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In Vivo Viscoelastic Response (VisR) Ultrasound For Characterizing Mechanical Anisotropy in Lower-Limb Skeletal Muscles of Boys With and Without Duchenne Muscular Dystrophy

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Abstract

Our group has previously shown *in silico* that mechanical anisotropy may be interrogated by exciting transversely isotropic materials with geometrically asymmetric acoustic radiation force excitations and then monitoring the associated induced displacements in the region of excitation. We now translate acoustic radiation force based anisotropy assessment to human muscle *in vivo* and investigate its clinical relevance to monitoring muscle degeneration in Duchenne muscular dystrophy (DMD). Clinical anisotropy assessments were performed using Viscoelastic Response (VisR) ultrasound, with degree of anisotropy reflected by the ratios of ViSR relative elasticity (RE) or relative viscosity (RV) measured with the asymmetric radiation force oriented parallel versus perpendicular to muscle fiber alignment. *In vivo* results from rectus femoris (RF) and gastrocnemius (GM) muscles of boys aged ~7.9 – 10.4 years show that RE and RV anisotropy ratios in RF muscles of boys with DMD were significantly higher than those of healthy control boys (RE: DMD = 1.51 ± 0.87 , Control = 0.99 ± 0.69 , $p=0.04$ Wilcoxon rank sum test; RV: DMD = 1.04 ± 0.71 , Control = 0.74 ± 0.22 , $p=0.02$). In the GM muscle, only the RV anisotropy ratio was significantly higher in dystrophic than control subjects (DMD = 1.23 ± 0.35 , Control = 0.88 ± 0.31 , $p=0.04$). In the dystrophic RF muscle, the RE anisotropy ratio was inversely correlated

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Conflict of Interest

The authors have filed a provisional patent for the techniques described in this work.

(Slope = -0.03 lbf^{-1} , $r=-0.43$ $p = 0.07$, Pearson Correlation) with quantitative muscle testing (QMT) functional output measures, but was not correlated with QMT in the dystrophic GM. These results suggest that VisR RE and RV measures reflect differences in mechanical anisotropy associated with functional impairment with dystrophic degeneration that are relevant to monitoring DMD clinically.

Keywords

Viscoelastic Response (VisR) Ultrasound; Acoustic Radiation Force; Duchenne Muscular Dystrophy; Viscoelasticity; Anisotropy; Transverse Isotropy

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder, which affects one in 3,500 – 4,500 male births each year. The disease causes muscle wasting and loss of ambulation at a young age, followed by pulmonary and cardiac compromise in the late teenage years, with death usually occurring in early adulthood (Emery 1991). Although a variety of DMD therapies are in development, no cure exists today, due in large part to inadequate outcome measures to support translation of new treatments to clinical trials.

Conventional outcome measures for assessing response to therapy in DMD include timed function tests and quantitative muscle testing, but these measures are nonspecific to a particular muscle, and they are subject to the volition of the patient, which may vary substantially in a child population (Bushby et al. 2010). Other outcome measures include magnetic resonance imaging (MRI) or ultrasound image metrics, such as T1 and T2 signal intensities or quantitative measures of ultrasound scattering characteristics, respectively (Mercuri et al. 2002; Rutkove et al. 2014). While these imaging approaches are advantageous in that individual muscles may be evaluated in isolation from other muscles in an objective manner, they are limited in that their outcome metrics primarily reflect muscle composition (Rutkove et al. 2014).

While compositional changes underlie dystrophic muscle degeneration, such changes are associated with corresponding structural and mechanical alterations, which are relevant biomarkers that offer insight to mechanisms of dystrophic muscle failure (Rutkove et al. 2014). To understand why, consider the composition and structure of healthy skeletal muscle. Individual muscle fibers are surrounded by protective connective tissue called endomysium. The muscle fibers exist in bundles, or fasciculi, surrounded by additional connective tissue called perimysium. Finally, more connective tissue, called epimysium, groups the fasciculi into an individual skeletal muscle, and muscles are separated from each other by connective tissue outside the epimysium known as fascia (Bilston and Tan 2015). This arrangement creates highly organized skeletal muscles that are structurally and mechanically anisotropic, i.e. the structural and mechanical properties of muscle vary with orientation. Structurally, muscle fibers are long and cylindrically shaped, with their long axes aligned along the long axis of the muscle. Mechanically, the longitudinal shear elastic and viscous moduli are greater than the transverse (Gennisson et al. 2010).

