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Validation of shear wave elastography in skeletal muscle

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ABSTRACT

Skeletal muscle is a very dynamic tissue, thus accurate quantification of skeletal muscle stiffness throughout its functional range is crucial to improve the physical functioning and independence following pathology. Shear wave elastography (SWE) is an ultrasound-based technique that characterizes tissue mechanical properties based on the propagation of remotely induced shear waves. The objective of this study is to validate SWE throughout the functional range of motion of skeletal muscle for three ultrasound transducer orientations. We hypothesized that combining traditional materials testing (MTS) techniques with SWE measurements will show increased stiffness measures with increasing tensile load, and will correlate well with each other for trials in which the transducer is parallel to underlying muscle fibers. To evaluate this hypothesis, we monitored the deformation throughout tensile loading of four porcine brachialis whole-muscle tissue specimens, while simultaneously making SWE measurements of the same specimen. We used regression to examine the correlation between Young's modulus from MTS and shear modulus from SWE for each of the transducer orientations. We applied a generalized linear model to account for repeated testing. Model parameters were estimated via generalized estimating equations. The regression coefficient was 0.1944, with a 95% confidence interval of (0.1463–0.2425) for parallel transducer trials. Shear waves did not propagate well for both the 45° and perpendicular transducer orientations. Both parallel SWE and MTS showed increased stiffness with increasing tensile load. This study provides the necessary first step for additional studies that can evaluate the distribution of stiffness throughout muscle.

1. Introduction

Normal skeletal muscle stiffness results from active tension produced by muscle contraction and passive tension produced largely by connective tissue (Hill, 1938; Huxley, 1957). While the manifestations of deficits in the active component are readily diagnosed and unmistakably detrimental to daily function (NIMSD Consortium, 1996; Fried et al., 2001), analogous alterations in the passive component are less understood. This apparent lack of information should not imply that passive skeletal muscle stiffness does not play a key role in skeletal muscle growth, metabolism, or function—as it is integral for all three (Jaspers et al., 1999; Kjaer, 2004; Tatsumi et al., 2006; Werle, 2008). Numerous *in vitro* studies have identified the connective tissue network of collagen within the extracellular matrix (ECM) as a key contributor to passive stiffness in a variety of skeletal muscles (Bensamoun et al., 2006; Borg and Caulfield, 1980; Brown et al., 2012; Rowe et al., 2010). It is becoming increasingly apparent that the ECM is vital for mechanotransduction, as well as muscle growth and adaptation (Jaspers et al., 1999; Kjaer, 2004; Tatsumi et al., 2006; Werle, 2008). Later research indicates that passive stiffness may also play a role in muscle performance and adaptation to exercise (Fouré et al., 2011). Increased collagen content and stiffness are seen in numerous musculoskeletal pathologies, including spasticity (Brown et al., 1999; Damiano et al., 2001; Gracies, 2005; Vaz et al., 2006), as well as typical aging (Alnaqeeb et al., 1984; Botelho et al., 1954; Larsson et al., 1979; Tomonaga, 1977). It is clear that muscle stiffness is closely related to joint constraint—increased muscle stiffness is associated with poor range of motion, while reductions in muscle stiffness may predispose to joint subluxation (Johns and Wright, 1962; Vandervoort, 1999). Unfortunately, *in vivo* passive stiffness measures historically focused primarily on either qualitative measures (Park and Kwon, 2012) or the stiffness of entire joints

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and muscle groups (Sinkjaer and Magnussen, 1994), thus limiting the advancement of our clinical and scientific understanding. Reliable, noninvasive, quantitative techniques for measuring and monitoring skeletal muscle stiffness are necessary not only to advance our understanding of the mechanism and effects of altered skeletal muscle stiffness but also to improve diagnosis and treatment following injury.

A number of techniques are currently available for monitoring muscle stiffness *in vivo*. Myotonometry is quick and inexpensive, but tends to be superficial or merely qualitative (Bizzini and Mannion, 2003; Park and Kwon, 2012). Stretch-release techniques elegantly distinguish the stretch reflex stiffness from intrinsic joint stiffness (Sinkjaer et al., 1988), but are unable to quantify the mechanical properties of individual skeletal muscles. Similarly, range of motion measures can quantify resistance to movement, but also evaluates the properties of entire joints and are unable to target individual muscles for assessment (Rabita et al., 2005). Magnetic resonance elastography shows great promise for quantifying the stiffness of whole muscles and muscle groups across a range of ages and contraction levels (Debernard et al., 2011a, 2011b), but is costly and lacks real-time application (Jenkyn et al., 2003). Ultrasound shows great utility in evaluating underlying architecture of more superficial muscles (Rutherford and Jones, 1992), and a variety of techniques have been used to evaluate muscle stiffness (Park and Kwon, 2012), even in concert with magnetic resonance elastography (Debernard et al., 2013), but, until recently, ultrasound has been purely qualitative. As skeletal muscle is a very dynamic tissue, an ideal technique would be capable of real-time measurement to quantify skeletal muscle stiffness throughout its functional range. Such a technique should be sensitive to small changes in stiffness and capable of determining material properties in light of the functionally relevant and often complex skeletal muscle architecture.

