Measurement of Passive Skeletal Muscle Mechanical Properties *In Vivo*: Recent Progress, Clinical Applications, and Remaining Challenges

LYNNE E. BILSTON[1,2] and KRISTY TAN[1,3]

[1]Neuroscience Research Australia, Randwick, NSW, Australia; [2]Prince of Wales Clinical School, University of New South Wales, Randwick, NSW, Australia; and [3]Graduate School of Biomedical Engineering, University of New South Wales, Randwick, NSW, Australia

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Abstract—The ability to measure and quantify the properties of skeletal muscle *in vivo* as a method for understanding its complex physiological and pathophysiological behavior is important in numerous clinical settings, including rehabilitation. However, this remains a challenge to date due to the lack of a "gold standard" technique. Instead, there are a myriad of measuring techniques each with its own set of pros and cons. This review discusses the current state-of-the-art in elastography imaging techniques, i.e., ultrasound and magnetic resonance elastography, as applied to skeletal muscle, and briefly reviews other methods of measuring muscle mechanical behavior *in vivo*. While *in vivo* muscle viscoelastic properties can be measured, these techniques are largely limited to static or quasistatic measurements. Emerging elastography techniques are able to quantify muscle anisotropy and large deformation effects on stiffness, but, validation and optimization of these newer techniques is required. The development of reliable values for the mechanical properties of muscle across the population using these techniques are required to enable them to become more useful in rehabilitation and other clinical settings.

INTRODUCTION

Skeletal muscles are the major actuator in the human body, and abnormal muscle mechanical function can both cause muscle disorders (e.g., disuse causes muscle atrophy) and arise as a result of disease processes (e.g., contracture after stroke). Such abnormalities can impair musculoskeletal function, and they thus provide major challenges for rehabilitation engineers aiming to design assistive technologies.

Non-invasive measurements of muscle mechanical properties in human subjects could be very valuable to rehabilitation clinicians, as they could provide a quantitative assessment of muscle status that is directly related to physiological function and muscle structure. There is also clear potential for clinical use of *in vivo* muscle mechanical property measurements to assist with diagnosis of muscle disorders, and also in monitoring disease progression and treatment response.

Our understanding of the mechanical behavior of muscles has come a long way since researchers first began trying to quantify the force required to elongate passive muscle.[7] It is now well recognized that skeletal muscles are mechanically anisotropic and nonlinearly viscoelastic.[21,76,77] However, quantifying these complex mechanical properties *in vivo* remains an open challenge in biomechanics, with no single method providing a comprehensive characterization of muscle properties. While it is relatively straightforward to measure the overall mechanical behavior of muscle–tendon groups that cross easily manipulated joints by measuring torque–angle relationships during passive joint movements it is much more difficult to obtain measurements of the mechanical behavior of individual muscles, and to separate the behavior of tendons from the muscle bellies. In this paper, we review recent developments in measuring muscle properties *in vivo*, and lay out some of the remaining challenges. While we focus here on passive properties, active contraction properties are obviously also important, but elastography and other methods for characterizing these *in vivo* are currently less well established. We primarily discuss the properties of the muscle belly, which we will refer to as 'muscle', and where we refer to the properties of the muscle–tendon unit, this will be noted explicitly.

Address correspondence to Lynne E. Bilston, Neuroscience Research Australia, Randwick, NSW, Australia. Electronic mail: l.bilston@neura.edu.au

PASSIVE MUSCLE MECHANICAL PROPERTIES

Passive muscle tissue, particularly skeletal muscle, is a hierarchically organized, anisotropic, nonlinearly visco-elastic soft tissue. Skeletal muscle is anisotropic due to its microstructural arrangement consisting of parallel fiber bundles. Skeletal muscle's hierarchical structure can be visualized at different length scales (see Fig. 1). Each muscle is surrounded by a connective tissue layer called epimysium, which is continuous with tendons and attaches muscles to bones. Each muscle consists of a bundle of muscle fascicles. Each muscle fascicle is surrounded by perimysium and contains many myofibers surrounded by endomysium, which are in turn made up of numerous myofibrils.[62] The myofibrils consist of actin, myosin and titin filaments in organized structures that move relative to each other during contraction.[43] This hierarchical structure gives rise to some of muscle's characteristic mechanical behavior, as different elements of the microstructure deform and interact under load to give rise to the complex overall mechanical response. Moreover, alterations in the structure of muscles, due to cell loss, fatty infiltration, and fibrosis in disease processes are reflected in the mechanical behavior of the tissue, as discussed below in the section on muscle disorders.

Muscles exhibit the typical nonlinear length–tension (or stress–strain) behavior of most soft biological tissues, whereby stiffness increases with applied stretch. Relaxation experiments show a broad spectrum of relaxation time constants (e.g., Kwon et al.[71]), likely indicating a range of different relaxation processes occurring within muscle tissue, perhaps related to its hierarchical structure, although this has not been proven.

Muscle has also been shown to be thixotropic,[61] where the passive behavior of a muscle exhibits a yield stress during small amplitude deformations after contraction. The apparent increase in stiffness is reduced by large passive movements, although the mechanisms are not completely understood.

Methods for measuring muscle mechanical properties must therefore take into account the complex mechanical behavior of these tissues, particularly when comparing measurements made in different joint positions or muscle lengths, where the nonlinear behavior will strongly influence any measures that do not capture the full nonlinear behavior. This includes the vast majority of elastography measurements, which estimate properties from small deformation loading.

