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Quantitative Muscle Ultrasound in Duchenne Muscular Dystrophy: A Comparison of Techniques

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Abstract

Introduction—Muscle pathology in Duchenne muscular dystrophy (DMD) can be quantified using ultrasound by measuring either the amplitudes of sound-waves scattered back from the tissue [quantitative backscatter analysis (QBA)] or by measuring these backscattered amplitudes after compression into grayscale levels obtained from the images (GSL).

Methods—We measured and compared QBA and GSL from 6 muscles of 25 boys with DMD and 25 healthy subjects, aged 2–14 years, with age and, in DMD, with function (North Star Ambulatory Assessment).

Results—Both QBA and GSL were measured reliably (intraclass correlation ≥ 0.87) and were higher in DMD than controls ($P<0.0001$). In DMD, average QBA and GSL measured from superficial regions of muscle increased (rho ≥ 0.47, $P < 0.05$) with both higher age and worse function; in contrast, GSL measured from whole regions of muscle did not.

Discussion—QBA and GSL measured from superficial regions of muscle can similarly quantify muscle pathology in DMD.

Keywords

ultrasound; muscle; myopathy; Duchenne muscular dystrophy; biomarker

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked disease of children characterized by progressive weakness and replacement of muscle with fat and connective tissue, resulting in disability, loss of ambulation, and ultimately death from respiratory or cardiac failure. With the advent of multiple potential therapies, there is a need for sensitive biomarkers to rapidly and reliably identify successful candidate therapies in DMD clinical trials. Standard outcome measures such as the six-minute walk test[1] and North Star Ambulatory Assessment (NSAA)[2] are constrained by subjective elements such as effort and mood of the patients and cannot be performed in boys of all ages and abilities.[3–5] Quantitative muscle ultrasound, in contrast, is a reliable technique[6] for objectively quantifying muscle pathology that can be performed at all ages and does not rely on patient effort. In boys with DMD, increased intramuscular fat and connective tissue results in higher echointensity in the ultrasound image[7,8] that increases with disease progression and with reductions in strength and function over time.[9]

Quantification of the muscle ultrasound echointensity can be performed using different techniques. Echointensity is determined by the amplitude of the acoustic energy reflected back from the tissue to the ultrasound transducer, known as backscatter. The amplitudes of the backscattered sound waves detected by the transducer are measured in decibels [quantitative backscatter analysis (QBA)] and then compressed using proprietary algorithms into 256 gray scale pixel levels [grayscale level (GSL)] for image display. During compression to GSL, data contained in the backscattered energy can be lost or skewed. This is because relatively large amounts of backscattered energy must be compressed into only 256 GSL pixel values and because the backscattered energy may not be uniformly, or linearly, compressed across the dark to bright GSL spectrum. Thus, measurement of GSLs may not yield the same range, distribution, or variation of the actual backscattered energy. GSLs are, however, more accessible than backscatter values. GSLs can be measured directly from the ultrasound images using any standard image-processing software, whereas obtaining backscattered energy information directly from the transducer requires advanced mathematical processing and access to data that is generally concealed by manufacturers from end users. It is unknown whether direct measures of backscatter, when compared to GSL, better quantify the presence and degree of muscle pathology. In this study, we compared measurement of backscatter to GSL from ultrasound images of muscle in healthy boys and those with DMD and assessed their relationships to age and function. We also expand on prior work by evaluating multiple muscles in both the upper and lower extremities and by comparing ultrasound echointensity quantified from different regions within the muscle.

MATERIALS AND METHODS

Subjects and Recruitment

The institutional review board of Boston Children’s Hospital approved the protocol. Informed written consent and verbal assent were obtained, respectively, from, parents and children. All subjects were male and aged 2 to 14 years. Subjects were not permitted to have a pacemaker or other electrical device for inclusion in the study. Boys with DMD were

recruited through the Neurology clinic and had genetic mutations and clinical presentation consistent with DMD. Boys with DMD were excluded if they were involved in an ongoing clinical therapeutic trial or if they had another neuromuscular or other medical condition that impacted health substantially. Healthy subjects were recruited by advertisement and via family members and did not have a history of neuromuscular or other disease that would substantially impact health.

