Contemporary image-based methods for measuring passive mechanical properties of skeletal muscles in vivo

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Authors: Lynne E Bilston[1,2], Bart Bolsterlee[1,3], Antoine Nordez[4,5], Shantanu Sinha[6]

Affiliations:

1. Neuroscience Research Australia, Randwick, Sydney, NSW 2031, Australia
2. Prince of Wales Clinical School UNSW Medicine, Randwick, Sydney, NSW 2031, Australia
3. Graduate School of Biomedical Engineering, UNSW, Kensington, NSW 2052, Australia
4. Health and Rehabilitation Research Institute, Auckland University of Technology, Auckland, New Zealand.
5. Movement, Interactions, Performance Laboratory (EA 4334), Faculty of Sport Sciences, University of Nantes, Nantes, France.
6. Muscle Imaging and Modeling Lab, Department of Radiology, University of California San Diego, San Diego, CA-92121, USA.

Corresponding author: Professor Lynne Bilston,
Neuroscience Research Australia
Barker St, Randwick, NSW 2031, Australia
Email: L.Bilston@neura.edu.au

BB, AN, and SS contributed equally to this manuscript.

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Abstract

Skeletal muscles' primary function in the body is mechanical – to move and stabilize the skeleton. As such, their mechanical behavior is a key aspect of their physiology. Recent developments in medical imaging technology have enabled quantitative studies of passive muscle mechanics, ranging from measurements of intrinsic muscle mechanical properties, such as elasticity and viscosity, to 3D muscle architecture and dynamic muscle deformation and kinematics. In this review we summarize the principles and applications of contemporary imaging methods that have been used to study the passive mechanical behaviour of skeletal muscles. Elastography measurements can provide in vivo maps of passive muscle mechanical parameters, and both MRI and ultrasound methods are available (magnetic resonance elastography and ultrasound shear wave elastography, respectively). Both have been shown to differentiate between healthy muscle and muscles affected by a broad range of clinical conditions. Detailed muscle architecture can now be depicted using diffusion tensor imaging, which is particularly useful for computational modeling of muscle, but also has potential in assessing architectural changes in muscle disorders. More dynamic information about muscle mechanics can be obtained using a range of dynamic magnetic resonance imaging methods, which characterize the detailed internal muscle deformations during motion. There are several MRI techniques available (e.g. Phase-contrast MRI, Displacement encoded MRI, and 'tagged' MRI), each of which can be collected in synchrony with muscle motion, and post-processed to quantify muscle deformation. Together, these modern imaging techniques can characterize muscle motion, deformation, mechanical properties and architecture, providing complementary insights into skeletal muscle function.

Introduction

As the primary actuator in the human body, research aimed at understanding the mechanical behavior of skeletal muscles has been performed for more than a century (e.g. 14). From this wealth of research, it is now clear that, even when passive, skeletal muscles have very complex mechanical behavior, and are anisotropic, nonlinearly viscoelastic tissues (e.g. 34, 87, 88). Skeletal muscle's anisotropy (different mechanical behavior in different directions) arises from its anisotropic microstructure, with the parallel myofibers arranged into fascicles. Nonlinear viscoelastic behavior in skeletal muscle can be observed from an increasing apparent 'stiffness' as muscles are stretched (i.e. nonlinear elasticity) and reduction in the force in a stretched passive muscle over time (i.e. viscoelastic relaxation). This behavior is thought to arise from similar sources as in other soft tissues, namely the inherent nonlinear viscoelasticity of constituents, interactions between these constituents, and progressive recruitment of these constituents under load.

While the focus of this mini-review is the passive properties of skeletal muscle, contractions alter the mechanical properties of muscle. Active muscle properties are beyond the scope of this review. However, muscle contractions can also alter the subsequent passive properties in some circumstances. For example, a muscle contraction can induce a small amount of thixotropy post-contraction, where a muscle appears stiffer until an apparent 'yield stress' is overcome (71) during subsequent passive elongation of the muscle.

In the last 30 years, researchers' attention has turned to methods to make measurements of muscle mechanics *in vivo*, as these are thought to better represent 'real world' conditions, and recently, imaging technology has begun to enable in vivo measurements. However, the complex mechanical behavior of muscles gives rise to several practical and methodological challenges for research in human muscle mechanics, particularly for the image-based methods that we focus on here. Many of these are discussed in more detail throughout this review, but they include the need to carefully control the deformation or loading state of a muscle and ensuring that a muscle is truly passive during measurements, as both make substantial differences to the mechanical parameters obtained.

