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Strain-induced damage reduces echo intensity changes in tendon during loading

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ABSTRACT

Tendon functionality is related to its mechanical properties. Tendon damage leads to a reduction in mechanical strength and altered biomechanical behavior, and therefore leads to compromised ability to carry out normal functions such as joint movement and stabilization. Damage can also accumulate in the tissue and lead to failure. A noninvasive method with which to measure such damage potentially could quantify structural compromise from tendon injury and track improvement over time. In this study, tendon mechanics are measured before and after damage is induced by "overstretch" (strain exceeding the elastic limit of the tissue) using a traditional mechanical test system while ultrasonic echo intensity (average gray scale brightness in a B-mode image) is recorded using clinical ultrasound. The diffuse damage caused by overstretch lowered the stress at a given strain in the tissue and decreased viscoelastic response. Overstretch also lowered echo intensity changes during stress relaxation and cyclic testing. As the input strain during overstretch increased, stress levels and echo intensity changes decreased. Also, viscoelastic parameters and time-dependent echo intensity changes were reduced.

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1. Introduction

To understand how tendons function to perform their roles in movement, stability, and shock absorption, it is necessary to investigate the biomechanical properties of tendon. These properties undergo changes when tendons are injured (Young et al., 1998; Brown et al., 1981; See et al., 2004), or when nearby connective tissues are injured (Perry et al., 2009; Soslowsky et al., 1994).

Though *in vitro* tendon studies of tissues provide some insight into the altered mechanics, they fall short of providing patient-specific metrics. Individualized mechanical properties of human pathologies (rather than animal pathology models) provided by noninvasive *in vivo* testing would provide better insight into the extent of injury, mechanical compromise, time course of healing, and efficiency of treatment for the exact injury or pathology. For example, changes in stiffness and viscoelastic properties could be analyzed to elucidate the microstructural processes (both damage and repair) occurring within the tendon, as well as provide a quantitative measure for the current state of tissue compromise. A noninvasive method by which to examine such mechanical properties would allow researchers to describe representative *in vivo* populations rather than animal models or cadaver tissues. While cadaver tissues are more convenient and available than other donor tissues, they are a clinically unreliable alternative to actual patient populations. Current models use carefully collected individual geometry but only use generalized tendon and ligament behavior; *in vivo* mechanical evaluation would allow for individual tendon properties to be combined with the geometry for more accurate models.

Because of their speed, safety, and affordability, ultrasound-based techniques are gaining popularity as a potential method of *in vivo* mechanical evaluation. Ultrasonic sound waves can be used not only to image tissues in the body, but they can also give information about the mechanical state of the tissue. For example, speckle tracking can be used to measure displacement and strain in the tissue (Heimdal et al., 1998; D'hooge et al., 2000). The resulting deformation information can be used to infer stiffness information using elastography (Ophir et al., 1991; Ophir et al., 1996); such stiffness information can be used to detect changes in tissue properties for diagnostic purposes (i.e. stiffening associated with tumor formation; Itoh et al., 2006). Wave propagation velocity through the tissue (i.e. speed of sound) can also be measured (Crevier-Denoix et al., 2009) to elucidate tissue properties, and measuring the propagation of shear waves through the tissue of interest can be used to estimate tissue elasticity (Arda et al., 2011).

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Acoustoelastic (AE) theory is based on the principle that a material's acoustic properties are altered as it is deformed and loaded (Hughes and Kelly, 1953), which can be measured as a change in wave velocity and amplitude (Kobayashi and Vanderby, 2005; Kobayashi and Vanderby, 2007). Previous work in this area has measured changes in both wave propagation speed and amplitude (Pastrone and Tonon, 1997; Kobayashi and Vanderby, 2005), but the focus has been primarily on measuring shear wave propagation speed (Rogerson and Scott, 1992; Sandiford and Rogerson, 2000; Catheline et al., 2003; Hamilton et al., 2004; Zabolotskaya et al., 2004; Destrade et al., 2010; Destrade et al., 2010; Destrade and Ogden, 2010; Gennisson et al., 2007) in order to precisely recover the higher order effects conducting the acoustoelastic effect in soft tissue phantoms (i.e. agar–gelatin or polyvinyl alcohol based phantoms).