A specially trained and experienced physical therapist (AP) administered the NSAA on all children with DMD who were capable of performing it (n=14, aged 4–13 years).

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 flexors, quadriceps, tibialis anterior, and medial gastrocnemius muscles (Table 1). Dominance was determined by report of the child and parent. When this was unknown, a ball was given to the child to throw to assess the throwing hand. Ultrasound gain, depth, focal points, and transducer frequency settings were kept constant for all image acquisitions. Research assistants trained by the senior investigator but who otherwise had no prior experience with ultrasound acquisition obtained all images.[6] Ultrasound probe placement was standardized for each muscle (table 1). Measurements were made with the subject seated with the knee bent at 90° and the arm extended at mid-chest height with the elbow straight and supported by the examiner or a pillow. A single image in the transverse plane was acquired to obtain each GSL or QBA value.

Image and Data analysis

Median GSL values (arbitrary units, a.u.) were measured using MATLAB ® from 2 regions of interests (ROI), a “superficial” ROI and a “whole muscle” ROI, placed within each muscle image (Figure 1). The superficial ROI was a 250 pixel by 50 pixel rectangle (1 cm × 0.5 cm) placed within muscle immediately below the layer of subcutaneous tissue and above bone. Rarely, the superficial ROI size was decreased to fit within the rectus femoris and avoid artifact from inter-muscular fascia. The whole muscle ROI was a rectangular ROI drawn to include as much muscle as possible from the superficial to deep fascia or bone while remaining within the lateral margins of the imaged muscle.

For quantitative backscatter analysis (QBA), each ultrasound image 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 “raw” ultrasound data and WERE used to create images. A superficial ROI was placed in each image as described above, and the median backscatter value [in decibels (dB)] within the ROI was measured; a whole muscle ROI was not performed for the QBA analysis.

For reliability testing, we performed repeated measures on a representative sample of 36 GSL and 36 QBA images that included each examined muscle region from healthy and dystrophic muscle of all levels of severity. Two raters (TG and IS) independently performed repeated superficial ROI placement and analysis; their data were compared to determine

inter-rater reliability. One rater (TG) repeated measurements twice to determine intra-rater reliability.

Results were evaluated for each individual muscle and for an average of all 6 muscles from each subject. Reliability was tested using intraclass correlation (ICC), percent variation, and Bland-Altman analysis. Mann-Whitney tests were performed for two-group comparisons. Wilcoxson signed rank test for paired samples was performed for comparison of different muscle groups. Spearman correlations were performed to determine the relationships between GSL, QBA, age, and NSAA. A Fisher r-to-z transformation was used to compare rho values. A *P*-value of <0.05 was considered significant.

RESULTS

Subject Demographics

We enrolled 25 boys with DMD and 25 healthy boys of similar (*P*>0.05) age [8.1 (2.2–13.4) vs. 7.0 (2.1–12.6) years] and weight [21.3 (12.0–65.0) vs. 28.1 (12.7–41.8) kg]. At the time of the study, 56% (14/25) of patients with DMD were on corticosteroids. Fourteen DMD subjects completed the NSAA.

Comparisons of dystrophic and healthy muscle

Both QBA and GSL from superficial ROIs in each muscle and on average were higher (*P*<0.0001) in DMD subjects than controls (Figure 2). GSLs from whole muscle ROIs in each muscle and on average were also higher in DMD than controls (*P*<0.0001). In boys with DMD, the quadriceps generally showed the highest echointensity. In DMD, the quadriceps had higher QBA and GSL from superficial ROIs than every other muscle (QBA: p<0.05, GSL: *P*<0.03, Figure 2), except for QBA of the medial gastrocnemius (*P*=0.06). Even in the youngest boys with DMD (age 8 years or younger, n=16), the quadriceps was brighter than every other muscle (QBA: *P*<0.032, GSL: *P*<0.021) except for GSL of the medial gastrocnemius (*P*=0.06).

