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Quantitative muscle ultrasound detects disease progression in Duchenne muscular dystrophy

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Abstract

Objective—We assessed changes in quantitative muscle ultrasound data in boys with Duchenne muscular dystrophy (DMD) and healthy controls to determine if ultrasound can serve as a biomarker of disease progression. Two approaches were used: grey scale level (GSL), measured from the ultrasound image, and quantitative backscatter analysis (QBA) measured directly from the received echoes.

Methods—GSL and QBA were obtained from six unilateral arm/leg muscles in 36 boys with DMD and 28 healthy boys (age 2–14 years) for up to 2 years. We used a linear mixed-effects model with random intercept and slope terms to compare trajectories of GSL, QBA, and functional assessments. We analyzed separately a subset of boys who initiated corticosteroids.

Results—Compared to healthy boys, increasing GSL in DMD boys >7.0 years was first identified at 6 months (e.g., anterior forearm slope difference of 1.16 arbitrary units/month $p=0.004$, 95% confidence interval (CI) [0.38,1.94]); in boys <7 years, differences in GSL first appeared at 12 months (0.82 arbitrary units /month, $p=0.04$ 95% CI [0.075,1.565] in rectus

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femoris). QBA performed similarly to GSL (e.g., DMD boys >7 years of 0.41dB/month, $p=0.01$, 95%CI [0.096,0.72] in anterior forearm at 6 months). US identified differences earlier than functional measures including 6-minute walk and supine-to-stand tests. However, neither QBA nor GSL showed an effect of corticosteroid initiation.

Interpretation—QBA performs similarly to GSL and both appear more sensitive than functional assessments for detecting muscle deterioration in DMD. Additional studies will be required to determine if quantitative muscle ultrasound can detect therapeutic efficacy.

Keywords

ultrasound; muscle; myopathy; Duchenne muscular dystrophy; biomarker

INTRODUCTION

Clinical trials in Duchenne muscular dystrophy (DMD) generally rely on measures of function and strength, such as the 6-minute walk test, as their primary outcome.^{1–3} Imaging modalities, however, may also be valuable in demonstrating progressive alterations in muscle over time and the effect of therapy. For example, recent work has shown that magnetic resonance imaging (MRI) of muscles is very sensitive to disease progression in DMD.^{4,5} Another imaging modality, quantitative ultrasound, has also been shown preliminarily to identify disease status and progression in DMD. Muscle echo intensity increases with greater muscle fat and fibrosis,^{6,7} worsening strength and function⁸ and, when measured by an experienced examiner, can detect disease progression over time in both very young (age less than three years)⁹ and older (aged 3–15 years) boys with DMD.¹⁰ Quantitative ultrasound also offers some practical advantages over MRI as it can be performed at the bedside, is relatively inexpensive, and is technically easier to evaluate both upper and lower extremities and subjects who cannot lie still or flat.

In this two-year, non-blinded longitudinal study, we evaluated alterations in quantitative muscle ultrasound in boys with DMD and compared them to healthy controls, along with the electrical impedance myography (EIM) measurements and functional assessments, as described in our companion paper.¹¹ Our goal was to determine if quantitative muscle ultrasound, when performed by trained evaluators in a clinical trial setting, could provide effective biomarkers of disease progression in DMD. We measured muscle echointensity in two ways. We utilized a direct analysis of the amplitudes of the reflected ultrasound echoes, measured in decibels, termed quantitative backscatter analysis (QBA), in addition to the more standard analysis of the grey scale pixel level (GSL) in a region of interest (ROI) within the fully processed image. The reason for evaluating QBA specifically is that the relatively large amounts of data contained in the raw backscattered energy may be lost or skewed when proprietary algorithms are used to compress the values into the 256 GSLs for image display. It is unknown whether QBA provides similar, better, or worse outcomes than using GSL for measuring disease progression in DMD.

