For example, dehydration of tendon has been shown to

cause a shortening of the collagen molecules, resulting in the generation of large stresses (Masic et al. 2015). Decreases in temperature make tendon behavior less viscous and more elastic (Huang et al. 2009). For these reasons, a water bath is typically used to immerse the tendon in order to control for hydration and temperature during testing. Before proceeding with testing, accurate measurement of tendon cross-sectional area is vital to ensure that material properties are properly reported. This is most robust when done through noncontact methods, such as laser-based systems, to reduce error (Favata 2006). Once cross-sectional area is measured, tendons are mounted in an anatomical orientation, where the fibers are loaded longitudinally, in order to model the *in vivo* scenario. Grips placed on the tendon ends properly are important to isolate the tendon and also prevent errors associated with slipping and stress concentrations. To reduce these errors, tendons are ideally tested with a high length versus width ratio. Furthermore, errors from grip slippage can be further reduced by using optical or other noncontact techniques to accurately calculate strain and measure finite levels of local deformation (e.g., finding the deformation at the insertion or midsubstance) (Peltz et al. 2009). During testing, the rate of loading also has an impact on data. For tendon, specifically, rate-dependent mechanical changes have been associated with altered uncrimping and volumetric contraction (Buckley et al. 2013).

2.6 Biological Factors Influencing Tendon Mechanics

Several non-modifiable and modifiable biologic factors significantly affect tendon mechanical properties. Non-modifiable factors include age, gender, and anatomic location. Advanced age is a risk factor for increased incidence of tendon injury (Abate et al. 2009; Langberg et al. 2001; Birch et al. 2016). Although there appears to be diminished mechanical properties with age, the

mechanisms behind these phenomena are still under investigation. Additionally, gender has also been shown to be a risk factor for injury susceptibility and altered healing characteristics (Pardes et al. 2016; Fryhofer et al. 2016). Sex hormones regulate the collagen composition leading to altered mechanical properties (Hansen and Kjaer 2016). Anatomic location of the tendon within the body is another non-modifiable factor that affects tendon mechanical properties. Similar to what is seen *ex vivo* in the laboratory, variations of the loading environment such as load cycles and local temperature in various anatomic regions of the body affect the mechanical properties (Maganaris 2002). In contrast with non-modifiable factors, modifiable factors offer greater potential for optimizing therapeutic strategies for tendon pathology. Modifiable factors include comorbidities and activity level. Comorbidities such as diabetes, hypercholesterolemia, tobacco use, and renal disease have all been shown to adversely affect tendon mechanics (Connizzo et al. 2014; Beason et al. 2013; Ichinose et al. 2010; Taşoğlu et al. 2016; Artan and Basgoze 2015). Exercise also profoundly affects tendon mechanics, with increased activity increasing modulus compared to age-matched control tendons (Arnoczky et al. 2008). Furthermore, return to early activity after acute tendon injury has been shown to improve mechanical properties (Freedman et al. 2016).

Conclusion

Tendon composition, structure, mechanical properties, and testing methods provide insight into the fundamental mechanisms that govern tendon function. The collagen matrix composition and crimp structure promote viscoelastic mechanical properties of tendons. By testing these mechanical properties, the physiologic and pathologic behavior of tendons is better understood. Translation of tendon biomechanics to the bedside aids clinicians in improving the care of patients.

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