This review outlines four key image-based methods that have been used to quantify skeletal muscle mechanics, and aims to provide critical evaluation of their strengths and weaknesses, and applications in both healthy subjects and patient groups. The first of these is magnetic resonance elastography (MRE), which relies on transmission of an external vibration to the muscle, using MRI to measure the resulting propagation of displacement waves in the tissue. Maps of shear modulus are then calculated. The second, ultrasound shear wave elastography, is based on similar principles, but uses ultrasound rather than MRI. Both these methods rely on the physics of wave propagation through elastic or viscoelastic media, where wave speed is related to the mechanical properties of the material, with waves traveling faster through stiffer material. In strain-based ultrasound elastography, the effect of the sonographer compressing the tissue with the transducer is used to create qualitative stiffness maps, relying on the principle that stiffer tissue regions will deform less than softer regions. We focus here on quantitative methods and will not further discuss strain elastography. Thirdly, diffusion tensor imaging gives insight into the microstructure of the muscle, by quantifying the diffusion of water molecules within the tissue, and relies on this being different along the direction of muscle fascicles than perpendicular to it.

Most of these methods provide quasistatic measurements of mechanical behavior as they require seconds to minutes to acquire, although some ultrasound shear wave elastography implementations can provide more rapid measurements. The fourth approach, dynamic imaging, aims to characterize the deformation of muscle during motion, and can be either ultrasound or MRI-based. Finally, imaging is often combined with other biomechanical measurements, such as the combination of ultrasound with dynamometry to measure muscle deformation to match with joint torques (e.g. 42), to give a picture of the mechanical behavior of muscle. These latter methods typically do not yield the intrinsic mechanical properties (e.g. shear modulus), of muscle and their variation across a muscle, but do provide whole muscle length-tension or fascicle-tension curves.

Magnetic Resonance Elastography of skeletal muscle

Methods. MRE of skeletal muscle involves applying an external sinusoidal vibration at a given frequency that is synchronized to a displacement-sensitive magnetic resonance imaging acquisition to the muscle. By varying the temporal offsets between the mechanical vibration and the imaging acquisition, the displacement of each pixel in the image domain throughout a vibration cycle is obtained. These data are then post-processed to calculate the shear modulus at each point in the tissue of interest, a measure of the resistance of the tissue to shear deformation. This post processing typically involves filtering the data, then applying an inversion algorithm that either solves the linear viscoelastic wave equation, or a simplified version thereof. A more detailed review of the technical details of MRE in muscle can be found in (8). Figure 1 shows a typical MRE setup, displacement wave data at 60Hz, and a calculated shear modulus elastogram from a healthy adult male volunteer.

<insert Figure 1 about here>

The majority of MRE studies of muscle properties assume that muscle is isotropic (e.g. 25, 39, 48, 75, 85), and thus the reported values represent an 'average' in all directions, and may not be as sensitive to physiologically relevant differences in muscle mechanical properties, such as those that may affect the muscle fiber direction more than the cross-fiber direction (e.g. 15). More recently, anisotropic MRE methods have been developed (e.g. 74) that consider muscle to be a transversely isotropic material. These methods have been used to study both healthy and damaged or disease-affected muscle (e.g. 38, 73) as discussed below. In order to extract anisotropic muscle properties, the most robust methods require knowledge of the muscle fascicle directions, which can be obtained from diffusion imaging (see the section devoted to this technique below). These directions, together with the displacement data, can be used to estimate the anisotropic elastic component of the shear modulus both along the muscle fascicle direction and perpendicular to it, as well as an estimate of the viscous behavior (e.g. 74).

While MRE can be used to measure both the elastic and the viscous properties of muscle and other tissues, many early studies reported only the elastic properties (e.g. 4, 6, 13, 18, 41). Multi-frequency MRE performs measurements using multiple vibration frequencies, which can give insight into the strain-rate sensitive viscoelastic behavior of muscle in order to assess the viscous nature of the tissue (e.g. 19).