Relationship between GSL, QBA, Age and NSAA

GSL and QBA from each muscle and on average (Figure 3) showed a linear compression throughout the range of values (rho 0.90–0.97, all *P*<0.001). In DMD, GSL and QBA from superficial ROIs increased similarly with age and the NSAA on average and in most individual muscles (Table 2, Figure 4). Both GSL and QBA from superficial ROIs from the deltoid, forearm flexors, and tibialis anterior but not from the quadriceps or medial gastrocnemius increased with age. QBA from the superficial biceps brachii also increased with age. Both GSL and QBA from superficial ROIs on average and from the tibialis anterior increased with worsening performance on the NSAA (Table 2). In contrast, neither average nor individual muscle GSL from whole muscle ROIs varied with age (*P*>0.06) or NSAA score (*P*>0.1). In healthy controls, neither QBA nor GSL from superficial ROIs varied with age.

QBA and GSL Reliability Analysis

In superficial ROIs from both GSL and QBA, Bland-Altman analyses revealed strong reproducibility (Figure 5). Both GSL intra-rater (0.96) and inter-rater (ICC 0.99) reliability and QBA intra-rater (0.91) and inter-rater (0.87) reliability were high. There was minimal variability between repeat values for both GSL intra-rater (8.67%) and inter-rater (4.86%) and QBA intra-rater (6.10%) and inter-rater (8.11%). Reliability of GSL from whole muscle ROI was reported previously [6] and was not repeated.

DISCUSSION

GSL performed comparably to QBA for quantifying the presence and degree of dystrophic pathology. This supports the basic premise of measuring GSL as a proxy for the actual backscattered energy when quantifying the underlying structural and compositional changes in DMD muscle. We found similarities between GSL and QBA likely because in the ultrasound system and settings used in our study, the backscatter values were compressed linearly and uniformly into grayscale pixel levels. Fortunately, some of the other commonly used ultrasound systems also provide linear compression settings.[10] However, it is possible that in ultrasound systems with non-linear compression, the relationship between GSL and QBA may differ across the range of values and this could confound measurements.[11] Even with a linear compression curve, GSLs represent only a portion of the acquired backscatter data. It remains possible that small changes in echointensity, as might be observed in dystrophic muscle over time, could be captured better through measurement of the uncompressed backscatter than the compressed GSL values.

The optimal muscle groups for detecting changes with worsening pathology and over time in DMD may differ depending on the age of the child and the severity of the disease.[9] We found GSL and QBA increased similarly in both proximal and distal arm and leg muscles in boys with DMD compared with healthy boys. However, correlations between GSL and QBA with age and function were not uniform in different muscles. We found a correlation between age and GSL or QBA measured from the deltoid, biceps brachii, forearm flexors, and tibialis anterior but not from the quadriceps or medial gastrocnemius. Prior work showed similar findings, with muscle echointensity increasing over time more in distal than proximal dystrophic muscles.[9] In DMD, more severely or earlier affected proximal muscle groups, such as the quadriceps, may suffer from a ceiling effect, in which the severity of the abnormality plateaus upon reaching a maximum. The gastrocnemius may also not be an ideal muscle group to study age-related changes with ultrasound in boys with DMD, since calf hypertrophy and pseudohypertrophy can alter the echointensity, obscuring a relationship between echointensity and age.[12] GSL and QBA levels averaged from multiple muscles, but not from most individual muscles, correlated with the NSAA. This may be because the NSAA is a composite functional assessment of multiple muscles or because variability in GSL and QBA measurements was decreased by averaging results from multiple muscles. Only 14 of 25 boys were able to complete the NSAA which may also have affected these results. Future longitudinal studies of young and older boys and men with DMD should include imaging multiple muscle groups and assessments of strength and function tailored to

the patient's age and ability to best determine how echointensity changes over time and with worsening pathology in DMD.