MATERIALS AND METHODS

Subjects and Recruitment

For detailed information on the demographics, recruitment strategies, initial sample size estimations, and overall study design see the companion paper.¹¹ All studies were approved by the Boston Children's Hospital Institutional Review Board.

Ultrasound Image Acquisition

Transverse US images were obtained using the Terason t3000 system (Teratech Inc., Burlington, MA) with a 10 MHz probe of the dominant side on deltoid, biceps brachii, anterior forearm compartment (a composite of all the muscles in this region), rectus femoris, tibialis anterior, and medial gastrocnemius muscles at predefined locations over each of the muscles (Table 1).¹² Ultrasound gain, depth, focal points, and transducer frequency settings were kept constant for all image acquisitions throughout the study (probe frequency range = 5–12 MHz, depth 5 cm, focal points of 0.6 cm and 1.3 cm). Six research assistants trained by CZ and JW but who otherwise had no prior experience with ultrasound acquisition obtained all images during the 3.5 year study course.¹³ A single image in the transverse plane was acquired to obtain each GSL or QBA value. The first and a second sonographer also each acquired a repeat image of the biceps brachii and rectus femoris to evaluate inter-rater reliability.

We obtained serial ultrasound images over a period of up to 24 months from unilateral proximal (deltoid, biceps brachii, quadriceps) and distal (anterior forearm, tibialis anterior, and medial gastrocnemius) muscles.

Image and Data analysis

Images were analyzed using MATLAB® (The Mathworks, Natick MA). We measured standard median GSL values (arbitrary units (arb.u.)) from a ROI within muscle immediately below the layer of subcutaneous tissue and above bone, from the most superficial 1/3 of the ROI of the muscle as previously described (Figure 1).¹⁰

For QBA, each raw ultrasound data file was exported into a MATLAB® file using software (Ult2Matlab®, provided by Teratech, Inc.). Using additional MATLAB® computer code (provided by Teratech, Inc.), backscatter intensity values were derived from the ultrasound data and used to create images. An identical ROI was placed in each image as for measurement of the GSL. We measured the median backscatter value (decibels (dB)) from the most superficial 1/3rd of the ROI of the muscle exactly as for measurement of the GSL.

For both GSL and QBA, authors CMZ and AP placed the region of interest and performed the GSL and QBA measurement. CMZ ensured quality control by review of the images and regions of interest.

Functional measurements

Two functional assessments, the 6-minute walk test (6MWT) and timed supine-to-stand test were performed, as described in the companion paper.¹¹

Data analysis

For each muscle group, we used separate linear mixed effects models with random intercept and slope terms to account for within-subject correlations and between-subject variability under the missing-at-random assumption to estimate and compare trajectories (slope) of each group. See the companion paper¹¹ for additional details, including reproducibility analyses, application of a false discovery rate analysis, sample size estimations, and the piecewise linear mixed effects model used for assessing the effect of corticosteroids.

RESULTS

Subjects

A total of 37 DMD boys and a total of 31 healthy controls were initially screened and enrolled. Of these, a number were excluded from the analysis or otherwise did not have sufficient data as shown in Consort Diagram in Figure 3 of the companion paper;¹¹ in addition, one healthy control, who only had one visit, had no adequate ultrasound data obtained and was excluded entirely from the analysis. Thus, a total 36 boys with DMD, mean age 7.3 years (range 2.2–13.3) and a total of 28 healthy controls, mean age 7.1 years, (range 3.3 to 14.6 years) had data that were included in the analysis; these ages were comparable ($p = 0.86$). However, 6 of the DMD boys did not return for follow-up visits and thus 30 contributed longitudinal data. 16 boys with DMD were taking steroids throughout the study, 7 started steroids during the study, and 13 were not on steroids. There were a total of 246 healthy subject and 211 DMD QUS assessments over the 2-year period; functional assessment data are provided in the companion paper.¹¹