All of the above methods provide only the linear viscoelastic properties, i.e. those at very small deformations. Physiologically, we are typically interested in the nonlinear behavior over the full

range of physiological deformations that a muscle undergoes. There is one recent study that has attempted to measure the nonlinear behavior of skeletal muscle, by conducting MRE at a range of quasistatic stretch levels (84). Another recent study has observed increased shear moduli when muscle is indented in order to induce damage (68). Further work is required to embed such experimental approaches in a robust theoretical framework that considers both muscle anisotropy, and the complexity of the nonlinear mechanics of muscle.

Critical Evaluation. The major strength of MRE is its capacity to provide 3D 'maps' of muscle viscoelastic properties in vivo, including variation in properties between muscles and within a muscle. With further development to reduce the impact of some of the current limitations, it has the potential to provide useful physiological and clinical information in skeletal muscle applications.

Many of the limitations of MRE are not specific to its application to muscle. These include a lack of standardization of either the imaging or the analysis methods, with different transducer types, vibration frequencies, and MR imaging sequences used. Analysis methods also remain a critical challenge for the field, as the calculation of the shear modulus from the image data is a numerically ill-conditioned problem, and is both sensitive to noise in the images, and low displacement wave amplitude (due to attenuation of the propagating vibration). Also arising from the numerical methods used is a limited spatial resolution, since at least 2, and often 4, adjacent pixels are used to calculate the shear moduli in any given pixel. These issues also apply to anisotropic MRE, and development of robust analysis methods for anisotropic tissues are still an open challenge.

Anisotropic MRE has been partially validated in muscle, via comparison of anisotropy measures against both phantoms and ex vivo muscle tissue (74), although quantitative comparisons of the anisotropic shear moduli and viscous components are few and far between, in part because of the differences in loading rate achievable with rheometers and MRE. As result of this wide variation in methods, and the use of relatively small samples in many studies, reported results can differ between research laboratories. Another key concern is that due to the nonlinear mechanical behavior of muscle, the loading state of the muscle can strongly affect the measured properties (68, 84). To compare results, it therefore is important to standardize the muscle passive tension, perhaps by ensuring that the muscle is completely slack, and also to ensure that the transducer does not load the muscle.

There is very limited data that suggests that the vibration needed for MRE does not invoke muscle activity in the tongue (15, 20), and some studies show that mild muscle contraction increases the measured shear moduli in MRE experiments (e.g. 41, 48), suggesting MRE may be sensitive to contraction-induced stiffness changes. The latter could mean that MRE could be useful for measuring stiffness changes due to low level contractions, but also means that if a muscle is not completely passive during an MRE measurement, this could introduction bias in the reported stiffness. Finally, MRE measurements take a few minutes to perform. It is thus not practical for dynamic studies, and is challenging to use for muscle contraction studies, as only low level contractions can be held constant for the duration of the acquisition, or repeated contractions must be used and data acquisition 'gated' to the contractions. For anisotropic MRE that also requires diffusion measurements to identify the muscle fiber directions, this adds another few minutes to each acquisition, further constraining its use to static measurements.

Applications. MRE has demonstrated potential for measuring mechanical properties in vivo, and results to date suggest that it has potential to be a useful clinical tool for tracking muscle degeneration (73), injury (39, 43), and the effects of hormonal and other disorders on muscles e.g. (13, 15, 63, 66). MRE has been applied in mostly small studies of clinical populations and in animal models of disease. One study showed that changes in the stiffness of the muscles of hypogonadal men could be detected using MRE (13). A small study of hyperthyroid patients showed lower stiffness before treatment, with muscle properties returning to control values after treatment (5). Green et al (39) showed that muscle stiffness followed the same time course as pain symptoms after eccentric muscle damage. Brown et al (15) studied obstructive sleep apnea patients, reporting lower tongue stiffness in the direction of the genioglossus muscle fibers than in matched healthy controls, consistent with greater upper airway collapsibility in patients. In a mouse model of muscular dystrophy, Qin et al (73) found that the mechanical anisotropy was a sensitive marker of muscle necrosis, detecting changes earlier than diffusion imaging. McCulloch et al used MRE in myositis patients, finding that relaxed thigh muscles of patients have significantly lower shear modulus than controls (63). See also (50) for a review of pediatric applications.