Kobayashi and Vanderby (2005) have previously derived the acoustic relationship between reflected wave amplitude and strain-dependent stiffness and stress in a deformed material using A-mode ultrasound (Kobayashi and Vanderby, 2005; Kobayashi and Vanderby, 2007); as the tensioning of tendon increases, the intensity of reflected ultrasonic echoes increases, which leads to a brighter B-mode ultrasound image (Duenwald et al., 2011). This AE effect may be amplified by tension-induced changes in the tendon microstructure. The effects of damage on ultrasound signals have yet to be explored. The purpose of this study, therefore, is to examine the effect of overstretch damage (overstretch defined as a strain state exceeding the elastic limit of the tissue) on ultrasonic echo intensity. Currently, it is known that overstretch results in mechanical compromise in tendon (Duenwald-Kuehl et al., 2012), but there is no method of *in vivo* evaluation or imaging that can estimate the damage and functional risk associated with continued activity. Such a method would allow for quantification of mechanical compromise following tendon injury and track mechanical improvement with healing.

2. Materials and methods

2.1. Specimen preparation

Porcine digital flexor tendons ($n=30$) were excised from lower legs obtained from a local abattoir with care to leave bony insertion sites intact. Specimens were kept hydrated in physiologic buffered saline (PBS) until loading into the test frame. Bony ends were potted in lightweight polyester resin filler molded to fit inside the bone grip. Unloaded cross-sectional area was measured assuming an elliptical shape by measuring the long and short axes at three points along the tendon and averaging.

2.2. Mechanical testing

Specimens were loaded into custom grips in the servohydraulic test system (Bionix 858, MTS, Minneapolis, MN). Grip-to-grip displacement was controlled by the servohydraulic machine and load was measured using a 1000 lb load cell (Honeywell, Morristown, NJ). Data were captured on a PC equipped with Labtech Notebook (Laboratory Technology Corporation, Fort Collins, CO).

Specimens were preloaded to 1 N, initial tendon length was measured, and tendons were preconditioned using a sinusoidal wave from 0% to 2% strain at 0.5 Hz for 20 s. Tendons were allowed to rest for 1000 s prior to further mechanical testing.

Stress relaxation (held 100 s) and cyclic testing (0.5 Hz for 20 s) to 4% were performed on tendons with 1000 s rest periods between each test. Two relaxation tests and two cyclic tests were performed on each specimen prior to inducing damage from an overstretch pull to 6.5% ($n=10$), 9% ($n=10$), or 13% ($n=10$) strain. These strains were chosen to fall outside of the normal maximum physiologic strain (5%–6%) (Gardiner et al., 2001; Lochner et al., 1980) but below reported failure strains (15%–20%; Johnson et al., 1994; Shadwick, 1990). Following overstretch, relaxation and cyclic tests were repeated.

Parameters calculated from the mechanical data were chosen to correspond with ultrasound-obtained information. Cyclic testing parameters included the peak stress reached during the first three (viscoelastic) cycles, the difference in peak stress from the first and third cycles, and the peak stress reached during the last three (elastic) cycles. Relaxation testing parameters included the maximum stress reached during the onset of relaxation and the change in stress during the first 5 s of relaxation. The ratio of post-damage values to pre-damage values was calculated and plotted.

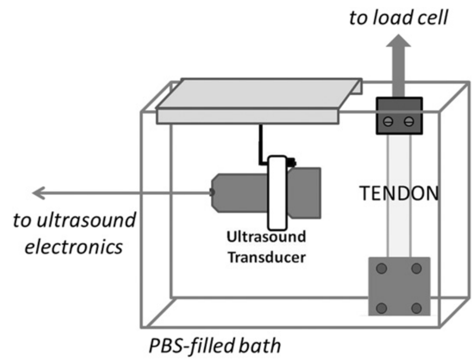

Fig. 1. Experimental setup to acquire mechanical and ultrasonic data simultaneously. All testing was conducted in the PBS-filled bath which acted to keep the tendon hydrated and transmit ultrasound waves to and from the tendon surface.

2.3. Ultrasound analysis

During mechanical testing, B-mode cine ultrasound was recorded (20 frames/s) testing using a GE 12L-RS Linear Array Transducer at 12 MHz and GE LOGIQe ultrasound (General Electric, Fairfield, CT). The ultrasound transducer was fixed in position using a custom platform which connected the testing bath (see Fig. 1). All ultrasound settings, including gain, time gain compensation (TGC), frequency, and distance between transducer and tendon front surface were optimized for the first specimen and then held constant between tests and across test specimens. The overall echo intensity (defined as the average gray scale brightness of the selected region in the B-mode image) of the tendon, averaged over the entire region of interest (ROI) between the grips, was calculated for each frame in order to record the echo intensity changes over time using EchoSoft post-processing software (Echometrix, Madison, WI). Echo intensity was recorded during the first 5 s (for a total of 100 images) of relaxation testing to record viscoelastic effects, as well as during the first three cycles of cyclic testing (120 images) to record additional viscoelastic effects and during the last three cycles of the cyclic testing (120 images) to evaluate elastic effects.