Alterations in QUS data over time

Of the all images collected, 76/2831 images (2.7 %) were deemed technically inadequate (poor image quality/artifact/technical error) and were excluded from analysis. Both GSL and QBA in DMD generally increased over time compared to controls (Table 2). Age impacted which muscles showed the largest effect over time, with larger effects in older than younger boys with DMD. In older boys with DMD, the GSL from the anterior forearm muscles (slope difference 1.16 arb.u./month $p=0.004$, 95% confidence interval (CI) [0.37,1.94] Figure 2) and upper extremity average (deltoid, biceps brachii, anterior forearm, slope difference 0.62, $p=0.03$ 95% CI [0.071,1.17]) best detected the increasing echo intensity compared to similar aged controls. QBA showed similar differences (See Table 2 for details). These differences increased in significance out to two years.

Changes in younger boys with DMD were subtler than in older boys. In boys 7 years with DMD, the GSL and QBA slope from the rectus femoris (Figure 2) and proximal muscle average (deltoid, biceps brachii, rectus femoris) best detected the worsening echo intensity compared to similar age controls (Table 2). This difference was present at 12 months from baseline (e.g. GSL proximal muscle average slope difference of 0.49 ($p=0.02$ 95% CI [0.098,0.88]) and increased in significance over time out to two years. No control muscle showed an increase in GSL or QBA over the 24-month period. Only the rectus femoris in younger controls showed a decreasing GSL (-0.52 arb.u./month, 95% CI $[-0.81,-0.23]$) and QBA (-0.19 dB/month, 95% CI $[-0.29, -0.083]$) over the 24 months. Application of a false

discovery rate analysis suggested reduced significance of these findings; however, most remained significant.

QBA vs. GSL

QBA did not confer any apparent advantage over GSL in detecting changes over time (Table 1). GSL and QBA slope in boys with DMD increased ($p = 0.03$) similarly over the 24-month period from the biceps brachii, deltoid, and anterior forearm muscles, in contrast to controls (Figure 1). In all boys with DMD, the mean increase in QBA was 0.83 dB/yr. from the biceps brachii, 0.80 dB/yr. from the deltoid, and 1.53 dB/yr. from the anterior forearm. Rates of progression for all muscles are shown in Supplemental Table 1.

Reproducibility assessments were also performed between baseline and 3–7 day visits and was generally good to excellent. Supplemental Table 2 provides intra-examiner reliability values in all muscles. Inter-examiner reliability, which was only measured on biceps and quadriceps, revealed similar values for DMD boys for both GSL and QBA. Specifically, for GSL, intraclass correlation coefficients (ICCs) were 0.89 and 0.59 for biceps and quadriceps, respectively; for QBA, these values were 0.90 and 0.58, respectively.

Effect sizes and clinical trial sample size estimations

We calculated effect sizes and sample sizes for hypothetical clinical trials based on rates of progression, as described in our companion paper.¹¹ Sample sizes necessary for clinical trials were generally smaller using QBA and GSL than with functional measurements, based on the observed effects in our study (see Table 3). For instance, at 12 months to detect a 50% treatment effect, 35 boys would be needed per arm if using GSL of the forearm, whereas 131 would be needed using 6MWT.

Effects of corticosteroids

Seven boys were placed on corticosteroids (including prednisone, prednisolone, and deflazacort) at varying doses at various time points in the study. In these boys, there was no difference in the six-muscle average GSL or QBA comparing slopes post-steroid initiation to those pre-steroid initiation or never on steroids, using a piecewise linear mixed-effects model (e.g., GSL with slope difference of -0.14 arb.u/month 95% CI $[-0.47, 0.19]$, $p=0.41$ and QBA with slope difference of -0.038 dB/month, 95% CI $[-0.14, 0.068]$, $p=0.48$).