Ultrasound shear wave elastography

Methods. As for MRE, ultrasound shear wave elastography (SWE) consists of applying a mechanical perturbation to induce the propagation of a shear wave. The different ultrasound SWE techniques have been described previously in detail (e.g. 35, 78) and some are available in commercial ultrasound scanners (e.g. Supersonic Imagine, Siemens, Toshiba). Briefly, the mechanical perturbation can be applied using an external mechanical actuator or remotely with ultrasound. Ultrasound is then used to track the shear wave propagation. Since ultrasound can provide a high temporal resolution (frame rate up to 20 kHz), it is possible to induce the shear wave propagation with a transient excitation (7). A transient excitation would provide an easier tracking of the shear wave because boundary conditions are less problematic (35). In addition, a transient excitation enables us to analyze the shear wave propagation as a function of the frequency (i.e. shear wave spectroscopy, (36)). Shear wave spectroscopy can be used to account for the viscosity (36) and the guided wave propagation that occurs in stiff and thin tissues (16) .

Critical evaluation. A previous study showed a good correlation (R^2=0.94) between the Young's modulus measured using a material testing machine during passive stretching of an animal muscle and the shear modulus measured using ultrasound SWE (27). It validated the shear modulus measurement as a relevant outcome for measuring muscle mechanical properties. In addition several studies showed that this technique is reliable at rest (e.g. 54) and during passive muscle stretching (e.g. (58), Figure 2). In comparison to MRE, this technique is relatively low cost and relatively easy to access. While spatial resolution (i.e. pixel size) in ultrasound SWE can be slightly better than for MRE, both require averaging over many pixels for reliable results. In addition, the measurement can be performed in less than 100 ms (7). Thus, the temporal resolution, which can reach up to 1-4 Hz, can enable tracking of the changes in muscle mechanical behavior during contractions and/or passive stretching in order to analyze the non-linear behavior of muscle. It is also easy to use in a biomechanics lab and to synchronize with other measurements (e.g. force and/or motion).

<insert Figure 2 about here>

As with all ultrasound measurements, ultrasound SWE requires appropriate practice to account for probe location, orientation and pressure. The practice is completed when the experimenter is able to perform reliable measurements (intra day or between days depending on the experimental design). In addition, it measures the shear wave velocity in 2D, providing an incomplete picture of the muscle mechanical behavior. Anisotropy can be easily measured in fusiform muscles because in that case the shear wave propagation can be analyzed with respect to the direction of muscle fibers (36). Because it is impossible to measure the shear wave velocity along the muscle fiber direction of pennate muscles (i.e. the large majority of human muscles), anisotropy is more problematic here than for fusiform muscles. In the case of a pennate muscle, we recommend performing the measurement along the shortening/lengthening axis of the muscle (27). Thus, the measurement reflects the muscle behavior that actually influences the motion of the joint, exactly as it can be done in an isolated muscle using a material testing machine (27).

A recent study shows the feasibility of performing 3D measurements in the muscle using matrix probes (37). In the future, this innovative technique will provide a better understanding of 3D muscle behavior during stretching and contractions. In addition, measurements of muscle viscosity would provide a deeper understanding of muscle behavior and could become a new relevant clinical outcome in several diseases.

Applications. In the last couple of years, there has been an explosion of publications using SWE to quantify human muscle behaviour, possibly thanks to commercialised devices that are accessible in many fields of research (biomechanics, sport sciences, physiotherapy, medicine). Thus the applications are very numerous and cannot be exhaustively reviewed here. The first field of application is the evaluation of muscle in diseases that increase muscle stiffness such as Duchenne Muscular Dystrophy (53) or contractures induced by stroke (26, 57). Interestingly , Le Sant et al. (56) have shown that it is possible to map the shear modulus of plantar flexors during a passive dorsiflexion, and thus provide a better understanding of their contributions to the passive stiffness at the joint level. This protocol could be relevant for the evaluation of contracture in a muscle group. Secondly, Hug et al. (46) showed that changes in muscle shear modulus are linearly related to the changes in both passive and active muscle force. This result was used to measure the muscle and tendon slack length (45), to analyze the acute effects of stretching (67), warm-up (65), massage (29), or eccentric exercise that induces muscle damage (55).