ELASTOGRAPHY AND IMAGING-BASED METHODS FOR MEASURING MUSCLE MECHANICS

Elastography encompasses a group of techniques that estimate the mechanical properties of a tissue based on the relationship between mechanical properties and the propagation characteristics of mechanical vibration waves, typically in the acoustic frequency range (10–1000 Hz). The wave propagation is usually measured using some form of medical imaging (ultrasound and MRI are the most common), and the tissue modulus estimated from the wave equation (see Eq. 1).

$$\rho\partial_t^2 \vec{u} = \mu\nabla^2 \vec{u} + (\lambda + \mu)\nabla(\nabla \vec{u}) + \eta\partial_t\nabla^2 \vec{u} + (\xi + \eta)\partial_t\nabla(\nabla \vec{u}), \tag{1}$$

where $\vec{u}$ is the displacement vector, ρ is the density of the tissue, μ is the shear modulus, λ the second Lamé coefficient, η the shear viscosity and ξ is the viscosity of the compressional wave. The subscript t refers to the time derivative. Despite some concerns over the validity of this approach,[68] a much simplified version is often used, that estimates local wave speed from the imaging data, and calculates the shear modulus from:

$$\mu = \rho v^2 \lambda^2 \tag{2}$$

where v is the frequency of the vibration and λ is the local wavelength. Development of robust inversion techniques to estimate the viscoelastic properties from elastography imaging data is an ongoing area of research, as this is an inherently ill-conditioned numerical problem. Poor quality datasets with low signal-to-noise ratio tend to provide unreliable tissue property estimates, with different biases depending on the inversion algorithms applied. While most studies use some form of direct numerical inversion, recent work is exploring the use of finite-element inversion techniques that have the potential to be more robust, e.g., Van Houten et al.[75]

The term elastography is also often applied to qualitative elastography, typically using ultrasound, where relative stiffness of different tissue regions is estimated from the effect of static mechanical compression on local tissue deformation.[37]

Ultrasound Methods

While not strictly an elastography technique, traditional B-mode ultrasonography has been used together with dynamometry measurements of joint torques to quantify the passive length–tension curves and strain in specific muscles[32] and muscle groups in vivo but this method is limited in its field of view and can only image superficial muscles.[35] Cross-correlation techniques can be used on the image sequences to quantify fascicle strains, which are combined with muscle length–tension data to estimate fascicle length–tension curves.[32] This has a key advantage that it gives large-deformation information about the muscle fascicles, and thus can detect differences in muscle properties in clinical groups where the nonlinear mechanical behavior is altered.[38] The reliability of this
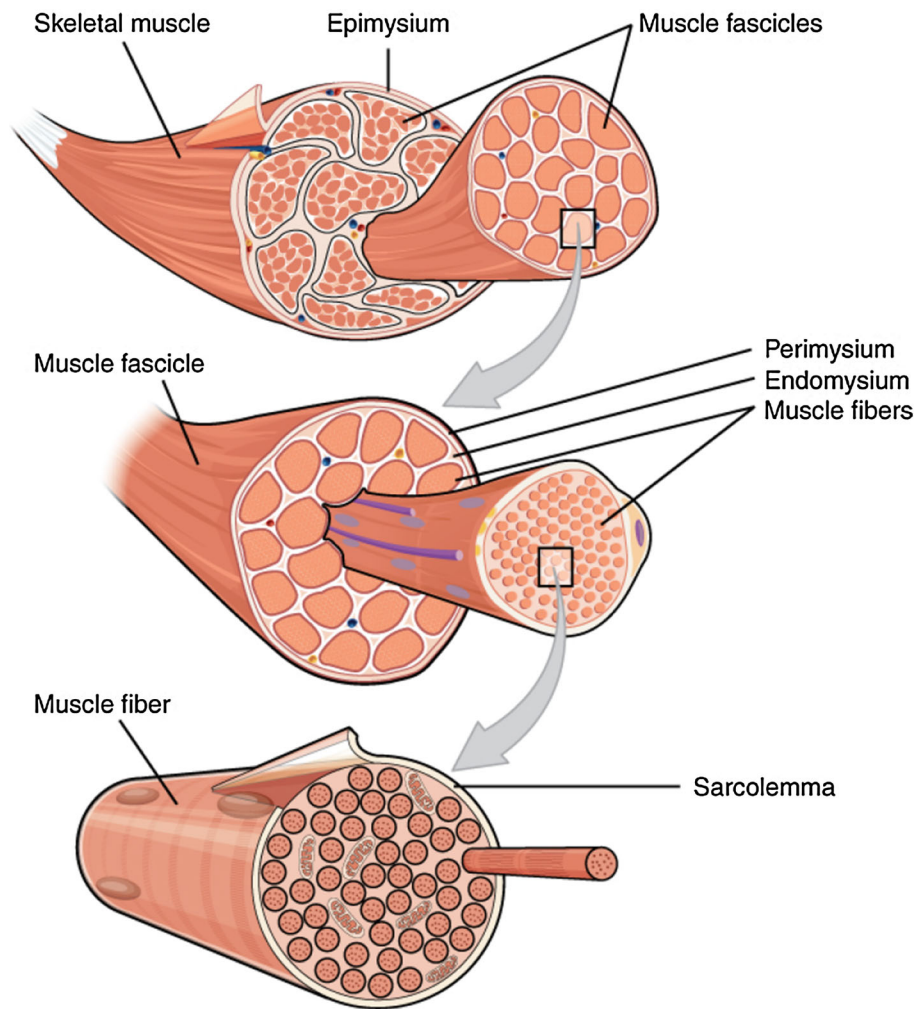
FIGURE 1. Hierarchical structure of skeletal muscle tissue (used with permission from OpenStax College[58]).

method was found to be good for same-day measurements within the same subject but measurements taken more than 1 week apart could not be reliably reproduced possibly due to misalignment of the ankle and knee joint axes with the axes of the apparatus.[32]