Skeletal muscle organization breaks down in DMD as muscles undergo necrosis and inflammation with muscle fiber fragmentation and fatty deposition (Cros et al. 1989; Grounds 2008; Kornegay et al. 2003; Li et al. 2015; Wang et al. 2013a). Further, collagen is deposited, primarily along the epimysium and perimysium between fasciculi. These changes, which occur in repeating cycles of progressive muscle wasting, alter both the structural and mechanical anisotropy in dystrophic muscle.

Structural and mechanical anisotropy in dystrophic muscle has been evaluated using MRI based diffusion tensor imaging (DTI) (Li et al. 2016) and magnetic resonance elastography (MRE) (Manduca et al. 2001). Notably, Manduca et al showed that in the mdx mouse, a mutant mouse model with an inactive dystrophin protein (Bulfield et al. 1984), DTI measures alone did not distinguish mdx from WT muscles, suggesting that mechanical anisotropy may be a more sensitive measure of dystrophic muscle degradation than structural anisotropy.

While the diagnostic relevance of DTI and MRE to DMD has been demonstrated clinically, and although MRI holds a pivotal role in the clinic, high cost and low portability generally limit accessibility to these modalities. Alternatively, low-cost and readily portable ultrasound based methods have been developed for interrogating structural and mechanical anisotropy in tissue. Among these approaches are elastographic ultrasound methods that ultrasonically track acoustic radiation force (ARF)-induced shear waves to estimate directionally dependent shear wave propagation velocities that, as in MRE, reflect anisotropy in elastic moduli (Aristizabal et al. 2014; Wang et al. 2013b). This approach is advantageous in that shear wave velocity can be related to material elasticity in a quantitative fashion (Wang et al. 2013b). However, the performance of shear-wave based approaches suffers in heterogeneous tissue and in tissue in which shear wave propagation is inhibited in at least one direction. In particular, in muscle, shear wave propagation is inhibited in the direction across muscle fibers (Eby et al. 2013; Gennisson et al. 2003).

Rather than relying on shear wave propagation, Viscoelastic Response (VisR) ultrasound interrogates tissue mechanical properties by observing ARF-induced tissue displacements in only the ARF region of excitation (ROE) (Hossain et al. 2016; Moore et al. 2017a). We herein present a pilot clinical investigation of VisR derived mechanical anisotropy evaluated in the lower-limb skeletal muscles of boys with DMD. Specifically, we examine the rectus femoris (RF) and gastrocnemius medial head (GM) muscles. These muscles were chosen because they are expected to exhibit different degrees of dystrophic degeneration within an individual (Cros et al. 1989; Torriani et al. 2012). We test the hypotheses: 1) VisR derived mechanical anisotropy measures in the RF and GM muscles of boys with DMD statistically differ from those in age-matched boys with no known neuromuscular disorders, and 2) VisR derived mechanical anisotropy measures reflect muscle functional output.

Materials and Methods

Viscoelastic Response (VisR) Ultrasound

A detailed description of VisR ultrasound can be found in (Selzo et al. 2016). Briefly, to perform VisR, two ARF excitations are delivered to the same region of excitation, and

displacements induced in the region of excitation are monitored over time (typically 1–5 ms) using conventional B-Mode type motion tracking pulses. Displacements are then measured using one-dimensional axial normalized cross-correlation (Luo and Konofagou 2010; Pinton et al. 2006) to yield displacement vs. time profiles that are fit to the solution of the mass-spring-damper (MSD) model. The MSD model is written as:

$$\frac{d^2}{dt^2}u(t) + \omega^2\tau\frac{d}{dt}u(t) + \omega^2u(t) = S\omega^2(H(t) - H(t - t_{ARF}) + H(t - t_{ARF} - t_s) - H(t - 2t_{ARF} - t_s)) \quad (1)$$

where u is displacement, t is time, ω is the natural frequency, τ is the relaxation time constant, and S is the static sensitivity of the system. The right hand side of Equation (1) is the forcing function of the system, where H is the unit step function, t_{ARF} is the duration of each ARF excitation, and t_s is the time between the two ARF pulses.