These data support that echointensity is best measured from the superficial region of the muscle when quantifying levels of dystrophic pathology using ultrasound. GSL measured from the superficial region of muscle correlated with age and function, whereas GSL measured from the larger, entire region of the muscle did not. Work by others has also shown that GSL measured from the superficial region of muscle increases over time, paralleling the development of worsening strength and function.[9] Limiting quantification to the superficial portion of muscle may be advantageous for several reasons. It minimizes effects of attenuation and reduces variability when quantifying echointensity between systems.[10] We also show that muscle echointensity from relatively small, superficial ROIs can be quantified reliably by trained research technicians without specific training in either ultrasound or radiology, similar to other recent work demonstrating high reliability measuring GSLs in images obtained by different examiners and between research technicians and a radiologist. [6] The approach of using trained research technicians closely parallels typical data collection of larger scale clinical trials, further supporting a role for quantitative muscle ultrasound for this purpose.

In conclusion, GSL of superficial muscle regions correlates with disease status in DMD and performs similarly to actual backscattered data when compression is linear. While GSL and backscatter in dystrophic muscle generally increase with higher age and worsening function, in individual muscles these relationships are less consistent. Longitudinal studies of multiple muscle groups are required to determine whether GSL is sufficient for the more challenging task of identifying disease progression over relatively short periods of time, a prerequisite for its incorporation into clinical therapeutic trials.

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ABBREVIATIONS

DMD	Duchenne muscular dystrophy
QBA	Quantitative backscatter analysis
NSAA	North Star Ambulatory Assessment
GSL	Grayscale level
ROI	Region of interest
dB	Decibels
ICC	Intraclass correlation
PV	Percent variation

References

1. Enright PL. The six-minute walk test. Respiratory care. 2003; 48:783–785. [PubMed: 12890299]
2. Mazzone E, Messina S, Vasco G, Main M, Eagle M, D'Amico A, Doglio L, Politano L, Cavallaro F, Frosini S. Reliability of the North Star Ambulatory Assessment in a multicentric setting. Neuromuscular Disorders. 2009; 19:458–461. [PubMed: 19553120]
3. Bushby K, Connor E. Clinical outcome measures for trials in Duchenne muscular dystrophy: report from International Working Group meetings. Clinical investigation. 2011; 1:1217–1235. [PubMed: 22639722]
4. Finkel R, Wong B, Bushby K, Reha A, Elfring G, Miller L, Babiak J, Morsy M, Peltz S, Welch E. P3. 51 Results of a Phase 2b, dose-ranging study of ataluren (PTC124®) in nonsense mutation Duchenne/Becker muscular dystrophy (nmDBMD). Neuromuscular Disorders. 2010; 20:656–657.
5. Aaron R, Esper GJ, Shiffman CA, Bradonjic K, Lee KS, Rutkove SB. Effects of age on muscle as measured by electrical impedance myography. Physiological measurement. 2006; 27:953. [PubMed: 16951455]
6. Zaidman CM, Wu J, Wilder S, Darras B, Rutkove S. Minimal training is required to reliably perform quantitative ultrasound of muscle. Muscle & nerve. In Press.
7. Pillen S, Arts IM, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. Muscle & nerve. 2008; 37:679–693. [PubMed: 18506712]
8. Pillen S, Tak RO, Zwarts MJ, Lammens MM, Verrijp KN, Arts IM, van der Laak JA, Hoogerbrugge PM, van Engelen BG, Verrips A. Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. Ultrasound in medicine & biology. 2009; 35:443–446. [PubMed: 19081667]
9. Jansen M, van Alfen N, Nijhuis van der Sanden MW, van Dijk JP, Pillen S, de Groot IJ. Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. Neuromuscular disorders. 2012; 22:306–317. [PubMed: 22133654]
10. Zaidman CM, Holland MR, Hughes MS. Quantitative Ultrasound of Skeletal Muscle: Reliable Measurements of Calibrated Muscle Backscatter from Different Ultrasound Systems. Ultrasound in medicine & biology. 2012; 38:1618–1625. [PubMed: 22763008]
11. Zaidman CM, Holland MR, Anderson CC, Pestronk A. Calibrated quantitative ultrasound imaging of skeletal muscle using backscatter analysis. Muscle & nerve. 2008; 38:893–898. [PubMed: 18563722]
12. Reimers CD, Schlotter B, Eicke BM, Witt TN. Calf enlargement in neuromuscular diseases: a quantitative ultrasound study in 350 patients and review of the literature. Journal of the neurological sciences. 1996; 143:46–56. [PubMed: 8981297]