The most common ultrasound elastography technique, strain elastography (or compression elastography), relies on manually deforming a tissue with the ultrasound transducer, and inferring the stiffness of the tissue from the deformation observed in the ultrasound images.[18] Localized stiff regions deform less than softer tissue regions. The local deformation can be used to construct a map of apparent tissue stiffness. A map of "Young's modulus" is sometimes constructed by assuming that the transducer applies a uniform stress to the imaged tissue, and that the modulus is therefore proportional to the local tissue strain. This technique is most useful for providing qualitative information about variation in stiffness of tissue within the imaging field of view, as the assumptions used for estimating tissue moduli are difficult to support, because the deformation of a tissue varies with depth, the stress induced by manual compression of tissue with an ultrasound transducer is not uniform, and the nonlinear mechanical properties of tissues mean that the modulus cannot be estimated simply from the observed deformation. Moreover, it assumes that all tissues in the imaging field of view are loaded in series by the transducer, and if they are loaded in parallel, then the assumption of the stress being uniform is invalid. Instead, the strain is more likely to be uniform, and the stress will vary with the modulus, so that different stiffnesses will not be detected by a variation in strain. Repeatability and operator-dependent measurements are also significant challenges for this technique. Nonetheless, this technique has been used to study muscle mechanical behavior in both healthy volunteers and patient populations.[6,17,55,78] It is currently unable to provide information on muscle anisotropy.

An extension of ultrasound strain elastography is acoustic radiation force impulse (ARFI) elastogra-
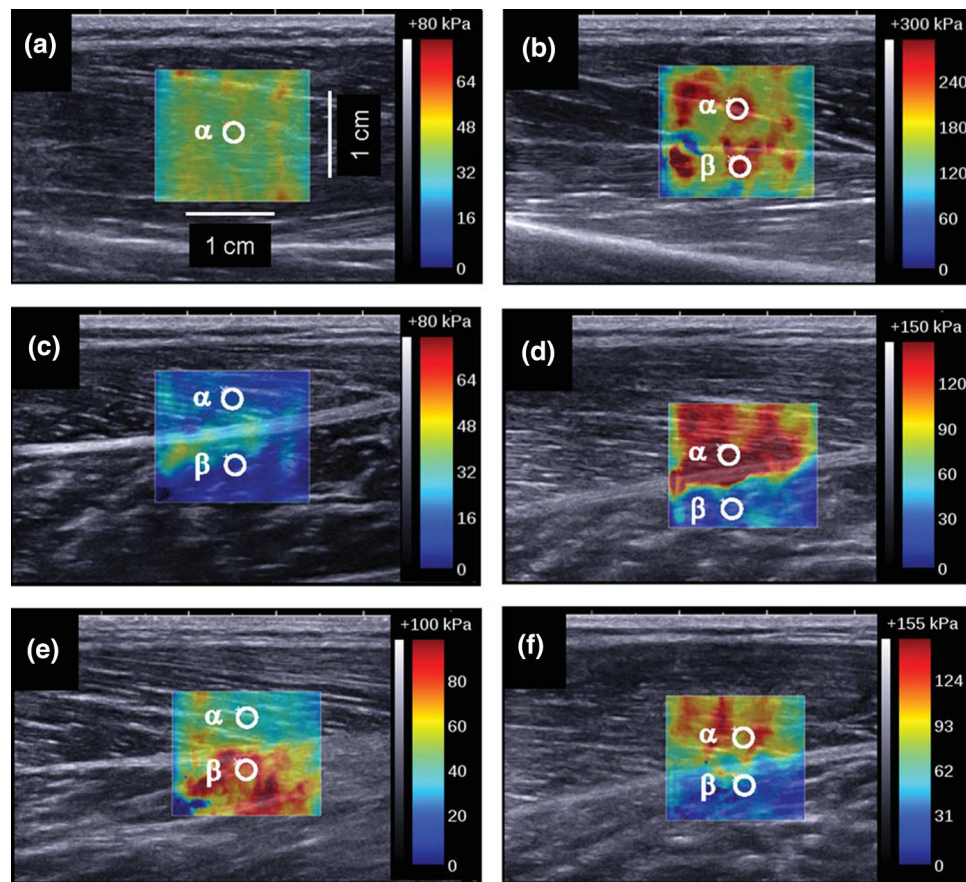
FIGURE 2. Supersonic shear wave ultrasound elastography of the muscles of the lower leg during standing. The colored region shows the calculated shear modulus, superimposed on a B-mode image. Localized stiffnesses can be calculated (small circles, α, β). (a, b) Tibialis anterior at rest and during contraction, respectively. (c, d) Medial gastrocnemius and soleus at rest and during contraction, respectively, with knee extended. (e) Medial gastrocnemius and soleus during contraction with knee flexed. (f) Medial gastrocnemius and soleus during quiet standing (used with permission from Shinohara *et al.*[67]).

phy.[54] Instead of manually compressing the tissue, ARFI creates a focused ultrasound pulse that travels through the tissue. Using rapid imaging ultrasound pulse echoes, the tissue deformation can be tracked, and analyzed to provide a qualitative stiffness map. Its main advantage over strain elastography is that it is not limited to superficial tissues that can be manually deformed,[20] although it is still limited by ultrasound penetration depths.

More recently, efforts to provide quantitative mechanical property data from ultrasound elastography led to the development of shear wave elastography, which relies on similar physical principles to MR elastography (see below), where propagation of an externally imposed acoustic wave is tracked, and used to estimate the tissue shear modulus. This stimulus can be generated by ARFI, and its propagation imaged with high frame rate ultrasound, as in the supersonic elastography technique.[67] An example of this technique applied to lower limb muscles is shown in Fig. 2. This system can provide elastograms at a frame rate of approximately 1 Hz, and is therefore suitable for studies of quasistatic muscle processes, such as slow or sustained contractions.[57] Measurements in muscle vary with transducer orientation with respect to the muscle fascicles, and anisotropic measurements are typically not available. It has only moderate repeatability and reliability in muscle applications.[81]

Transient elastography was originally developed to image liver fibrosis, and works by the same principle as shear wave elastography. However, only a single impulse vibration is delivered, and the shear modulus is estimated from the propagating and reflected ultrasound echoes.[10,19] It has been used in a small number of muscle studies to provide an estimate of "muscle hardness". One example is estimating muscle stiffness changes during passive stretching.[56] It provides rapid results, but is essentially a one-dimensional technique, so is prone to bias as a result of device placement and angulation with respect to the muscle fascicles. Each measurement is acquired over 50–100 ms,[66] so that, in principle, near real-time acquisition is possible, but in practice, a single dataset requires 8–10 repetitions to provide a reliable result, so that measurements require

8–10 min to perform.[1] Reproducibility is limited,[56] limiting its utility for robust measurements in both clinical and scientific rehabilitation settings.