The solution to (1) is based on parameters ω , τ and S , which are materially dependent. The solution can be written in terms of the elastic constant, μ , the viscous constant, η , the mass, m , and the ARF amplitude, A .

$$\omega = \sqrt{\frac{\mu}{m}}; \tau = \frac{\eta}{\mu}; S = \frac{A}{\mu} \quad (2)$$

Equation (2) can be rearranged to form more intuitive viscoelastic parameters evaluated relative to the applied ARF amplitude, A . Specifically, VisR Relative Elasticity (RE) is evaluated as:

$$RE = \frac{1}{S} = \frac{\mu}{A} \quad (3)$$

and VisR Relative Viscosity (RV) as:

$$RV = \frac{\tau}{S} = \frac{\eta}{A} \quad (4)$$

where A is generally unknown (Palmeri and Nightingale 2011) but is assumed to be constant over a limited spatial extent.

Assessing Mechanical Anisotropy in Muscle using Viscoelastic Response (VisR) Ultrasound

The mechanical properties of skeletal muscle can be described as transversely isotropic (TI) (Eby et al. 2013; Genisson et al. 2003; Morrow et al. 2010; Qiang et al. 2015; Qin et al.

2014; Urban et al. 2009), meaning that the mechanical properties are consistent within but differ between planes oriented parallel and perpendicular to an axis of symmetry (AoS). These directional differences in mechanical property are interrogated in VisR ultrasound by using a spatially asymmetric ARF excitation impulse. Hossain et. al. (Hossain et al. 2017) demonstrated *in silico* that when the long axis of a spatially asymmetric ARF spatial force profile (SFP) is oriented along the AoS, the induced displacements primarily reflect the transverse shear elastic modulus. Further, when the long axis of a spatially asymmetric ARF SFP is oriented across the AoS, the induced displacements predominantly represent the longitudinal shear elastic modulus. Finally, the ratio of displacements achieved with the long axis of the spatially asymmetric ARF SFP aligned along versus across the AoS reflect the degree of elastic anisotropy (Hossain et al. 2017).

In the context of VisR imaging, RE and RV measures obtained using spatially asymmetric ARF SFPs with long axes oriented along the AoS reflect transverse shear elastic and viscous moduli, respectively. Similarly, VisR RE and RV measures made with spatially asymmetric ARF SFPs with long axes directed across the AoS reflect longitudinal shear elastic and viscous moduli. Finally, by Equations 3 and 4, the ratios of transverse to longitudinal RE and RV measures reflect the ratios of transverse to longitudinal elastic and viscous moduli, respectively, provided that the ARF amplitude, A , remains constant for both orientations of the spatially asymmetric ARF SFP:

$$R_{\mu} = \frac{RE_t}{RE_l} \propto \frac{\mu_t}{\mu_l} \quad (5)$$

and

$$R_{\eta} = \frac{RV_t}{RV_l} \propto \frac{\eta_t}{\eta_l} \quad (6)$$

where subscripts t and l represent the transverse and longitudinal directions, respectively. From here forth, we will refer to the elastic and viscous moduli oriented parallel to the AoS as the “longitudinal” moduli and the elastic and viscous moduli oriented perpendicular to the AoS as the “transverse” moduli.

Viscoelastic Response (VisR) Imaging

All procedures were approved by the institutional review board of the University of North Carolina at Chapel Hill (IRB). Informed, written consent was gathered from the subject and guardian in accordance with IRB guidelines. VisR imaging was performed on the RF and GM muscles of 6 boys with DMD and 5 control boys with no known neuromuscular disorders ranging in age from 7.9 to 10.4 years. Control and dystrophic subjects fell within this average age range, and were not age matched. For some subjects (7 dystrophic, 5 control), imaging was performed at multiple time points but with no less than three months between imaging sessions. This yielded 22 DMD and 12 control data sets for evaluation.

Using a Siemens Acuson Antares ultrasound scanner and a VF 7–3 linear array transducer (Siemens Healthcare, Ultrasound Business Unit, Issaquah, WA, USA), a custom VisR beam sequence was implemented using the system parameters reported in Table 1. The focal depth was adaptively set near the bottom of the muscle of interest in the center of the imaging frame, generally between 15 and 23 mm. A spatially asymmetric ARF, with a larger elevational than lateral aspect at the focal depth, was delivered to the muscle with the long axis of the ARF oriented first along and then across the long axis of the muscle fibers. Muscle fiber orientation was assessed using B-Mode guidance by a trained sonographer with 15 years' experience. First, the sonographer manually aligned the ultrasound probe across muscle fibers in the center of the muscle lengthwise, and VisR data were collected. Then, the sonographer manually rotated the ultrasound probe about its center axis to be positioned along the muscle fibers, and VisR data were again collected.

