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Ultrasound echo is related to stress and strain in tendon

Sarah Duenwald [a], Hirohito Kobayashi [b], Kayt Frisch [a], Roderic Lakes [a,c], Ray Vanderby Jr. [a,b,*]

[a] Department of Biomedical Engineering, University of Wisconsin—Madison, 1550 Engineering Drive, Madison, WI 53706, USA
[b] Department of Orthopedics, University of Wisconsin—Madison, 600 Highland Avenue, Madison, WI 53792, USA
[c] Department of Engineering Physics, University of Wisconsin—Madison, 1500 Engineering Drive, Madison, WI 53706, USA

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ABSTRACT

The mechanical behavior of tendons has been well studied *in vitro*. A noninvasive method to acquire mechanical data would be highly beneficial. Elastography has been a promising method of gathering *in vivo* tissue mechanical behavior, but it has inherent limitations. This study presents acoustoelasticity as an alternative ultrasound-based method of measuring tendon stress and strain by reporting a relationship between ultrasonic echo intensity (B-mode ultrasound image brightness) and mechanical behavior of tendon *in vitro*. Porcine digital flexor tendons were cyclically loaded in a mechanical testing system while an ultrasonic echo response was recorded. We report that echo intensity closely follows the applied cyclic strain pattern in time with higher strain protocols resulting in larger echo intensity changes. We also report that echo intensity is related nonlinearly to stress and nearly linearly to strain. This indicates that ultrasonic echo intensity is related to the mechanical behavior in a loaded tissue by an acoustoelastic response, as previously described in homogeneous, nearly incompressible materials.

Acoustoelasticity is therefore able to relate strain-dependent stiffness and stress to the reflected echo, even in the processed B-mode signals reflected from viscoelastic and inhomogeneous material such as tendon, and is a promising metric to acquire *in vivo* mechanical data noninvasively.

1. Introduction

Tendons translate muscular contraction into skeletal movement, storing and releasing energy during motion (Ker et al., 1987). Understanding tendon mechanics is therefore essential to understand normal and pathologic movement and for analyzing the structural and mechanical consequence of injury. Thus far, tendon mechanical data have been obtained largely through *in vitro* experimentation (Abrahams, 1967; Cohen et al., 1976; Ker, 2007; Rigby et al., 1959; Woo et al., 1982). Though such studies provide essential basic mechanical information about the tissue, a noninvasive method to acquire mechanical data would allow patient-specific analysis and direct analysis of human pathologies that are poorly modeled by animals (e.g., rotator cuff). *In vivo* tendon loads have been computed using force plates or dynamometers and geometrical information (Maganaris and Paul, 1999, 2000; Riemersma et al., 1988) and/or with EMG studies of muscle (Lloyd and Besier, 2003). Direct studies of tendon are typically invasive and often rely on implanted strain gauges, providing only local measure of strain and a partial measure of mechanical behavior. EMG estimates contractile force in muscle (and thus in tendon), but it has a number of limiting factors such as cross talk from different muscles, passive tissue effects, different muscle lengths during contraction, and dynamic motion.

Recently, ultrasound imaging has been used to evaluate tissue strain and other mechanical properties (Samani et al., 2007; Skovorada et al., 1994, 1995). Many researchers have tried to evaluate tissue strain and mechanical properties using elastography, a technique originally proposed by Ophir et al. (1991), which maps strain distributions in tissues resulting from compression on the surface; since strain is inversely related to stiffness, elastography is an indirect method to estimate tissue stiffness (Doyley et al., 2000; Kallel et al., 1996; Kallel and Bertrand, 1996; Ponnekanti et al., 1992, 1995). Though researchers have used this technique to identify tissue properties, current elastographic methods have some inherent limitations.