Magnetic Resonance Elastography

The most common alternative to ultrasound elastography is Magnetic Resonance Elastography (MRE), which is a magnetic resonance imaging technique that works by measuring the shear wave propagation through soft tissues.[53] The shear wave is generated by an MR-compatible mechanical transducer that is synchronized to the MR image acquisition. The displacement data are captured by using motion sensitive gradients in the imaging sequence. MRE can acquire displacement data throughout cyclic deformations in three orthogonal directions. The data can then be used to numerically solve the wave equations (Eqs. 1, 2) to provide estimates of the linear viscoelastic properties of tissues. Early studies focused on the elastic properties only, but more recent studies have also been able to estimate the viscous properties of soft tissues using this technique. The majority of MRE studies obtain data at a single vibration frequency, but there is increasing interest in obtaining more information by measuring the responses at multiple frequencies, e.g., Bensamoun et al.[36] This can be done by repeating measurements at different frequencies, or by using an input vibration stimulus consisting of multiplexed single frequency inputs[23] and using post-processing to extract the response at each of the component frequencies.

For applications in muscle mechanics, a few different approaches to generating the vibration have been used. One approach is to use a pneumatic driver secured around the muscle of interest.[69] While this is easy to use and is reasonably free of transducer-associated imaging artefacts, there are two key limitations. The first is that to achieve good wave transmission into the tissue the transducer must be place firmly on the muscle, and this tends to deform the muscle locally, and can alter the local mechanical properties, due to muscle's inherent nonlinearity. That is, as muscle is compressed, its stiffness increases. The second is that the pneumatic technique makes it more difficult to achieve tight control over the input waveform, making multi-frequency measurements substantially more difficult. An alternative approach is to use an electromechanical driver that creates vibration by coupling a coil carrying current synchronized to the imaging gradients to the primary (B0) field of the MRI. In studies of the lower limb in our group, we apply this technique, coupling the transducer to the tibia, which then transmits vibration into the muscles of the calf without compressing them.[27,28] The primary disadvantages of this method are a more complex setup to ensure that any stray magnetic field from the transducer does not impinge on the area of interest, and the need to carefully control the electrical components for safety. This method is amenable to multi-frequency measurements. Other approaches place drivers outside the MRI fringe field and use mechanical linkages to connect to the subject.

Thus far, MRE has successfully been used to measure the shear modulus of a range of tissues in vivo, including muscle,[5,24,27,28] breast,[68,74] brain[26,31,49] and liver,[33,34] in both healthy and clinical populations.

Methods to measure anisotropy in soft tissues using MRE have been recently developed[64] and applied to muscle tissue. Most of these methods assume transverse isotropy of the shear moduli to simplify parameter estimation (see Eq. 3). The earliest method used numerical optimization in the inversion process to identify symmetry axes, and solved a transversely isotropic version of the linear viscoelastic wave equation, to estimate the shear moduli parallel and perpendicular to the material symmetry axis (in this case, aligned with the z-coordinate axis).

$$-\rho\partial_t^2 \mathbf{u} \cong \mu_\perp \nabla^2 \mathbf{u} + (\lambda + \mu_\perp)\nabla(\nabla \cdot \mathbf{u})$$
$$+ (\mu_{//} - \mu_\perp)\begin{pmatrix} \frac{\partial^2 u_x}{\partial_z^2} + \frac{\partial^2 u_z}{\partial_x \partial_z} \\ \frac{\partial^2 u_y}{\partial_z^2} + \frac{\partial^2 u_z}{\partial_y \partial_z} \\ \nabla^2 u_z + \partial_z(\nabla \cdot \mathbf{u}) \end{pmatrix} + \zeta\partial_t\nabla^2\mathbf{u} \quad (3)$$

$\mu_\perp$ and $\mu_{//}$ are the elastic components of the shear modulus perpendicular and parallel to the axis of symmetry respectively. Other symbols are the same as in Eq. 1.

We have recently developed and validated a method that uses diffusion tensor imaging (DTI) to identify the tissue structural symmetry axis, and combined this with numerical solution of the anisotropic wave equation to estimate tissue anisotropy. The degree of mechanical anisotropy can be quantified by the ratio of the shear moduli parallel and perpendicular to the symmetry axis. We have applied this to healthy human calf muscles[27] (See Fig. 3), and have demonstrated its potential utility in assessing muscle degenerative conditions (see "Muscle Degeneration" section).

Another open challenge for elastography techniques, both ultrasound and MR-based, is that they are generally not able to measure large-deformation tissue mechanics, due to both the magnitude of the vibration stimulus (usually in the order of tens of microns) and the theory used to estimate the shear modulus. This is because elastography methods rely on the theory of wave propagation through a linear viscoelastic medium, and are thus limited to small deformation (linear viscoelastic regime). For most soft

tissues this is $\ll 1\%$ strain. However, new developments are beginning to address this challenge. For example, research is currently underway in our group to superimpose MRE measurements on static large deformations of lower limb muscles to estimate the large-deformation, anisotropic properties of human muscles *in vivo*. Tissue quasistatic deformations can be measured using a range of MRI techniques, including tagging methods.[11,59,80] Proof of principle has been demonstrated in isotropic tissues.[13]