DISCUSSION

Muscle echo intensity, measured by two different approaches (GSL and QBA) increases over time in boys with DMD compared to controls; this effect is more apparent in older boys (age >7 years) than younger boys with DMD. As anticipated, the application of a false discovery rate analysis reduced the significance of these results to some extent, yet many remained significant. This parallels the clinical course of boys with DMD, who may not show functional decline until approximately age 7,² and this is similar to prior longitudinal studies of GSL ultrasound and MRI in DMD, as well as to EIM as described in the companion paper.^{4, 5, 10, 11} The muscles in our study that were most sensitive to disease progression in DMD compared to healthy boys included the anterior forearm and upper extremity average

in older boys and the rectus femoris and proximal muscle average in younger boys. This is consistent with well-described clinical patterns in DMD, with the earliest weakness detectable in the proximal arm and leg muscles followed by progressive weakness of the distal upper extremities. Our GSL findings are supported by a prior, smaller study by Jansen et al, which found the largest increase in GSL values over time in the biceps brachii in older boys (8–15 years) and in the rectus femoris in younger boys (4–7 years) with DMD.¹⁰ In addition to studying QBA for the first time, our study differs from prior studies^{9, 10} in that we compare DMD and controls over time and that research assistants and study coordinators, rather than expert neuromuscular sonographers, obtained all images. Our study and prior investigations show that trained examiners can reliably obtain the ultrasound image and measure muscle echointensity.^{12, 15} We were also able to detect increased echointensity over time in DMD despite using six different trained research assistants to acquire the ultrasound images. This supports the feasibility of using trained examiners to perform quantitative ultrasound as would be likely required in a longitudinal, multicenter clinical trial.

A major question that this study addressed was whether QBA would outperform GSL as a detector of disease progression. Our results demonstrated similar performance for both approaches. One reason for evaluating this possibility is that QBA data is derived from the generated image prior to transformation into GSL by a variety of proprietary algorithms used by ultrasound manufacturers to improve image quality. Using QBA would allow for improved ability to compare images acquired across clinical trial sites with devices produced by different ultrasound manufacturers, potentially simplifying clinical trial application. Nonetheless, for the time being, since GSL is ultimately easier to measure and more accessible to the general user than QBA, this will likely remain the preferred measure. Moreover, it is possible to estimate backscatter values based on GSLs using a phantom-based calibration method.¹⁴ Supporting this approach, we detected a mean increase in QBA from the biceps brachii of 0.83 dB/year in boys with DMD. This is nearly identical to a predicted increase of 0.8 dB/year from a prior cross-sectional study of backscatter calibrated from GSLs of the biceps brachii imaged using a different ultrasound system.⁸

Thus, both muscle GSL and QBA appear to be potential biomarkers for DMD clinical trials, particularly in older boys. Both ultrasound outcomes detected changes in DMD over time as early as 6–12 months and, similar to EIM and to quantitative muscle MRI,^{4, 5} changes most in boys age 7 years and older. In a recent study, MRI detected progressive changes in DMD as early as 3 months from baseline, suggesting that it is more sensitive than quantitative ultrasound.⁵ Nonetheless, ultrasound, EIM, and MRI all show deterioration earlier in muscles than currently employed functional assessments in ambulatory boys.^{4, 5, 10} Still, additional studies comparing all of these biomarkers to functional assessments in *non*-ambulatory boys and young men with DMD are needed.^{15, 16}

Neither GSL nor QBA changed following initiation of corticosteroids, a proven clinically effective treatment. As GSL and QBA increase with increasing muscle fat and fibrosis,^{6, 7} it might be expected to change following treatments that reduce the dystrophic pathology but not to corticosteroids, which may have limited direct impact on fat or connective tissue

deposition. Additional studies would be needed to determine the accuracy of this concept and whether the quantitative ultrasound would be sensitive to more effective therapies.