Elastography tracks inhomogeneous echo reflections (“speckles” resulting from tissue heterogeneities) in ultrasound images during loading (usually using the transducer to apply compression); strain information calculated using distortions of these reflectors is related to mechanical properties via post hoc mechanical analysis. A limitation of elastography is that it is inherently linear, assuming that the material properties as well as ultrasound wave velocity do not change during strain measurement, which restricts analyses to small increments of compression. When soft tissues were tested under larger deformations and were therefore nonlinear in stiffness, significant errors occurred (Itoh et al., 2006; Zhi et al., 2007). This is problematic in soft tissues such as tendons

* Correspondence to: Room 5059, 1111 Highland Avenue, Madison, WI 53705, USA. Tel.: +1 608 263 9593; fax: +1 608 265 9144.
E-mail address: vanderby@ortho.wisc.edu (R. Vanderby Jr.).

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as they are nonlinear in stiffness and undergo relatively large deformations during activity, reaching strains of several percent (elastography works best when strain increments are restricted to less than 1%). Another limitation is the commonly used method of compressive testing; tendon is loaded in tension *in vivo*, so interesting mechanical information is lost when only considering compressive transverse loads. These restrictions associated with standard elastographic methods limit its applicability to tendon. Finally, elastography measures strain. More data are needed, either stress or stiffness, to completely describe the mechanical behavior.

The theory of acoustoelasticity, developed byHughes and Kelly (1953) is based on the principle that the acoustic properties of a material are altered as the material is deformed and loaded, similar to a change in tension alters the pitch of a guitar string. Changes in acoustic properties caused by elastic deformation can be measured as a change in wave propagation velocity or reflected wave amplitude (Kobayashi and Vanderby, 2005, 2007). Kobayashi and Vanderby (2005, 2007) derived the acoustoelastic relationship between reflected wave amplitude and mechanical behavior (strain-dependent stiffness and stress) in a deformed, nearly incompressible material using A-mode 1-D ultrasound. Despite signal processing, this phenomenon is also manifested in B-mode 2-D ultrasound, as the tensioning of tendon increases the intensity of the reflected ultrasonic echoes, leading to a brighter ultrasound image in B-mode. Examples of this acoustoelastic effect in soft tissues have been reported in the literature. For example, mean echogenicity has been correlated to the elastic modulus of the equine superficial digital flexor tendon (Crevier-Denoix et al., 2005), and softening of the human extensor tendons has been detected with sonoelastography (De Zordo et al., 2009). Pan et al. (1998) reported increasing echo intensity when skin underwent increase in strain (13–53%). The present study examines the relationship between ultrasonic echo intensity from standard clinical B-mode images and the stress–strain behavior of tendon during controlled loading *in vitro*. Its purpose is to examine whether this acoustoelastic phenomenon has the potential for the functional evaluation of tendon *in vivo*.

2. Materials and methods

2.1. Specimen preparation

Eight porcine digital flexor tendons were carefully extracted from porcine legs (aged six months, sacrificed for an unrelated study) for *ex vivo* testing, leaving the bone and insertion site intact at the distal end. Bony ends were embedded in lightweight filler (Evercoat, Cincinnati, OH, USA) for ease of gripping. Specimens were kept hydrated in physiologic buffered saline (PBS) solution throughout preparation.

2.2. Mechanical testing

Mechanical testing was performed using a servohydraulic mechanical test system (Bionix 858; MTS, Minneapolis, MN, USA) with the custom bath shown in Fig. 1. The bony end of the tendon was secured in the metal block, attached to the bath, while the soft tissue end was secured in a moving grip, attached to the load cell. Grip-to-grip displacement was controlled by the servohydraulic system, and load was measured via a 50 lb load cell (Eaton Corporation, Cleveland, OH, USA). Once secured in the system, tendons were preloaded to 1 N to remove slack. Following preloading, tendons were preconditioned in a sinusoidal wave to 2% strain for 20 s and allowed to rest for 1000 s. Tendons were then subjected to cyclic mechanical testing to 2%, 4%, or 6% strain for 10 cycles at 0.5 Hz and allowed to rest for 1000 s to ensure complete recovery occurred between tests. The process was repeated until each specimen was tested at each of the three strain levels two separate times (in random order), for a total of six tests per tendon. To avoid strain history transient effects, the last three cycles were used for analysis (see Fig. 2) as tendon loads had effectively reached steady state.