Non-elastographic Methods

In addition to ultrasound and magnetic resonance elastography, other techniques have been used to measure mechanical properties of muscles. For example, myotonometry estimates mechanical properties by applying an impulsive compressive load to the tissue, and measuring the resulting muscle tissue motion, which is a damped free oscillatory decay. The oscillation frequency and the decay constant are interpreted as reflecting elastic and viscous components of the muscle mechanical properties.[73] A handheld device based on these concepts (the Myoton-3) has been shown to provide reliable measures of muscle 'tone' (stiffness arising from a combination of passive properties and muscle contraction) in controls and various patient groups.[12,41] While it does not provide objective viscoelastic properties that could be used in modeling or rehabilitation engineering design applications, myotonometry has been used clinically for assessing spasticity and tissue compliance in patient groups with a range of neuromuscular disorders, e.g., Leonard *et al.*,[42] Marusiak *et al.*,[48] and Rydahl and Brouwer.[65]

Table 1 summarizes the key features of these different methods for measuring muscle mechanical properties *in vivo*.
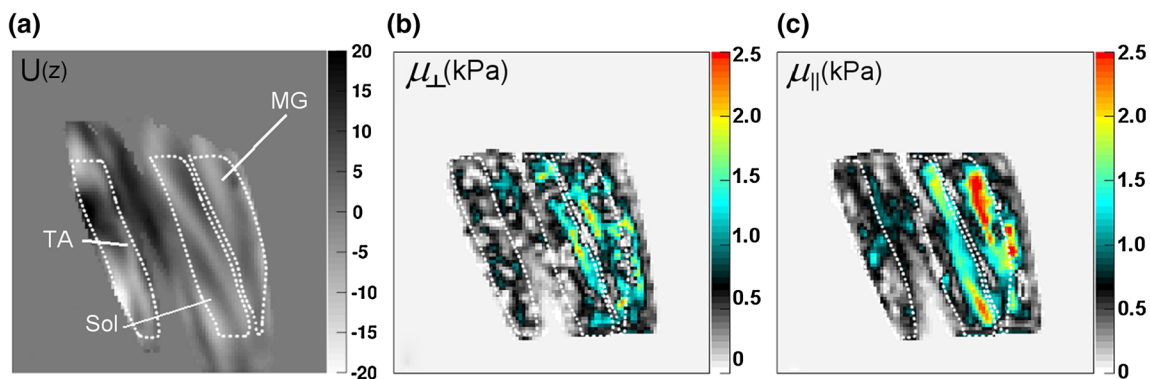

FIGURE 3. Anisotropic lower limb muscle MRE measurements from Green *et al.*[27] used with permission. (a) Wave image data, showing the three muscle regions of interest (tibialis anterior, TA; medial gastrocnemius, MG; and soleus, Sol) outlined in white dashed line. (b) Shear modulus perpendicular to the direction of the muscle fascicles as identified by diffusion tensor imaging. (c) Shear modulus parallel to the direction of the muscle fascicles as identified by diffusion tensor imaging.

TABLE 1. Comparison of different methods for measuring *in vivo* muscle mechanical properties.

	MRE	Shear wave ultrasound	Strain ultrasound elastography	Myotonometry
Temporal resolution (approximate)	Minutes	Seconds	Real-time	Seconds
Reliability testing done	No	Yes[32]	Yes[1,56,81]	Yes[12,41,65]
Limitations	Limited to linear viscoelastic region	Limited to linear viscoelastic region	Assumes tissue isotropy and limited to superficial tissue	Unsuitable for deep muscles
Cost	Very high	Moderate	Moderate	Low
Relevant references	5,11,13,23,24,26–28,31,33,34, 36,49,53,59,64,68,69,74,80	32,35,38	1,6,10,17–20,40,46,54–57,66,67,78,81	12,41,42,48,65,73
Conditions studied	Muscular dystrophy,[63] hyperthyroid patients,[4] obstructive sleep apnoea,[8] delayed onset muscle soreness syndrome[28]	Ankle contracture vs. fascicular stiffness after stroke[16,38,55]	Bethlem myopathy,[17,18] contracture in cerebral palsy,[40] muscle crush injury,[46] delayed onset muscle soreness syndrome[55]	Spasticity and tissue compliance,[12,41,48,65] lingual musculature in obstructive sleep apnoea[79]

TABLE 2. Selected quantitative muscle data from *in vivo* muscle studies.

Reference	Muscle group	Modality	Frequency	Quantitative data	Comments
Qin *et al.*[63]	Lateral gastrocnemius and plantaris	MRE	1000 Hz	Gastrocnemius $G'_{\parallel}/G'_{\perp}$ wild type $= 1.25 \pm 0.17$ $G'_{\parallel}/G'_{\perp}$ MDX $= 0.96 \pm 0.08$ Plantaris $G'_{\parallel}/G'_{\perp}$ wild type $= 1.19 \pm 0.08$ $G'_{\parallel}/G'_{\perp}$ MDX $= 1.20 \pm 0.10$	Anisotropic ratio decreased with muscle necrosis in mdx mice. Anisotropic ratio a potential biomarker to distinguish between healthy and dystrophic muscle
Green *et al.*[27]	Medial gastrocnemius, soleus, tibialis anterior	MRE	60 Hz	Medial gastrocnemius $\mu_{\parallel} = 0.83 \pm 0.22$ kPa $\mu_{\perp} = 0.65 \pm 0.13$ kPa Soleus $\mu_{\parallel} = 0.83 \pm 0.22$ kPa $\mu_{\perp} = 0.65 \pm 0.13$ kPa Tibialis anterior: $\mu_{\parallel} = 0.78 \pm 0.24$ kPa $\mu_{\perp} = 0.66 \pm 0.16$ kPa	Shear modulus measured in the direction parallel is greater than modulus measured perpendicular to the muscle fibers demonstrating importance of accounting for anisotropy in *triceps surae* muscles
Basford *et al.*[3]	Medial gastrocnemius	MRE	150 Hz	Shear modulus of control group $= 16.16 \pm 00.19$ kPa Shear modulus of disease group $= 38.40 \pm 00.77$ kPa	Muscle stiffness was found to increase with load in healthy subjects
Hoang *et al.*[32]	Medial and lateral gastrocnemius	Dynamometry	quasistatic	Standing Stiffness $= 2540 \pm 460$ N/m Sitting Stiffness 530 ± 100 N/m	Reliability of this method found to be better if performed on the same day
Niitsu *et al.*[55]	Elbow flexor muscles	Ultrasound elastography	quasistatic	Mean strain ratio pre-exercise 83.3 ± 2.07; peak 2 days after exercise 50.2 ± 3.29 (a.u.)	After eccentric exercise, muscle strain ratio decreased, indicating stiffening, peaking 2 days after exercise
Gennisson *et al.*[24]	Biceps brachii	Transient elastography	5 MHz	Shear modulus $= 0.92 \pm 0.55$ kPa	A linear relationship was found between the transverse shear moduli and the corresponding maximal myoelectric activity