For both transducer positions, VisR beam sequencing was as follows. First, a conventional B-Mode image was acquired with lateral beam spacing of 0.17 mm spanning 40 mm laterally. Then, ensembles of two 70 μ s ARF pushes, separated in time by four tracking beams. Immediately following the second ARF push, 50 additional tracking beams were acquired. This ensemble was delivered in 40 lateral positions spaced 0.5 mm apart spanning 20 mm and centered in the B-Mode imaging frame. The VisR sequence was acquired using wiper blading to reduce likelihood of local tissue heating from the excitations and tracking pulses. Radio frequency data from the tracking pulses was recorded to the scanner's memory and subsequently transferred to a PC for post-processing. The beam parameters of the ARF push and tracking pulses are reported in Table 1.

VisR RE and RV were computed by first estimating time-dependent tissue displacement from the acquired raw RF data using one-dimensional axial normalized cross-correlation with a kernel size of 3λ and a search size of 0.1 mm (Luo and Konofagou 2010; Pinton et al. 2006). The resulting displacement versus time profiles from each pixel within the 2D VisR imaging field of view were then fit to the MSD model described in (1–2) using a custom C++ implementation of the Nelder-Mead (Lagarias et al. 1998) minimization algorithm. The resulting fits were used to estimate RE and RV, as described in Equations (3–4). Parametric VisR RE and RV images were rendered, and the RF or GM muscle in each image was manually segmented using B-mode guidance, for visual review of VisR outcomes. Finally, RE and RV ratios were calculated using (5–6) in regions of interest (ROIs) positioned laterally in the center of the imaging field of view and axially at the focal depth. The ROIs spanned 2 mm laterally and 2 mm axially. The position and lateral size of the ROIs were chosen to encompass the region of muscle overlapped in cross-sectional and longitudinal muscle views, as determined by the elevational extent of the ARF SFP (Figure 1). Note from Figure 1 that the lateral aperture is translated to span a lateral range that matches the elevational extent of the SFP at the focal depth, such that the same volume of tissue is interrogated in both cross-sectional and longitudinal muscle views. For the VF7–3, the elevational extent of the SFP is ~2 mm; therefore, the lateral aperture is translated over 2 mm, as demonstrated by the multiple lateral apertures (labeled 1–3) in Figure 1. The axial size of the ROIs was chosen to encompass a region of consistent degree of spatial asymmetry in the lateral-elevational ARF SFP, which varied over axial range due to electronic and geometric focusing.

The extent to which ARF SFP spatial asymmetry varied over depth was evaluated *in silico* using Field II (Jensen and Svendsen 1992) simulations of ARF excitations generated using the system parameters described in Table 1 in simulated tissue with attenuation of 1.0 dB/cm/MHz. To estimate degree of spatial asymmetry in the simulated ARF lateral-elevational SFPs, an ellipse was fit to the SFP isobaric -6 dB contour, and the ratio of the elevational to lateral extent of the ellipse was evaluated. Figure 2 shows this ratio at the ARF focal depth plotted for focal depths ranging from 14 to 40 mm. The axial range over which the degree of asymmetry varies by less than 10%, and is asymmetric (ratio different from 1) spans from 14 mm to 23 mm. Note that all DMD and control data sets included in this study were acquired with focal depths falling within this range. For each focal depth in the 14 to 23 mm span, the degree of asymmetry remains approximately the same (<10% variation) over a range of at least 2mm around the focus, so 2 mm was chosen as the axial range for the anisotropy ROI.

Quantitative Muscle Testing

In addition to VisR, quantitative muscle testing (QMT) was performed at the time of imaging by a licensed physical therapist using a handheld dynamometer (HHD) (Stuberg and Metcalf 1988). Maximal output, in units of pounds of force, was measured for the knee extensor group, of which the RF is a primary muscle. The test was performed with the subject in a seated position with the knee in 90 degrees of flexion. The HHD was positioned at the distal lower leg, and the patient was instructed to extend his knee, pressing against the HHD, with as much force as possible. The output of the GM was similarly measured by QMT. For this test, the patient was in a supine position with the ankle at neutral dorsiflexion. The HHD was placed at the head of the metatarsus, and the subject was asked to press to plantar flexion with as much force as possible. For both the knee extension and plantar flexion exams, two trials were performed with a one-minute rest between trials.