2.4. Statistics

To compare echo intensity change parameters following overstretch at different strain levels (6.5%, 9%, and 13%), a repeated measures ANOVA was used. $p<0.05$ was used as the criterion for statistical significance in all comparisons.

3. Results

Diffuse damage caused by subjecting the tendons to overstretch strain levels resulted in decreased stress (Fig. 2) and echo intensity change (Fig. 3) during cyclic (Figs. 2(a) and 3(a)) and relaxation (Figs. 2(b) and 3(b)) testing.

The peak stresses reached during the first three and last three cycles of cyclic testing were decreased following damage (each level significantly different, $p<0.001$ in each case), as was the decrease in peak echo intensity from the first cycle to the third (Fig. 4(a); each level significantly different, $p<0.001$). Maximum stress reached during stress relaxation was decreased following over-stretch damage (Fig. 4(b); each level significantly different, $p<0.001$), as did the stress decay during the first 5 s of relaxation (Fig. 4(b); each level significantly different, $p<0.001$).

Likewise, the peak echo intensity reached during the first three and last three cycles of cyclic testing was decreased following overstretch (each level significantly different, $p<0.001$ and $p=0.002$, respectively), as was the increase in peak echo intensity from the first to third cycle (Fig. 5(a); each level significantly different, $p<0.001$). Max echo intensity reached during stress

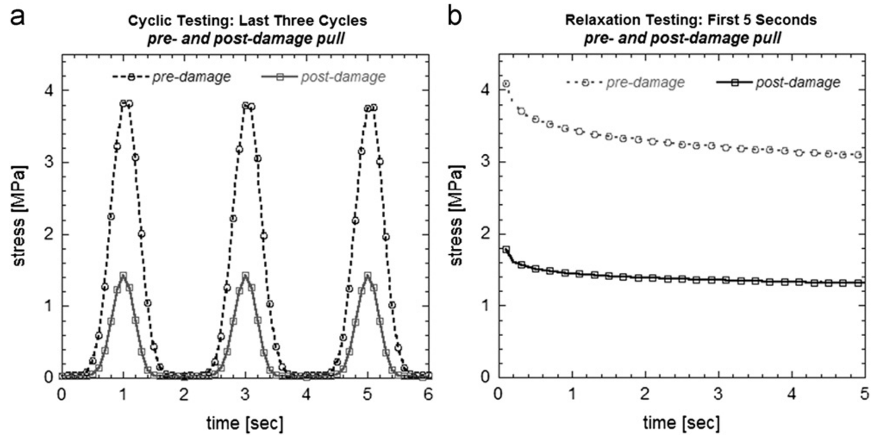

Fig. 2. Stress vs. time before and after diffuse damage induced by overstretch during (a) cyclic testing (cycles 8–10) and (b) stress relaxation testing (first 5 s) from one representative specimen. Note that the overall stress is decreased following damage, as is the stress decay during the first 5 s of relaxation. The relaxation curve shows 50% of the relaxation points for clarification.