In conclusion, quantitative muscle ultrasound can be performed by trained examiners without additional oversight and detects increasing echo intensity over time in boys with DMD compared to controls. Since ultrasound can be used in boys of all ages, it offers additional advantages to functional assessments, which typically must be applied in age- and developmentally appropriate populations. Muscle echo intensity increases in DMD over time differently depending on age and body region studied and, like functional measures, MRI, and EIM, is more sensitive to change in older than younger boys with DMD. More studies will be needed, however, to determine whether quantitative ultrasound, using either GSL or QBA, is also responsive to therapies that provide long-term functional benefit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Ultrasound images used to measure grey scale levels (GSL, left panels) and reconstructed backscatter (QBA, right panels) levels from the biceps brachii of a 7-year-old boy with Duchenne muscular dystrophy (top panels) and an 8-year-old healthy control (bottom panels). Both GSL and QBA increase over time in DMD but not in controls. We measured GSL and QBA from the most superficial 1/3 (dotted line) of muscle region of interest (solid line), demonstrated in the baseline control images.

Figure 2.

Example of 6-month and 1-year GSL data from longitudinal mixed-effects model in older boys with and without DMD. A, B. Averaged upper extremity GSL mean trajectory for each cohort along with its respective 95% confidence interval. C, D show the models with the baseline differences removed to emphasize the observed longitudinal changes.

Table 1

Locations of transducer placement

Muscle	Location
Deltoids	One-fifth distance from acromion to lateral epicondyle
Biceps Brachii	Arm supine, two-thirds distance from acromion to antecubital fossa
Wrist/Finger Flexors	Arm supine, one-third distance from the medial epicondyle to base of thumb
Quadriceps	Two-thirds distance from inguinal crease to superior aspect of patella, seated knee bent
Tibialis Anterior	One-fourth distance from fibula head to lateral malleolus midpoint, seated, ankle neutral
Medial Gastrocnemius	One-third distance from inferior aspect of popliteal fossa to medial malleolus, seated, ankle neutral

Table 2

Grey scale level (GSL)^{*}, quantitative backscatter (QBA)[†], and functional slope differences (Duchenne – controls) over time.

	N	6 months			12 months			24 months			
		Boys > 7.0 years	Slope Difference	p-value	95%CI	Slope Difference	p-value	95%CI	Slope Difference	p-value	95%CI
Ultrasound		16, 13									
Upper Extremity	GSL		0.62	0.03	[0.071,1.17]	0.50	0.0009\$	[0.23,0.77]	0.41	0.0001\$	[0.21,0.61]
	QBA		0.16	0.12	[-0.036,0.36]	0.17	0.002\$	[0.072,0.27]	0.15	0.0002\$	[0.072,0.23]
Anterior Forearm	GSL		1.16	0.004\$	[0.38,1.94]	0.68	0.0004\$	[0.33,1.03]	0.43	0.0004\$	[0.21,0.65]
	QBA		0.41	0.01	[0.096,0.72]	0.25	0.0006\$	[0.11,0.39]	0.17	0.0001\$	[0.092,0.25]
Proximal Average	GSL		0.21	0.56	[-0.50,0.92]	0.24	0.27	[-0.17,0.65]	0.30	0.02\$	[0.045,0.56]
	QBA		-0.01	0.91	[-0.27, 0.25]	0.06	0.4	[-0.077,0.20]	0.10	0.03\$	[0.022,0.18]
Rectus Femoris	GSL		0.40	0.6	[-1.07,1.87]	0.06	0.87	[-0.72,0.84]	0.06	0.78	[-0.37,0.49]
	QBA		0.07	0.8	[-0.44,0.58]	-0.01	0.92	[-0.28, 0.26]	0.02	0.77	[-0.12,0.16]
Six-muscle average	GSL		-0.43	0.09	[-0.92, 0.06]	0.11	0.49	[-0.20,0.42]	0.22	0.05\$	[0.004,0.44]
	QBA		0.09	0.39	[-0.11,0.29]	0.06	0.29	[-0.058,0.18]	0.09	0.02\$	[0.012,0.17]
6MWT	6, 13		11.9	0.18	[-5.15,29.0]	6.13	0.29	[-4.89,17.15]	-1.74	0.48	[3.02, -6.50]
STST	7, 13		0.84	0.11	[-0.16,1.84]	0.38	0.33	[-0.37,1.13]	0.40	0.12	[-0.07,0.87]
Boys 7.0 years											
Ultrasound		18, 15									
Upper Extremity	GSL		-0.19	0.45	[-0.68, 0.30]	0.16	0.23	[-0.095,0.42]	0.20	0.06	[0.004,0.40]
	QBA		-0.08	0.36	[-0.26, 0.096]	0.02	0.69	[-0.078,0.12]	0.06	0.13	[-0.018,0.14]
Anterior Forearm	GSL		-0.56	0.12	[-1.25, 0.13]	0.13	0.52	[-0.26,0.52]	0.26	0.11	[-0.054,0.57]
	QBA		-0.13	0.27	[-0.37, 0.11]	0.05	0.53	[-0.087,0.19]	0.09	0.14	[-0.028,0.21]
Proximal Average	GSL		-0.27	0.4	[-0.90, 0.36]	0.49	0.02\$	[0.098,0.88]	0.45	0.001\$	[0.20,0.71]
	QBA		-0.11	0.36	[-0.33, 0.11]	0.14	0.05	[0.003,0.28]	0.14	0.003\$	[0.062,0.22]
Rectus Femoris	GSL		-0.81	0.23	[-2.10, 0.48]	0.82	0.04	[0.075,1.57]	0.85	0.0007\$	[0.40,1.30]
	QBA		-0.38	0.1	[-0.83, 0.071]	0.27	0.05	[-0.004,0.54]	0.28	0.001\$	[0.12,0.44]
Six-muscle average	GSL		-0.43	0.09	[-0.92, 0.06]	0.11	0.49	[-0.20,0.42]	0.22	0.05	[0.004,0.44]