Statistical Analyses

Wilcoxon rank-sum statistical tests were performed to evaluate the null hypotheses of equal median VisR anisotropy estimates in DMD and control muscles. P-values of < 0.05 were considered significant in the rank-sum test. Linear relationships between anisotropy estimates and QMT outcomes were assessed visually using scatter plots, and quantified using Pearson correlation. For correlation results, P-values of < 0.1 were considered significant. Results from this work are reported as median $\pm$ median absolute deviation, unless otherwise noted. All statistical analyses were carried out using MATLAB 2017a (Mathworks, Inc., Natick, MA, USA).

Results

Figure 3 shows example parametric RE (a-b, e-f) and RV (c-d, g-h) images in cross-sectional (a, c, e, g) and longitudinal (b, d, f, h) views of an RF muscle of an 8 year-old boy with DMD. The muscle is outlined by the dashed line, and the rectangular box indicates the ROI for RE and RV anisotropy calculations. The values for the ROI are reported for each panel in Table 2. Recall that for the employed implementation of VisR imaging, viewing the muscle in cross-section means that the elevational (long) axis of the ARF SFP is oriented along the

muscle fibers, and as a result, the VisR RE and RV measures primarily reflect the transverse shear elastic and viscous moduli, as described by Hossain et. al. 2017 . Similarly, viewing the muscle longitudinally means that the elevational (long) aspect of the ARF SFP is oriented across the muscle fibers, and VisR RE and RV measures primarily reflect the longitudinal shear elastic and viscous moduli. These results suggest that in this dystrophic RF muscle, the transverse shear elastic modulus is greater than the longitudinal shear elastic modulus. Panels (e–f) and (g–h) of Figure 3 show the cross-sectional and longitudinal RE and RV images, respectively, from a control RF muscle of a 9-year-old boy. The values for the ROI of each panel are reported in Table 2. This result agrees with previous studies in healthy muscle, which found that the longitudinal shear elastic and viscous moduli were greater than the transverse moduli.

Figure 4 shows RE and RV images in the cross-sectional (panels a, c, e, g) and longitudinal (panels b, d, f, h) views of a dystrophic (panels a–d) and a control (panels e–h) GM. RE, RV and anisotropy ratios for each are reported for the GM in Table 3. Note that the control ratios in the GM, like the RF, agree with previous work, which suggests the longitudinal elastic and viscous moduli are greater than their transverse counterparts, in healthy muscle. In the dystrophic GM, however, it is observed that the anisotropy ratio is inverted compared to the expectation for healthy muscle.

Combined results of all VisR RE and RV anisotropy ratios from both dystrophic and control RF muscles are shown as boxplots in Figure 5. Panels (a) and (c) show the RE and RV anisotropy ratios in the RF, respectively. The RE anisotropy ratio is greater than one for the DMD group (Median [IQR] -1.51 [0.87]) and approximately one for the control group (0.99 [0.69]), and the median RE values are significantly different ($p = 0.04$) between DMD and control cohorts. Similarly, control RV anisotropy ratios (0.74 [0.22]) are less than one and statistically different from DMD RV anisotropy ratios (1.04 [0.71]) ($p = 0.02$). Panels (b/d) of Figure 5 show combined VisR RE (b) and RV (d) anisotropy ratios in control and DMD GM. Panel (b) shows that RE anisotropy values in control (1.14 [0.50]) and DMD (1.32 [0.47]) are not statistically different ($p=0.12$). However, RV anisotropy ratios are significantly larger in DMD (1.23 [0.35]) than control (0.88 [0.31]) groups ($p = 0.04$).

Figure 6 shows scatter plots of VisR RE and RV anisotropy ratios versus QMT outcomes, recorded as mean value of the two QMT trials, in units of pounds of force (lbf). For RF muscles, panels(a) and (c) show RE and RV anisotropy ratios versus knee extension QMT, respectively. Note that higher RE anisotropy ratios in the dystrophic RF correlate to lower QMT force output (slope = -0.03 lbf^{-1} $p=0.07$), with DMD RF muscles exhibiting lower QMT output than control. For GM muscles, panel (b) illustrates that VisR RE anisotropy ratios are similarly valued between control and DMD boys despite higher plantar flexion QMT force output in the control cohort. However, higher RE and RV anisotropy ratios in the control gastrocnemius are correlated in with higher QMT force output (RE slope = 0.01 lbf^{-1} , $p = 0.05$; RV slope = 0.01 lbf^{-1} , $p = 0.07$). Panel (d) suggests that higher VisR RV anisotropy values correspond to lower QMT force output in DMD boys, but the correlation is not statistically significant.