Quantitative ultrasound elastography is beginning to emerge as a promising diagnostic tool for evaluating the mechanical properties of skeletal muscle. Unlike earlier qualitative ultrasound elastography techniques, shear wave elastography (SWE) is an ultrasound-based technique that can characterize tissue mechanical properties based on the propagation of remotely induced shear waves (Bercoff et al., 2004; Chen et al., 2009; Palmeri et al., 2008; Sarvazyan et al., 1998). Shear modulus can be readily calculated from the measured shear wave propagation velocity and tissue density (Yamakoshi et al., 1990). A variety of ultrasound-based elastography techniques have been compared with magnetic resonance elastography, with good agreement in a variety of tissues and phantoms (Bensamoun et al., 2008; Dutt et al., 2000; Oudry et al., 2009). Several investigators have begun to apply similar ultrasound techniques and it is clear that increased skeletal muscle force production is associated with increased stiffness (Gennisson et al., 2005, 2010; Shinohara et al., 2010; Zhao et al., 2009). While these experiments are of great value, up until now no study has compared ultrasound elastography results with traditional materials testing for skeletal muscle. To ensure investigators are obtaining robust and meaningful results, baseline reliability and validity information must be obtained for the application of such novel techniques to anisotropic and inhomogeneous skeletal muscle tissue. Gennisson et al. (2003) found that shear waves propagate much more readily along beef muscle fibers longitudinally, as compared to perpendicularly or any interval of rotation therein. Later investigations support these initial findings, as parallel transducer orientations obtained the most reliable measures of muscle elasticity (Gennisson et al., 2010). Despite the recent advances applying ultrasound elasticity imaging to skeletal muscle, these techniques are yet to be validated taking into account the mobility and dynamic properties of skeletal muscle. The purpose of this study was to validate SWE throughout the functional range of motion of skeletal muscle. We hypothesized that combining traditional materials testing techniques with SWE measurements will show increased stiffness measures with increasing tensile load, and will correlate well with each other throughout the tensile range when the ultrasound transducer is oriented parallel to the muscle fibers.

2. Methods

2.1. Specimen preparation

We obtained four right brachialis whole-muscle samples immediately post-mortem from 6- to 9-month old female swine. All animal care was in accordance with the Mayo Clinic Institutional Animal Care and Use Committee guidelines. We completed all muscle testing within 5 h of sacrifice, so rigor mortis was not expected to play a significant role in the mechanical testing (Van Ee et al., 2000). At the time of sacrifice, all animals were euthanized with injections of telazol (2.5 cm^3, 100 mg/ml), heparin (10 cm^3, 1000 units/ml), and Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI; 15 cm^3, pentobarbital sodium: 390 mg/ml, propylene glycol: 0.01 mg/ml, ethyl alcohol: 0.26 mg/ml, benzyl alcohol 0.2 mg/ml). Following dissection and visualization of the intact brachialis, we flexed the forelimb *in situ* to 90° and fully extended to 180° at the elbow to establish the muscle's initial (L_0) and final (L_1) lengths, respectively. Both lengths were obtained by measuring from origin, just under the humeral head, to insertion, on the fused radius and ulna. We then harvested the intact brachialis from origin to insertion, preserving and harvesting both bony attachments to facilitate later mechanical testing. In the interest of facilitating material testing and to control for individual variability between swine, we did not retain surrounding soft tissues (adjacent musculature, overlying adipose tissue and skin, *etc.*) Following harvest, we kept the muscle tissue cool and moist with chilled saline. Prior to testing, both the proximal humeral attachment and distal radioulnar attachment were placed in collars and fixed with bone cement (polymethylmethacrylate).

2.2. Materials testing

We mounted the specimen on a materials testing machine (model 312; MTS, Minneapolis, MN) for simultaneous tensile testing and SWE ultrasound evaluation, as indicated in Fig. 1. The collars holding the humeral and radioulnar bone segments were fixed vertically on the actuator using custom-designed attachments, replicating the normal anatomic relationship between the two bones. We used the materials testing system (MTS) to stretch the tissue specimen to its initial length, as previously measured *in situ* at 90° flexion. We manually measured the thickness and width of the muscle at mid-belly, assuming an ellipse, to estimate cross-sectional area (CSA) in the location where SWE measurements would be taken. The same individual obtained all measurements throughout the study to reduce variability. Each specimen underwent displacement-controlled tensile testing from initial length to final length, at ~1.15% L_0 per second, with simultaneous ultrasound measurements. A load cell (model 3397; Lebow Products, Troy, MI; Accuracy: 0.05%) measured force throughout the testing procedure. We collected all MTS force and displacement data at 20 Hz. The specimen was preconditioned for six cycles prior to tensile testing using the same loading protocol. We conducted all testing at room temperature (22 °C) and kept the specimen moist with saline and held at L_0 between each tensile test, as previously documented for similar studies (Gottsauner-Wolf et al., 1995). A digital output signal sent from the MTS to the Verasonics ultrasound system (Verasonics Inc., Redmond, WA) triggered and synchronized ultrasound SWE measures with MTS data acquisition. The digital output signal began with the start of MTS displacement and immediately triggered the first Verasonics acquisition, which we programmed to automatically acquire additional measures every 1.5–2.0 s, depending on the loading rate and overall displacement. We obtained 10 ultrasound measures at regular, prescribed intervals throughout each continuous tensile test.

From the collected MTS data, we calculated strain by dividing the displacement data by initial length, and plotted the stress–strain curve, using CSA calculated from the initial measurements. We defined Young's modulus as the slope between ten consecutive MTS data points from the stress–strain curve. Using this approach, Young's modulus was calculated for each of the ten SWE time points.

2.3. Shear wave elastography

Prior to tensile testing, we fixed a linear-array ultrasound transducer (L7-4, center frequency = 5 MHz, Philips Healthcare, Andover, MA) over the middle third of the muscle specimen. We attached the transducer to a custom-built device that maintained constant, minimal pressure between the ultrasound transducer and the underlying muscle tissue throughout each tensile test. Since all mechanical testing was well-within the submaximal range, we tested multiple transducer orientations for each muscle specimen. By gross inspection and brief B-mode ultrasound examination, we determined that the majority of fibers in the swine brachialis