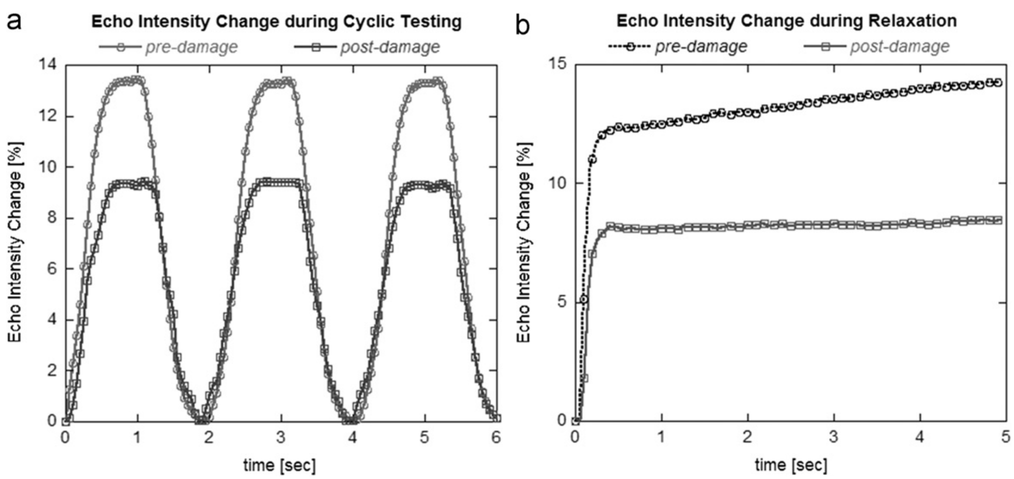

Fig. 3. Echo intensity change before and after diffuse damage induced by overstretch during (a) cyclic testing (cycles 8–10) and (b) stress relaxation testing (first 5 s) from one representative specimen. Note that the overall echo intensity change is decreased following damage, as is the increase in intensity during 5 s of relaxation (see Fig. 2 for related force vs. time data). The relaxation curve shows 50% of the relaxation points for clarification.

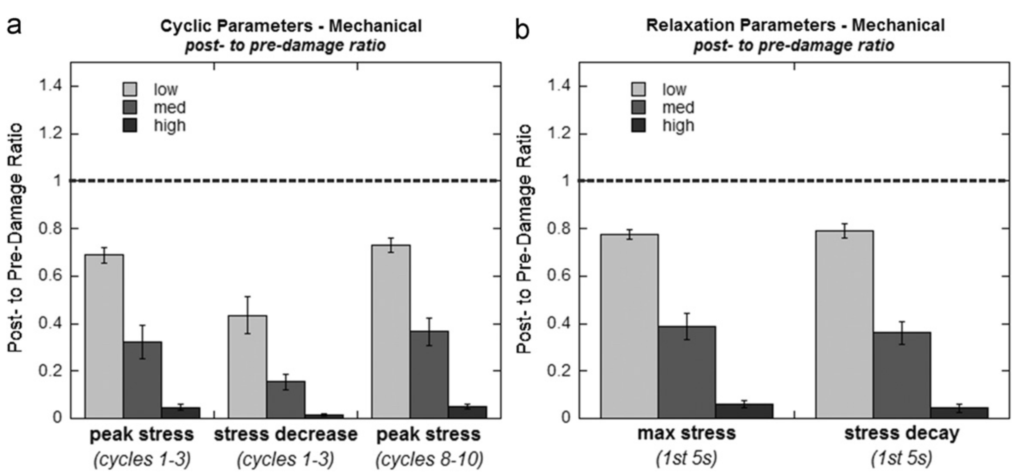

Fig. 4. Post- to pre-damage ratios of mechanical parameters from (a) cyclic testing and (b) stress relaxation testing for specimens. As strain during overstretch increases, post-damage parameter values decrease. Error bars indicate one standard deviation.

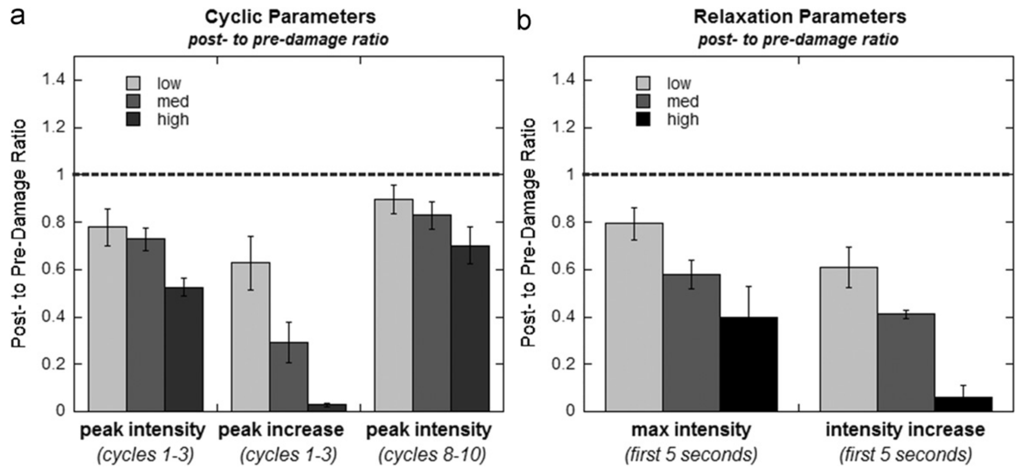

Fig. 5. Post- to pre-damage ratios of ultrasound parameters from (a) cyclic testing and (b) stress relaxation testing for specimens. As strain during overstretch increases, post-damage parameter values decrease. Error bars indicate one standard deviation.

relaxation testing, as well as the echo increase during the first 5 s of relaxation at the input strain, were decreased following overstretch (Fig. 5(b); each level significantly different, $p < 0.001$ for each case).