Discussion

VisR RE and RV anisotropy ratios less than one were expected based on the results of previous studies, which found that the longitudinal shear elastic and viscous moduli are greater than the transverse in healthy skeletal muscle (Bilston and Tan 2015; Cortez et al. 2016; Eby et al. 2013; Gennisson et al. 2010; Levinson et al. 1995; Qin et al. 2014; Wang et al. 2013b). However, RE and RV anisotropy ratios greater than one in DMD RF suggest that the transverse shear elastic and viscous moduli are greater than the longitudinal. The cause of the inverted relationship between transverse and longitudinal moduli with DMD may be related to underlying changes in the dystrophic RF composition. Recall that Cros et al showed that dystrophic RF is infiltrated by over 35% fibro-fatty tissue in the age range of the boys included in this study (Cros et al. 1989). Infiltration of fibro-fatty tissue occurs after necrosis of the muscle fibers, with necrosis causing muscle fiber fragmentation that could lower the longitudinal shear modulus (Bilston and Tan 2015). Post necrosis, fibro-fatty tissue replaces damaged muscle fibers with fat and a collagen (Cornu et al. 2001). Deposition of collagen matrix in the epimysium and perimysium may increase stiffness between muscle fibers and effectively increase the transverse modulus. With the coupled effects of decreased longitudinal modulus due to fragmentation and increased transverse modulus due to fibrosis, inversion of the RE anisotropy ratio may be explained. Similarly, inflammation and infiltration of fat along muscle fiber boundaries may underlie increased RV anisotropy ratio in the DMD RF. We note that while RE anisotropy ratio was statistically significantly greater in DMD than control RF muscles, the ratio values overlapped between groups, which could indicate that VisR's ability to detect early-stage dystrophic degeneration in the RF is limited. Additional studies are needed to establish VisR's clinical relevance to staging muscle wasting in DMD patients.

The finding of no difference in VisR RE anisotropy ratio between control and DMD GM is in agreement with previous findings, which showed the GM is less severely affected by fibro-fatty infiltration than the RF (Cros et al. 1989). In a clinical, biopsy based study of dystrophic skeletal muscle degeneration, Cros. et. al. found that 38% of dystrophic RF muscles in boys aged 7–10 years was fibro-fatty. In comparison, the GM muscle from the same study population had only 25% fibro-fatty composition, which was significantly ($p<0.05$) less than the RF (Cros et al. 1989). Similarly, Hollingsworth et. al. showed in an MRI based study of boys with DMD that the RF rapidly increased in fatty content in the age range of 7–10 years while the GM remained relatively unchanged (Hollingsworth et al. 2013). Instead of fatty and fibrous changes, the GM primarily undergoes inflammation and hypertrophy in the age range of the boys included in this study (Jones et al. 1983; Kornegay et al. 2003; Torriani et al. 2012). Interestingly, while hypertrophy increases the size of the GM muscle, VisR does not detect an associated change in elastic anisotropy. Although VisR RE anisotropy values were not different between control and DMD in the GM, the significant difference found between control and DMD RV anisotropy ratios suggests that RV anisotropy ratio may be more sensitive than RE anisotropy ratio to inflammation, hypertrophy, and/or lipidic changes in muscle composition.

The finding that higher VisR anisotropy ratio corresponds to lower QMT force output in the RF muscle of DMD patients suggests that reduction in muscle function with dystrophic

degeneration may be attributable to changes in transverse and/or longitudinal shear elastic and viscous moduli. In the GM, little variation in RE anisotropy ratio with QMT output suggests that although dystrophic changes degrade muscle function, the functional changes are not due to alterations in elastic anisotropy. Rather, functional deterioration may be related to changes in viscous anisotropy associated with inflammation, hypertrophy, and/or lipid infiltration. It is important to consider that while VisR mechanical anisotropy measurements are specific to the examined RF and GM muscles, QMT outcomes reflect the overall functional performance of a group of muscles. Therefore, the observed QMT performance cannot be attributed singly to the RF or the GM, and the potential impact of synergistic and antagonistic muscles on QMT output is unknown.

It is important to consider that by the implemented experimental protocol, VisR data were acquired while the subjects lied at rest in supine (RF) or prone (GM) position, with the RF and GM relaxed. However, previous work has shown that active loading increases the degree of mechanical anisotropy in healthy muscle (Deffieux et al. 2009; Eby et al. 2013; Gennisson et al. 2010; Hoyt et al. 2008; Levinson et al. 1995). In the future, we will implement VisR anisotropy assessment with active loading to more fully interrogate the impact of dystrophic muscle degeneration on mechanical anisotropy.