Dynamic MRI methods

Methods: The objective quantification of regional muscle deformation is a valuable clinical tool to evaluate normal and diseased muscle. Magnetic Resonance Imaging has been used, in muscle tissue, to quantify either displacement or velocity in a single 2D slice, with some of the earliest development of the cine phase-contrast technique reported in (3), with the quantification of the motion of the rectus femoris after tendon transfer surgery(2), and subsequently extended to multi-slice, or 3D volumes (49, 59, 61, 92). Strain and strain rate are kinematic properties that can be derived from the displacement (strain) and velocity (strain and strain rate) encoded magnetic resonance (MR) images and have been used to characterize myocardial, lingual, skeletal deformation (30, 31, 52, 77). Strain describes how the tissue is deformed with respect to a reference state and requires tissue tracking. Strain rate describes the rate of regional

deformation and does not require tracking or a reference state since it is an instantaneous measure. A positive strain or strain rate indicates a local expansion while a negative strain or strain rate indicates a local contraction.

Dynamic Imaging Sequences: There are three motion encoding sequences commonly used to monitor skeletal muscle dynamics: velocity encoded phase contrast (VE-PC)(59), displacement encoded imaging (DENSE)(49), MR tagging where the tagged lines/grid are tracked to quantify strain (28). In addition, HARP analysis of spin tagged lines and direct strain encoding, SENC have been applied to track cardiac motion (52) (91). The sequences are acquired gated to the muscle motion to study muscle tissue deformation during passive and active muscle activation (92) (59) (28). In VE-PC MRI, flow encoding gradients are incorporated into a gradient echo to encode the velocity in the phase; a 3-directional velocity encoding sequence is used to monitor the velocity vector. DENSE MRI directly encodes the displacement as the phase of a voxel but since it is a stimulated echo acquisition, suffers from a loss in SNR by a factor two compared to the VE-PC method. However, DENSE-MRI is useful for encoding displacements over long time intervals compared to T1 relaxation times. MR tagging techniques allow lines or grids to be superposed on the anatomical image; these lines and grids are created by specially designed RF pulses to saturate the protons with desired pattern (Spatial Modulation of Magnetization, SPAMM). The tracking of the tag lines is used to quantify muscle deformation. However, manual or semi-automated tracking is usually tedious and time-consuming. Further limitations of this technique are that tag lines fade due to T1 effects, spacing of the tag lines/grids is much bigger than the voxel resolution and tagging is usually done in 2D rather than in 3D.

Dynamic MR Imaging Hardware: Several groups have developed MR compatible hardware to execute repeated passive or active motion (49, 59, 61, 92) (28). One such foot pedal design has been used in several studies that cover the dynamics of calf muscle under passive plantarflexion, active isometric, plantar and dorsiflexion to evaluate normal, atrophied, and aging muscle (59) (81) (82) (21). In this design, the foot rests on a carbon-fiber plate with an embedded optical force transducer. For passive plantarflexion, the pedal is attached to a piston-cylinder device, controlled by computer-driven servo-motor and connected by tubing to the piston-cylinder. The angular motion of the foot-pedal is monitored by the digital output from the computer, which also generates an R-wave-like trigger pulse used to gate the acquisition.

Analysis: The calculation of strain or strain rate tensor is similar with the former using the displacement vector and the latter using the velocity vector (32) (81). In brief, for VE-PC data, phase images are corrected for phase shading artifacts and denoised with an anisotropic diffusion filter. The 2D spatial gradient of the velocity vector, L is calculated from the spatial gradient of the velocity components v_x *and* v_y. The symmetric part of the strain rate tensor is calculated from $D=0.5(L+L^T)$ and diagonalized. The negative eigenvalue during passive plantarflexion is denoted as SR_{fiber} since its eigenvector is closest to the muscle fiber direction and in the dorsiflexion stage, the negative eigenvalue changes to the orthogonal direction (Figure 3). The maximum shear strain is determined by rotating the 2D-SR tensor in the principal basis by 45^0.

<insert Figure 3 about here>

Critical evaluation. Dynamic imaging is not a real-time technique and relies on the repeatability of the motion. This limits acquisition to single 2D slice imaging. The clinical application of 3D

strain tensors awaits the development of fast 3D, 3-directional encoded sequences; a recent compressed sensing 3D, 3 directionally encoded VE-PC approach which allows acquisition in ~2 minutes shows promise (61).