Table 2 summarizes quantitative muscle properties obtained from studies of *in vivo* muscle mechanical properties, including in selected muscle disorders addressed in the following section.

IN VIVO MUSCLE MEASUREMENTS IN MUSCLE DISORDERS

Many of the techniques outlined above have been used in preclinical or early clinical research, in both human patients and animal models of these disorders, with promising results. Changes in the mechanical properties of skeletal muscle have been found after exercise[28] and in a wide range of metabolic and neuromuscular disorders such as spastic paraplegia, stroke and diabetes.[3,25,30,50] Mechanical property changes could also be a useful indicator for the progression of a disease, such as in muscular dystrophies[63] or pressure ulcers,[45] thus providing useful information on supportive technologies and other treatments. Here, we review the applications of these techniques across a range of disorders affecting muscle tissues.

Muscle Degeneration

Degenerative muscle disorders cause changes in muscle structure, fatty infiltration, and atrophy.[29,60,72] Current methods of tracking such changes in patients are somewhat limited, and typically clinical functional tests are used. However, early signs of disease progression are often missed by such tests. While it has long been thought that muscle degeneration is accompanied by mechanical changes,[14] attempts to quantify these changes have only been successful more recently. Drakonaki and Allen,[17] using strain elastography to examine the thigh muscles of a single Bethlem myopathy patient. They reported that affected areas of the muscle (based on traditional ultrasound and MRI images) were stiffer than surrounding healthy muscle tissue.[17] More recently, Qin *et al.*,[63] showed that mechanical anisotropy (the ratio of the shear modulus parallel and perpendicular to the muscle fiber axis, see above for details of the theory underpinning this method) is altered even in mildly affected muscles in a rodent model of muscular dystrophy, the mdx mouse.[63] Moreover, this method was able to detect

degeneration that was not apparent on anatomical or diffusion-weighted images, indicating that this has potential for tracking early disease progression. The mechanical anisotropy was correlated with muscle necrosis and reflected changes in the structural arrangement of muscle fascicles in the degenerating muscle. It remains to be seen whether the technique is equally useful in human muscular dystrophy patients.

Neuromuscular Disorders

Neurological disorders such as stroke, spinal cord injury and cerebral palsy often cause contractures (loss of passive joint range of motion). The mechanisms of con-tracture, which are a major source of disability for patients with neuromuscular disorders, are still not known.[44] However, these muscle–tendon units appear stiff, and it has long been hypothesized that the shortened muscle–tendon unit length is accompanied by an increased stiffness in the muscle and/or tendons across the affected joint. Relatively few studies have quantified mechanical properties of the muscles in these conditions. However, using a combination of ultrasound and dyna-mometry, it has been shown that in stroke and spinal cord injury patients with contractures, the length of the gas-trocnemius was reduced and the fascicular stiffness was increased in patients compared to controls across the range of motion.[16,22,38] More specifically, the lower limb
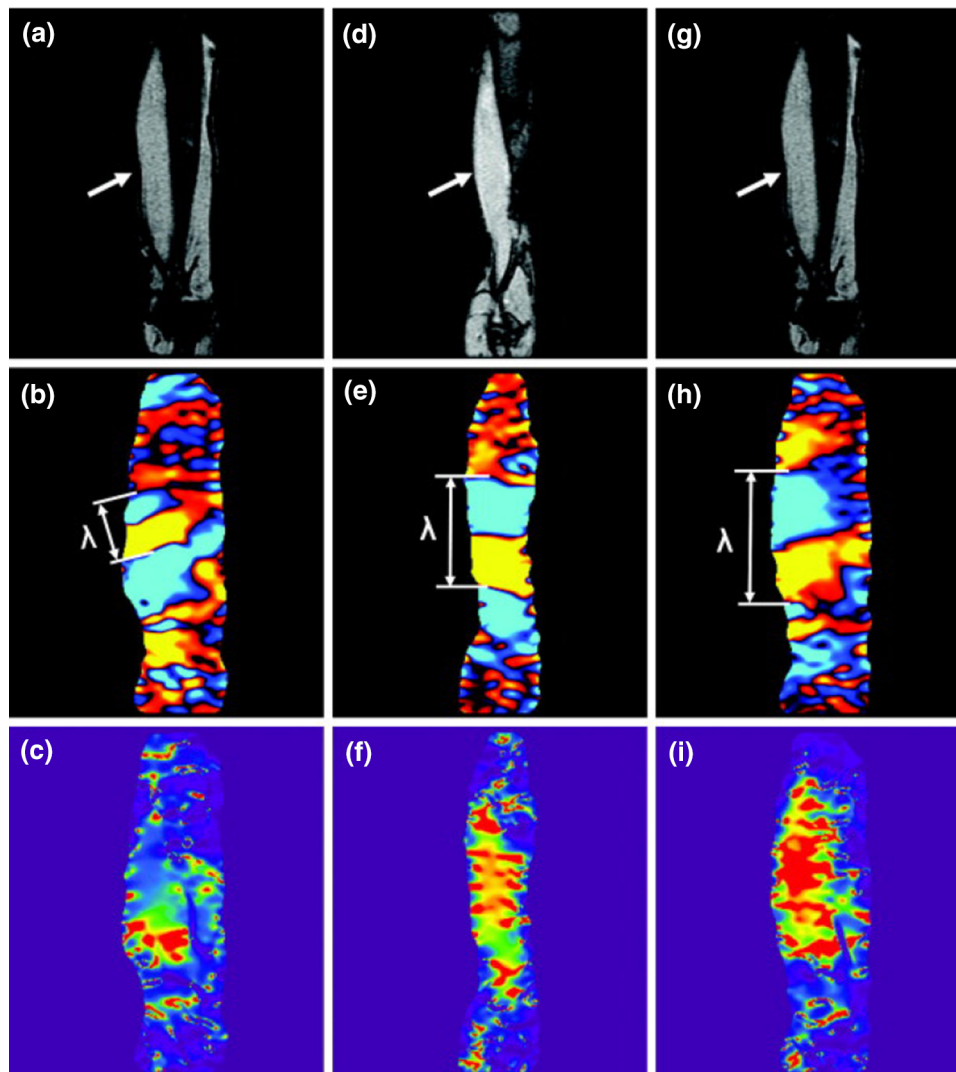