4. Discussion

In this study, the effect of overstretch damage on mechanical and echo intensity behavior in tendon during cyclic and stress relaxation testing was examined. Overstretch damage causes non-recoverable deformation (defined as requiring higher strain to reach a given stress) in the tissue, manifested in lower stresses during both cyclic and relaxation testing (Duenwald-Kuehl et al., 2012). The resulting decrease in echo intensity change is likely due to this non-recoverable deformation in the tissue. As echo intensity changes were previously found to increase with increased strain (Duenwald et al., 2011), it naturally follows that higher strains are required to reach the same echo intensity level following damage. This hypothesis is further verified by the fact that as the overstretch strain level increases (thus resulting in increased laxity), the echo intensity changes decrease. The time-dependent echo intensity changes were also reduced, which would be anticipated based on the reduction in viscoelastic parameters seen following diffuse damage.

The results of this study indicate overstretch damage is manifested in a reduced echo intensity change for similar levels of applied strain, which may be expanded upon in the future to identify diffuse damage in tissue. Though many imaging modalities are capable of detecting focal damage such as tears, diffuse damage is generally not detectable. An ultrasound-based method could potentially provide information regarding mechanical compromise in tendon tissue in a nondestructive, noninvasive method, particularly when results can be compared to a healthy normal (*i.e.* using the tendon from the uninjured limb as a contralateral control).

The ability to noninvasively measure tendon mechanics, including damage mechanics, *in vivo* is beneficial in both a clinical and a scientific setting, and has the potential to greatly improve the accuracy of subject-specific models of musculoskeletal biomechanics. Once the method is validated *in vivo*, any tendon that can be scanned with ultrasound can be evaluated. Potential uses include: rotator cuff studies (e.g. why some patients can cope with tears), patellar tendon studies (e.g. patellar tendon mechanics following ACL repair), lateral epicondylosis studies (e.g. injured tendon mechanics), rehabilitation monitoring (e.g. compare mechanical properties of tendon to the diseased and normal state), surgical follow-up (e.g. repaired tendon loading) and sports-related studies (e.g. how biceps tendons of baseball pitchers in pitching arm compare to non-pitching arm).

The ability of this method to focus on a particular area in addition to the tendon as a whole makes it possible to study the mechanics of specific locations (e.g. insertion sites, tears, repairs). It may be possible to use local analysis of stress and strain to compare normal and pathologic tendons, allowing researchers and clinicians to evaluate the types of loading profiles that can lead to the development of pathologies. Abnormal regions with high stress and strain concentrations are more susceptible to tear propagation than homogeneously loaded/deformed regions. Identifying these mechanical signatures on the tissue, along with defect size, may allow prediction of which tendons will tear or rupture, or which repaired tendons are likely to re-rupture, in a way that a standard physical examination cannot. Investigating load distribution through the tendon will give insight into why certain regions of the tendon, such as the calcaneal insertion site of the Achilles tendon, are more prone to tears than other regions, and what interventions may help prevent them.

The present paper study is correlative and not analytical, but we observe ultrasound echo intensity (an acoustoelastic-like effect related to increased wave reflection), and we observe predictable alterations in this behavior after overstretch damage. Our observations are phenomenological and do not consider microstructural changes, which may result from micro-damage that occurs during overstretch and lead to a local rearrangement of structure. Furthermore, the echo intensity changes demonstrated in this study may be affected by fluid shifts during loading, transverse compaction of fibers (Poisson effect) during stretch, and stretch-induced reductions of crimping. Future acoustoelastic characterization will require additional experimentation and theoretical development of an appropriate hyperelastic model for a transversely isotropic composite material allowing three-dimensional characterization of dilatational and shear waves. Finally, experiments in this study were performed *ex vivo* under displacement control. Such displacement control would be difficult to recapitulate *in vivo*, so a reformulation of this concept under load control remains for future studies.

In conclusion, the diffuse damage caused by overstretch in the tendon (lowering the stress at a given strain in the tissue and decreasing viscoelastic response), which resulted in lower echo intensity changes during stress relaxation and cyclic testing.

The reduced echo intensity for similar changes in strain is indicative of the mechanical state of the tissue, detects the diffuse damage in a way static image modalities cannot, and demonstrates a possible application for ultrasound as a tool for non-invasive tissue analysis.

Conflict of interest statement

Ray Vanderby holds intellectual property on some aspects of the ultrasound technique. Other authors have no conflict of interest to report.

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