2.3. Ultrasound

Cine ultrasound was recorded using a GE LOGIQ*e* ultrasound machine with GE 12L-RS Linear Array Transducer (General Electric, Fairfield, CT, USA). The ultrasound transducer (operating at 12 MHz) was held in fixed position in the custom bath (see Fig. 1) using a custom-built clamp fixed to the side of the bath to record cine B-mode ultrasound (20 frames per second) of the tendon during the mechanical testing outlined in the previous section. To avoid strain history transient effects and to correspond to the mechanical data, only the last three cycles were used for analysis. The overall echo intensity (i.e., the average gray scale brightness in a selected region of interest in a 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.

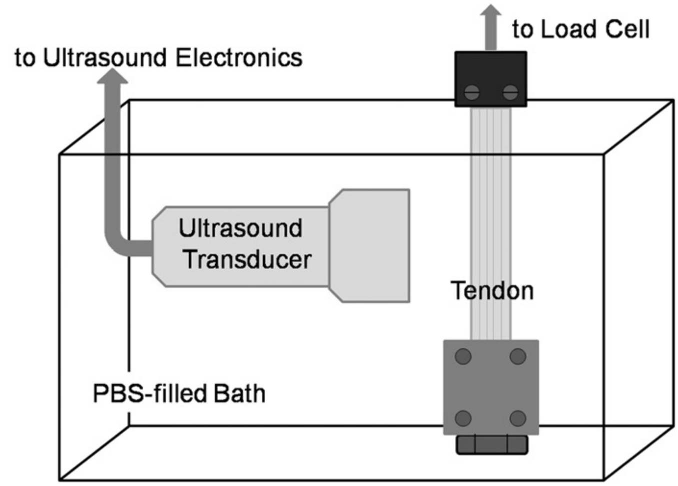

Fig. 1. Test setup. The tendon is held in place by a stationary metal block and a moveable soft tissue grip, which is connected to the load cell. The ultrasound transducer is submerged in the PBS bath and held in place to image the tendon during mechanical testing.

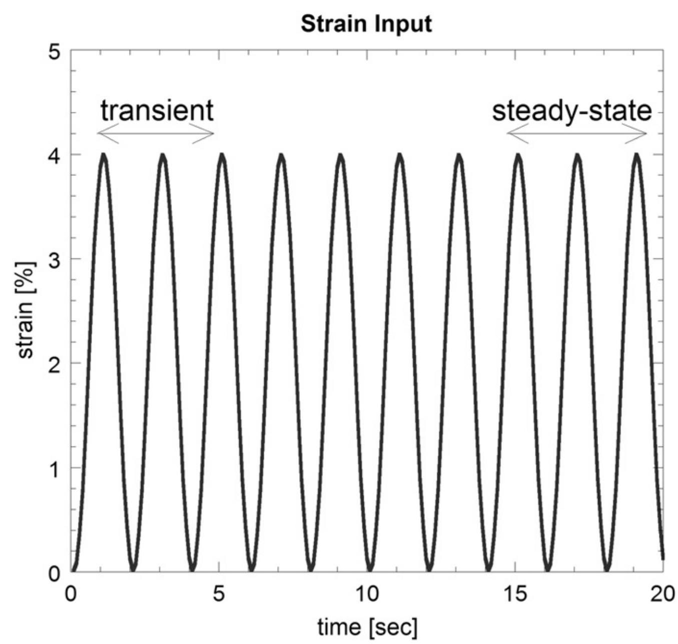

Fig. 2. Strain input for mechanical testing, shown for the 4% strain test. The first three cycles give rise to history dependent transient effects. Tissue has generally reached steady-state by the final three cycles. Data from the final three cycles are used in this study.

2.4. Region of interest tracking

In order to measure echo intensity changes over the ROI for the entire test, a region-based optical flow matching technique (Anandan, 1989; Barron et al., 1994) was used to track the movement of "speckles" (patterned echo reflections caused by tissue heterogeneity) in the ultrasonic echo that defined the ROI. This algorithm evaluated the speckle displacement between consecutive image frames by finding