FIGURE 4. Sample MR image (upper row, a, d, g), shear wave image (middle row, b, e, h) and elastogram (bottom row, c, f, i) for the biceps muscle in a human volunteer. Right column (g, h, i) are from an arm that was immobilized for X. The left column (a, b, c) images are from the contralateral limb when the position of the arm was matched to the experimental limb after immobilization. The middle column (d, e, f) images are from the contralateral limb when the joint torque at the elbow was matched to the experimental limb. λ: wave length. Longer wavelengths indicate higher stiffness (depicted as warmer colors in elastograms in the bottom row). Note that the proximal (upper) part of the biceps muscle in the limb that had been immobilized is stiffer than the contralateral limb (used with permission from Muraki *et al.*[52]).
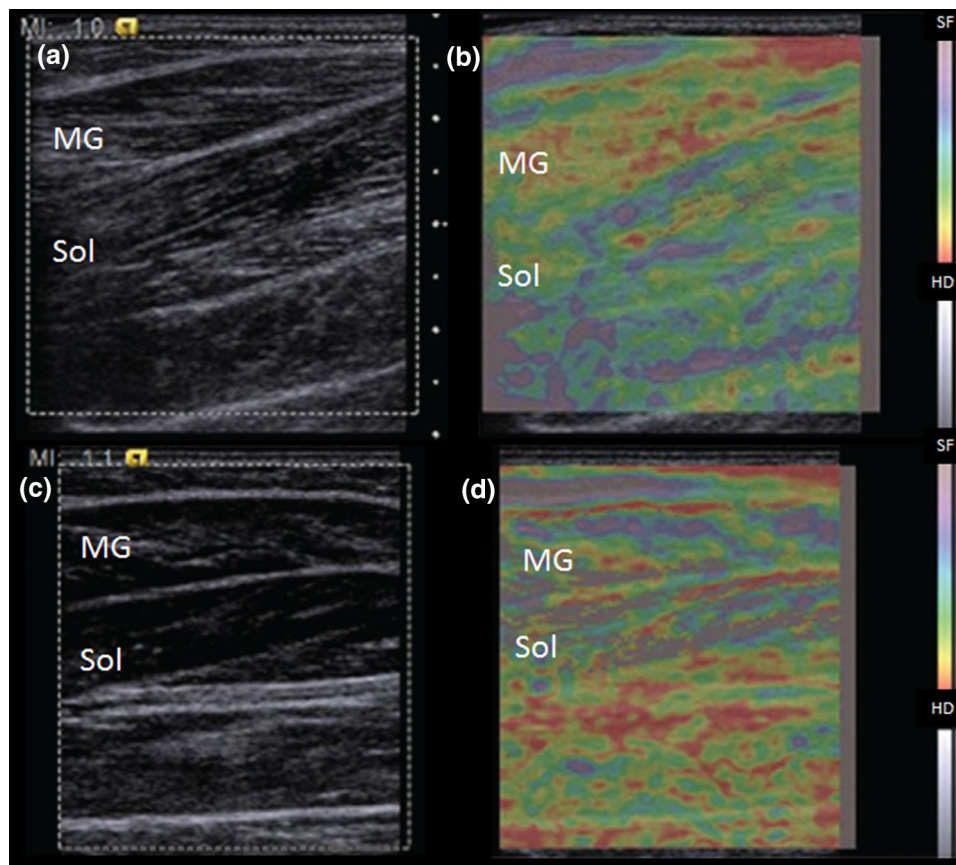
FIGURE 5. Sample B-mode ultrasound images (left column, a, c) and shear wave elastography images (right column, b, d) from the lower leg of a child with spastic cerebral palsy (upper row, a, b) and a healthy control (lower row, c, d). MG, medial gastrocnemius; Sol, soleus muscle. In the elastograms, the warmer colors indicate stiffer tissue (HD), and the cooler colors (blue and purple) indicate softer tissue (SF). Note that the medial gastrocnemius in the cerebral palsy patient appears stiffer (warmer colors) than in the healthy control subject. Adapted from Kwon *et al.*[40] used with permission.