A potential source of error in VisR mechanical anisotropy assessment is misalignment. If the ARF is misaligned across and/or along the muscle fiber axis, the measured RE or RV anisotropy ratio will be underestimated, as demonstrated in (Hossain et al. 2017). Similarly, transducer translation could introduce error into VisR RE and RV anisotropy assessment. If the transducer is translated during rotation between cross-sectional and longitudinal orientations, the tissue excited by the ARF will be different between the two imaging planes. To the extent that the underlying elastic and viscous properties vary spatially, the resulting VisR RE and RV anisotropy ratios may not reflect the true ratios in the desired measurement region. Misalignment and translation were mitigated in the presented work by the experienced sonographer, who used B-Mode guidance and anatomical markers to position the transducer, but methods to more systematically decrease the potential for misalignment and translation are in development (Moore et al. 2017b).

Another potential source of error in VisR mechanical anisotropy assessment is variation in the degree of ARF spatial asymmetry with depth. As Hossain et. al. describe, measured anisotropy ratio changes with degree of ARF spatial asymmetry (Hossain et al. 2017). In this study, variation in degree of ARF asymmetry was minimized by analyzing VisR data only within the limited axial range in which ARF asymmetry was consistent. However, a trade-off to this approach is that only a small portion of the muscle was considered. Future work will develop ARF excitation methods that expand the usable axial range.

A third error source is complex system inertia, as explained in detail in (Selzo et al. 2016). Because system inertia is impacted by both elasticity and viscosity, it will be directionally dependent in a TI material such as muscle. Such directional variation in complex system inertia would obfuscate observed directional differences in VisR RE and RV measures and complicate anisotropy ratio values. Quantifying the impact of and developing methods to

control for complex system inertia on VisR mechanical anisotropy measurement in muscle is a topic of ongoing investigation.

Our investigation had limitations that warrant careful consideration. First, our study was performed by a single sonographer for all patients; therefore, interobserver agreement could not be evaluated. Future studies will use multiple sonographers to determine if VisR outcomes are repeatable across users. Second, our study included a small number of subjects. Future work will increase patient and control cohort sizes to better assess differences in VisR mechanical anisotropy measures and confirm VisR's relevance to delineating dystrophic degeneration clinically.

Conclusion

This manuscript describes VisR RE and RV based mechanical anisotropy assessment in the RF and GM muscles of age-matched boys with and without DMD. The results suggest that VisR RE and RV anisotropy ratios detect changes in elastic and viscous anisotropy that are associated with dystrophic muscle degeneration and degradation in muscle function. This outcome is impactful in two primary ways. First, delineating changes in mechanical anisotropy with DMD progression could help to elucidate the complex and poorly understood pathophysiology of dystrophic degeneration, which could lead to development of improved approaches to inhibiting muscle degradation. Second, if validated as outcome measures for clinical trials, VisR RE and RV anisotropy ratios could help to translate novel DMD therapies into clinical implementation. A meaningful advantage to the VisR approach is that the method does not rely on shear wave propagation across muscle fibers. Another advantage is that anisotropy measurements are made focally and reflect the properties of a specific muscle. An important disadvantage to the approach as it is currently implemented is the limited spatial range over which anisotropy ratios are calculated. Ongoing work aims to expand the usable range. Overall, this pilot *in vivo* clinical feasibility study demonstrates VisR mechanical anisotropy assessment in dystrophic skeletal muscle and supports VisR's relevance to monitoring dystrophic degeneration in boys with DMD.

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Figure 1.

– Representation of the shape of the acoustic SFP from a lensed linear array ultrasound transducer, in lateral (a) and elevational (b) orientations. Panel (c) shows the region (yellow) about the axial focal depth where the two SFP orientations will overlap when the transducer is rotated 90 degrees about its center. Note that in the lateral orientation the aperture can be shifted laterally (denoted by subscripts 1–3 and different gray levels) to span a range similar to that of the elevational orientation. SFP = Spatial Force Profile

Figure 2.

– Plot of degree of asymmetry in the ARF SFP as a function of axial focal depth (mm).