Applications: Huijing et al showed in cadavers using such MRI techniques, the presence of local strains within calf muscles even when global strain was not evident (47). Yaman et al demonstrated, using high-resolution 3D MRI and calculations of large deformations using non-rigid transformations, significant heterogeneous local strains in all muscles of the lower leg concomitant to global length changes in the gastrocnemius muscle-tendon complex (90). Strain rate tensor imaging of the lower leg was used to study age related differences between younger and older subjects. Under passive plantarflexion, no significant differences with age were seen in strain indices in the medial gastrocnemius; however, regional differences were found (81). Maximum shear strain was shown to correlate with force in the same cohort of young and old subjects (82). Strain rate tensor imaging of disuse atrophy also identified maximum shear strain as a significant predictor of force loss with disuse (59). The authors speculate that the dependence of force on shear strain may be related to the mechanical properties of the extracellular matrix that may get stiffer with age (59) (82).

Recently, the feasibility of volumetric strain mapping in the calf muscles during passive plantarflexion using multi-slice VE-PC imaging was demonstrated which may have application as a surrogate measure of intramuscular pressure (49). DENSE imaging was used to explore the effect of activation and morphology on strain patterns in the hamstring muscles and this study showed that localized elevated strains and a narrow proximal aponeurosis may be risk factors for hamstring injury (32).

Diffusion Imaging

Methods. Diffusion tensor imaging (DTI) is an MRI technique that provides a measurement of the extent and direction of diffusion of water molecules. DTI has long been used to reconstruct the neuroanatomy of the brain. Its application to muscle fibers is rendered more difficult because of the much shorter T2 relaxation times with consequent decrease in signal-to-noise ratio (SNR) compared to the brain. But recently, DTI has been applied successfully to characterize muscle structure in terms of various DTI parameters, such as its fractional anisotropy (a longitudinal to cross-sectional ratio of diffusion within the fiber), primary, secondary and tertiary eigenvectors. The diffusion properties allow the measurement of three-dimensional (3D) fascicle length and orientation of muscle fibers in skeletal muscles and, potentially, the fiber length to fiber cross-section ratio and ellipticity of the cross-section. The fiber orientation measurements are based on the principle that diffusion of water molecules occurs primarily in the axial direction of muscle fibers because radial direction diffusion is obstructed by (presumably) cell membranes and intra-cellular obstructions (51). Previous studies have shown that DTI measurements of the primary direction of diffusion are aligned with the long axis of muscle fascicles (23, 76). The theoretical basis of DTI and the details of the data acquisition and post-processing procedures are beyond the scope of this review, but can be found in two recent review papers (24, 69).

DTI-based measurements of fiber orientations can be made at a great number of locations in a muscle and so provide useful information for characterizing the anisotropic mechanical behavior of muscle tissue (73, 74). The 3D fiber orientations can also be used to generate curves that follow the fiber orientation throughout a muscle. This procedure is called DTI tractography and the curves are called fiber tracts. Fiber tracts are not direct representations of muscle fibers or

muscle fascicles (which are bundles of muscle fibers), as the number of fiber tracts generated within a muscle can be arbitrarily chosen and the curves are infinitely thin (i.e. one-dimensional). However, fiber tracts follow the direction of fibers throughout a muscle, so their lengths and orientations reflect the fascicle lengths (defined as the length of a bundle of fibers from its origin to insertion on tendons or aponeuroses) and pennation angles of the muscle. Tractography has been used successfully to measure the 3D architecture of various human muscles *in vivo* (e.g. 9, 33, 93) (see Fig 4, which shows examples of muscle fibers tracked in the lower leg (a) and in the human female pelvis (b)).

<insert Figure 4 about here>

Critical evaluation. The major strength of DTI is that it provides *in vivo* fiber orientation measurements at high resolution, in whole human muscles and in 3D. This information is difficult or impossible to obtain with other imaging methods such as ultrasonography, which is primarily 2D and provides images that are smaller than most human muscles, or conventional MRI scans, which lack the resolution to discern individual muscle fibers. On clinical 3-Tesla MR scanners, DTI scans of whole human limb muscles take around ~8-15 minutes to obtain (depending on resolution, details of the protocol and scan volume), which makes DTI feasible for both basic physiological studies as well as clinical research on human muscles *in vivo*. These scan times limit the application of DTI to measurements of static muscle structure. Static DTI measurements, however, have also proven useful in investigations of anisotropic dynamic muscle behavior by combining measurements of muscle deformation patterns from dynamic MRI with fibre orientation measurements in a reference (undeformed) state from DTI (28, 38). A challenge in this approach is proper alignment of images obtained with DTI and dynamic sequences.