muscles of spinal cord injured patients with contractures have been studied using dynamometry combined with ultrasound imaging.[15,16] Subjects who have had a limb immobilized also can develop contractures. Patients with contractures were shown to have substantially stiffer (~50%) gastrocnemius muscle–tendon units than control subjects. These changes were well established at 3 months after injury and did not change between 3 and 12 months.[15] In cerebral palsy patients with contracture, similar methods demonstrated that increased joint stiffness was accompanied by a reduction in muscle fascicle elongation, indicating an increased stiffness in the muscle–tendon unit. One study of cerebral palsy patients using strain elastography and ARFI shear wave elastography found that both techniques indicated an increase in gastrocnemius stiffness in cerebral palsy patients with contracture compared to age matched healthy controls,[40] however the leg positions were not reported, so it is not known whether there was any bias introduced by different muscle lengths (arising from the nonlinear muscle length–tension properties). Figure 5 shows some sample data from that study. The same research group has shown that Botulinum A toxin injection (used to treat spasticity in cerebral palsy patients) reduced muscle stiffness[39] in one patient, although other studies have seen no change in stiffness using passive ankle flexion measurements over 10° of ankle dorsiflexion.[2] Muraki *et al.*[52] showed in an experimental study of dog forelimb immobilization that the biceps muscle is stiffer after 6 weeks of immobilization, using MR elastography (See Fig. 4). There is clearly additional research needed to determine the best protocols and methods for tracking the full range of nonlinear muscle property changes in neuromuscular disorders such as these.

Metabolic and Other Systemic Disorders

Muscle mechanics can also be affected by metabolic and other systemic disorders. Hyperthyroid patients are thought to have weak muscles. One MRE study found that patients prior to treatment had significantly less stiff thigh muscles than healthy controls.[4] The

stiffness returned to normal after treatment for the thyroid condition.

Obstructive sleep apnoea (OSA) is a common respiratory condition associated with increased collapsibility of the upper airway during sleep. Repeated airway collapses result in regular arousals, daytime sleepiness leading to increased risk of accidents, and cardiovascular sequelae. The mechanical behavior of the upper airway muscles, particularly the tongue and airway lateral wall dilator muscles, is thought to contribute to the increased risk of airway collapse, especially in obese individuals. Veldi et al.[79] used myotonometry to estimate tongue stiffness, and showed that despite reports of increased muscle activity in the tongue during wakefulness,[51] the tongues of OSA patients were less stiff than healthy control subjects. More recently, Brown et al.,[9] using MRE, reported that OSA patients have tongues that had an approximately 10% lower shear modulus than age and BMI matched healthy controls. Moreover, by using anisotropic analysis of the MRE data, they showed that the change in stiffness occurred in the direction of the muscle fascicles in the genioglossus. These studies suggest that there is potential for non-invasive measurements of muscle mechanical properties to provide insight into other disease processes affecting muscles.

Injury

Increased muscle stiffness is often reported after eccentric exercise, as part of the "Delayed Onset Muscle Soreness" syndrome. This has been studied in vivo using MRE by Green et al.,[28] who showed that 48 h after a bout of eccentric exercise induced by walking backwards on a treadmill, the calf muscles of human volunteers had a shear modulus that was an average of 21% higher than that pre-exercise. The shear modulus returned to pre-exercise levels by 7 days after exercise.

Muscle crush injury has been studied using supersonic shear imaging[46] over the first 72 h after injury in a rabbit model. The injured muscle region was significantly stiffer than the surrounding uninjured tissue at all time points. The technique was able to distinguish between injured and uninjured regions, with an area under the receiver-operator curve of >0.9 up to 2 h after injury.

FUTURE DIRECTIONS

To date, in vivo muscle mechanical measurements have been primarily part of preclinical evaluation studies in small study groups. Only a small number of studies have examined reliability and reproducibility of the methods, and few studies have been replicated in larger and/or independent populations. Moreover, the utility of these measures in making clinical decisions for an individual patient, including for assessment of rehabilitation strategies and treatment response, requires quantification of the sensitivity and specificity of the measures. Such studies are essential before such measures can be deployed routinely in the clinic. Moreover, many of the techniques described in this review are currently implemented in research settings only, and equipment and techniques need further refinement for practical clinical workflows. However, some of the existing data are likely to be useful for rehabilitation engineers by giving some indication of the likely changes in muscle mechanics in patients with a range of muscle disorders.

There are also several areas where technical improvements in the methods would be beneficial. This includes improved temporal resolution of many elastography techniques, in order to open up the possibility of dynamic (or even real time) quantitative elastography. MR elastography datasets typically require a few minutes to collect, and even in shear wave elastography, the temporal resolution is of the order of seconds, limiting the capacity to study dynamic processes, or to capture the nonlinear behavior of a muscle across its full length range. This is particularly relevant for muscles that undergo cyclic contraction, such as cardiac and respiratory muscles. Spatial resolution improvements might also allow more localized measurements of mechanical properties. Currently, MR elastography in particular is limited to providing regional data, due to a trade-off between better wave penetration (low frequencies) and spatial discrimination (at higher frequencies). While average muscle properties, and even regions of focal damage of the order of centimeters can be detected, smaller lesions and gradations in mechanical properties associated with focal loading are currently not reliably detected, or require long scan times. The ability to measure large deformation properties is also currently limited. While dynamometry-based methods can characterize the whole length–tension curve of some muscle groups (e.g., Hoang et al.,[32] Kwon et al.[70]), most elastography measurements are conducted at a single muscle length and reflect the local shear modulus at that particular muscle length. Although there is promising research using multiple elastography measurements to estimate the shear modulus in muscles across a range of muscle lengths,[47] this requires further development to properly characterize the anisotropic nonlinear viscoelastic properties in a robust and repeatable manner.

Significant challenges exist for studying active muscle tissue also, including how to characterize the combined effects of muscle contraction and nonlinear elasticity, both of which increase the apparent stiffness of muscles. In neuromuscular disorders, where muscle structural changes such as contracture may be accompanied by spasticity, this is particularly important. Applications to heart muscle are similarly challenging.

CONCLUSIONS

In vivo methods for measuring muscle mechanical properties are developing rapidly, but there are still significant challenges to overcome for these techniques to become easy, fast, accessible and affordable in the clinical setting. However, these techniques clearly have the potential to be useful in the context of rehabilitation engineering, opening up the possibility of making patient-specific measures of muscle mechanics and function to assist in tailoring rehabilitation equipment, and also for monitoring treatment.

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