Boys > 7.0 years	N	6 months			12 months			24 months		
		Slope Difference	p-value	95%CI	Slope Difference	p-value	95%CI	Slope Difference	p-value	95%CI
QBA		-0.18	0.05	[-0.36, -0.004]	0.03	0.63	[-0.088, 0.15]	0.08	0.04	[0.002, 0.16]
6MWT	10, 11	1.56	0.86	[-15.94, 19.06]	1.24	0.82	[-9.56, 12.04]	-1.13	0.61	[3.18, -5.44]
STST	16, 15	0.088	0.81	[-0.62, 0.79]	0.19	0.49	[-0.34, 0.72]	0.062	0.71	[-0.25, 0.38]

* GSL values are in arbitrary units/month;

[†] QBA values are in decibels/month; values are mean (+/- standard error);

[§] FDR q-value < 0.1;

N, Number of DMD boys, Number of healthy controls; 6MWT, 6-minute walk test; STST, Supine-to-stand test

Effect and sample size estimates for a treatment trial using GSL and QBA as the outcome in boys with DMD compared to functional assessments

Table 3

Study Length		6 months			12 months		
Boys > 7.0 years		Treatment effect	Effect size	Sample	Effect size	Sample	Sample
GSL Anterior Forearm	100%	1.10	14	1.35	9		
	50%		53		35		
QBA Anterior Forearm	100%	0.96	18	1.55	10		
	50%		69		38		
6MWT	100%	0.73	30	0.69	33		
	50%		117		131		
Supine-to-stand test	100%	0.87	21	0.44	82		
	50%		83		325		
Boys 7.0 years							
GSL Rectus Femoris	100%	0.43	87	0.78	27		
	50%		347		105		
QBA Rectus Femoris	100%	0.58	48	0.72	31		
	50%		189		121		
6MWT	100%	0.096	1702	0.089	1963		
	50%		6805		7851		
Supine-to-stand test	100%	0.09	1878	0.27	223		
	50%		7510		892		

These results assume 80% power and monthly evaluations; GSL, grey scale level; QBA, quantitative backscatter analysis; 6MWT, 6-minute walk test