Although there is strong indirect evidence DTI can provide valid measurements of muscle architecture, quantitative validity has not yet been established in human muscles. DTI measures of muscle architecture have acceptable repeatability (40, 79), and DTI-based reconstructions of muscles exhibit remarkable similarity to the structure of dissected muscles (e.g. 10, 33). Furthermore, DTI-based measurements of muscle architecture of the human gastrocnemius are similar, on average (though not in individual measurements), to measurements made with ultrasound (11). Other data show realistic fascicle lengthening in the gastrocnemius and the soleus muscles when DTI scans are obtained at different muscle-tendon lengths (9, 10).
A challenge for accurate measurements of muscle architecture using DTI tractography is that fiber tracts (the curves that are obtained through DTI tractography) often terminate intramuscularly or cross-over to adjacent muscles. There is evidence that (real) muscle fibers can terminate intramuscularly (72). However, it is unlikely that intramuscular endpoints of fiber tracts accurately reflect these intramuscular endpoints, because the location of fiber tract endpoints depends strongly on the stopping criteria used in DTI tractography and the noise level of the scan. Intramuscular endpoints of fiber tracts are thus more likely an artefact of DTI tractography, which can be remedied by the application of anatomical constraints which ensure that fiber tracts originate and insert on tendinous structures (9). More thorough validation of DTI-based measurements, especially when made on human muscles using the same scanners and protocols as used for *in vivo* studies, will likely increase the application of DTI in muscle mechanics research.

A potential limitation of DTI in clinical studies is the effect of intramuscular fat on diffusion properties of muscle tissue. The presence of fat leads to higher uncertainty in fiber orientation

measurements (22, 89), and may limit the accuracy of DTI-based reconstructions of fiber architecture of diseased muscles, in which fatty infiltration can be significant. It is likely that with improved fat suppression techniques (17), filtering the DTI data (60), and excluding regions with high fat content from the analysis (44), the confounding effects of fatty infiltration can be alleviated.

Applications. DTI measurements of fiber orientations have been used to identify differences in mechanical properties (38, 74) and deformation patterns (28, 70) in directions parallel and perpendicular to fibers. On the whole-muscle level, DTI has been used in combination with tractography algorithms to measure changes in muscle architecture with passive length changes (9, 10, 62, 83). DTI has also proven useful in biomechanical modelling studies, where information about fiber orientation is essential to accurately simulate the anisotropic behavior of muscle tissue (1, 12, 86). DTI has also been used to study changes in muscle fiber structure (DTI indices) and architecture associated with aging (80). In both the lateral and medial gastrocnemius, fiber lengths were shown to be significantly reduced, while the primary, secondary and tertiary diffusion tensor eigenvalues increased with age, underlining the complex dependence of these parameters on fiber atrophy and increased fibrosis.

Only two studies have used DTI to identify differences in muscle properties between healthy and diseased muscles in humans in vivo (44, 94). Both these studies focused on differences in diffusion properties and not on differences in muscle architecture. Patients with Duchenne Muscular Dystrophy were found to have different diffusion properties of some calf muscles compared to a control group (44). It is largely unknown how diffusion properties relate quantitatively to the muscle's microstructure and composition, which strongly limits the interpretation of group differences in diffusion properties. This study also highlighted the potential confounding effect of intramuscular fat and signal to noise ratio on the measurement of diffusion properties. While Mittal et al (64) present visualization of the external anal sphincter muscle and their hypothesized crossing in normal females, Zijta and colleagues (94) applied DTI to study pelvic floor muscles in women with pelvic floor organ prolapse or related symptoms and a control group. They did not identify differences in diffusion properties in pelvic floor muscles. These studies were performed on small numbers of patients, rendering the results only exploratory.