Degree of asymmetry was calculated by fitting an ellipse to the -6 dB contour of a simulated ARF SFP, at each focal depth from 14 mm to 40 mm in 1 mm steps, and taking the ratio of the elevational divided by the lateral dimensions of the SFP. The red vertical line demarcates the maximum focal depth for which the SFP is sufficiently asymmetric for anisotropy estimation. ARF = Acoustic Radiation Force, SFP = Spatial Force Profile

Figure 3.

– VisR RE images of dystrophic (a–b) and healthy (c–d) RF muscles in cross-sectional (a/c) and longitudinal (b/d) views. The dotted contours outline the muscle boundary and the white box shows the region of interest for the VisR anisotropy ratio calculation. Color encodes VisR RE in units of (1/mm). VisR = Viscoelastic Reponse Ultrasound, RE = Relative Elasticity, RF = Rectus Femoris

Figure 4.

– VisR RE images of dystrophic (a–b) and healthy (c–d) GM muscles in cross-sectional (a/c) and longitudinal (b/d) views. The dotted contours outline the muscle boundary and the white box shows the region of interest for the VisR anisotropy ratio calculation. Color encodes VisR RE in units of (1/mm). VisR = Viscoelastic Reponse Ultrasound, RE = Relative Elasticity, GM = Gastrocnemius Medial Head

Figure 5.

– Boxplots of RE (a–b) and RV (c–d) anisotropy in the RF (a/c) and GM(b/d). Control and dystrophic groups are represented by individual box and whisker plots, and the vertical axis shows the RE or RV anisotropy ratio. These ratios are taken as the transverse divided by the longitudinal RE or RV values. Significant differences are denoted by a black bar and asterisk. RE = Relative Elasticity, RV = Relative Viscosity, RF = Rectus Femoris, GM = Gastrocnemius Medial Head, DMD = Duchenne Muscular Dystrophy

Figure 6.

– Scatter plots of RE anisotropy ratio (a–b) and RV anisotropy ratio (c–d), plotted against the QMT functional output (lbs), in the RF (a/c) and GM (b/d) muscles. Orange circles represent dystrophic muscle results, and blue circles represent healthy muscle results. RE = Relative Elasticity, RV = Relative Viscosity, QMT = Quantitative Muscle Testing, RF = Rectus Femoris, GM = Gastrocnemius Medial Head, DMD = Duchenne Muscular Dystrophy

Table 1:

VisR imaging parameters

Parameter	Value
Transducer	Siemens VF7–3 linear array
Bandwidth	53%
Lens focal depth	37.5 mm
RF Excitation center frequency	4.21 MHz
ARF Lateral F/#	3.0
ARF Elevation F/#	5.0
ARF Duration (t_{ARF})	70 μs
Time between ARF Excitations	0.4 ms
Tracking Frequency	6.15 MHz

Table 2:

Median VisR RE, RV, and anisotropy values for dystrophic and control rectus femoris example images

Dystrophic Rectus Femoris	Cross-Sectional	Longitudinal	Ratio	Control Rectus Femoris	Cross-Sectional	Longitudinal	Ratio
Relative Elasticity	82.3 ± 3.5 (mm ⁻¹)	25.8 ± 3.5 (mm ⁻¹)	3.2 ± 0.2		51.9 ± 5.4 (mm ⁻¹)	63.7 ± 10.4 (mm ⁻¹)	0.8 ± 0.2
Relative Viscosity	107.7 ± 8.1 (ms·mm ⁻¹)	46.8 ± 3.5 (ms·mm ⁻¹)	2.4 ± 0.3		61.9 ± 17.2 (ms·mm ⁻¹)	86.7 ± 29.6 (ms·mm ⁻¹)	0.7 ± 0.6

Table 3:

Median VisR RE, RV, and anisotropy values for dystrophic and control gastrocnemius example images

Dystrophic Gastrocnemius	Cross-Sectional	Longitudinal	Ratio	Control Gastrocnemius	Cross-Sectional	Longitudinal	Ratio
Relative Elasticity	97.7 ± 19.6 (mm ⁻¹)	72.5 ± 7.2 (mm ⁻¹)	1.4 ± 0.4		114.0 ± 7.0 (mm ⁻¹)	132.7 ± 14.8 (mm ⁻¹)	0.83 ± 0.1
Relative Viscosity	216.2 ± 41.2 (ms·mm ⁻¹)	147.9 ± 15.1 (ms·mm ⁻¹)	1.3 ± 0.3		100.6 ± 21.4 (ms·mm ⁻¹)	181.5 ± 43.5 (ms·mm ⁻¹)	0.6 ± 0.3