Summary and conclusions

From this brief review, it is clear that modern medical imaging can provide a wealth of diverse information about muscle mechanics, ranging from muscle architecture through kinematics to intrinsic mechanical properties, both in health and disease. While MR elastography and ultrasound shear wave elastography can provide maps of muscle mechanical properties, each has their strengths and weaknesses. MRE can provide 3D maps of muscle elastic and viscous properties, including anisotropic properties, when combined with diffusion imaging. It is also showing promise for measuring nonlinear mechanical properties. However, MRE is expensive, is currently a research technique and is therefore somewhat difficult to access; data analysis methods vary considerably between researchers, and each dataset takes minutes to acquire, limiting its use for studying anything but quasistatic processes. Ultrasound shear wave elastography provides 2D maps of muscle properties, and acquires data much more quickly than MRE, although not yet fast enough for dynamic studies. It was also used to analyze the nonlinear behavior of muscle in passive and active conditions (46) and is more widely available due to commercialization of the technique by several ultrasound vendors. However, it is limited to 2D

maps, doesn't provide viscous parameters, is somewhat limited in its ability to quantify muscle anisotropy, and is more operator-dependent. Diffusion imaging provides detailed information on muscle architecture, and the acquisition methods are fairly readily available on clinical 3T MRI systems; however it shares the cost and temporal resolution limitations of MRE. These static techniques are complemented by dynamic MR imaging which enables measurements of dynamic muscle motion and kinematics, and several techniques are available. Diffusion imaging is also somewhat expensive and more difficult to access, as some imaging sequences are specialized, and analysis is labor-intensive. Future development to improve the temporal resolution of these imaging methods will expand the range of physiological questions they can be used to study. However, the combination of these methods has already provided major new insights into muscle function, and each has demonstrated potential for use in better understanding changes in muscle passive properties in a broad range of disorders that affect skeletal muscle.

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Figure Captions:

Figure 1. Typical dataset and MRE setup for measuring calf muscle mechanical properties. (a) T2-W (weighted) anatomical image indicating the tibialis anterior (TA), soleus, and medial gastrocnemius (MG) muscles from an oblique slice through the lower leg. (b) Sample displacement wave image for displacements in the through plane direction (U_z). C. Calculated isotropic shear modulus map. G* is the magnitude of the linear viscoelastic shear modulus. D. MRE setup used to obtain the above dataset. Figure copyright A. Hatt & L. Bilston. Used with permission.

Figure 2. Typical example of changes in shear modulus of the gastrocnemius medialis during passive dorsiflexion performed at 2°/s (Adapted from Maïsetti et al (58)). Le Sant et al (56) replicated this measurement on 13 locations among all the plantar flexor muscles.

Figure 3: Eigenvectors (lines) corresponding to the eigenvalues of the strain rate (SR) tensor are shown superposed on the eigenvalue images for a young subject (81). A zoomed area of the medial gasctrocnemius is shown during passive joint rotation (L->R): (a) $SR_{in\text{-}plane}$, negative eigenvalue, dorsiflexion, (b) SR_{fiber}, positive eigenvalue, dorsiflexion, (c) SR_{fiber}, negative eigenvalue, plantarflexion, and (d) $SR_{in\text{-}plane}$, positive eigenvalue, plantarflexion. The pixel color is assigned according to the magnitude of the eigenvalue and ranges from 0 s^{-1} (green) to -800 s^{-1} (blue) for the negative eigenvalue images and from 0 s^{-1} (red) to 800 s^{-1} (yellow) for the positive eigenvalue images (note: values were scaled by 1000). In dorsiflexion, the negative strain rate direction is approximately perpendicular (a), while the positive strain rate is approximately parallel to the fiber direction (b), while the reverse is true for the plantarflexion phase (c,d). $SR_{in\text{-}plane}$ is the strain of the fiber cross-section in the plane of the image (the third component in the slice direction is not calculated here as only one slice is acquired).

Figure 4. Example of a three-dimensional reconstruction of the architecture of the human (a) medial gastrocnemius muscle and (b) pubo-rectalis and anal sphincter muscle (with different relevant structures shown, (64), using information from anatomical scans and DTI tractography. A three-dimensional surface model of the muscle, created from a T1-weighted anatomical scan (one transverse slice is depicted), is shown as a transparent overlay in (a). Fiber tracts, which were generated from DTI data co-registered with the anatomical image, were fitted with polynomial curves and extrapolated to ensure that fascicles attach to tendinous structures (see (9) for more details about these procedures). In (b), color coding of the fiber tracts follows DTI convention and indicates direction of the tract.