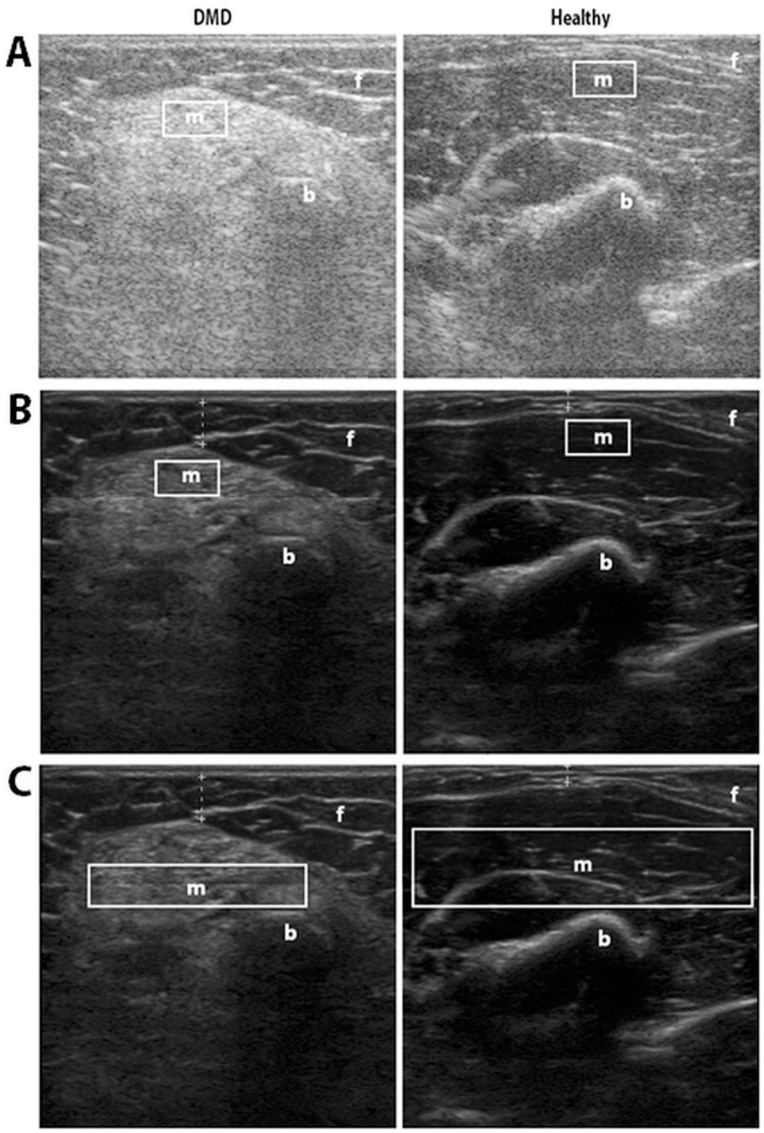

FIGURE 1. QUANTITATIVE BACKSCATTER ANALYSIS (QBA) AND GRAYSCALE LEVEL (GSL) REGIONS OF INTEREST

Echointensity is higher in ultrasound images of biceps brachii muscles from an 11 year-old boy with Duchenne muscular dystrophy (left panel) compared with a same aged healthy control (right panel) in both images created directly from the received backscatter (A) and GSL images generated by the ultrasound system (B). QBA and GSL measured from superficial regions of interest (A and B) generally increased with higher age and worse function; GSL measured from larger regions of interest encompassing the whole muscle (C) did not. White rectangle: region of interest. f: subcutaneous fat, m: muscle, b: bone.

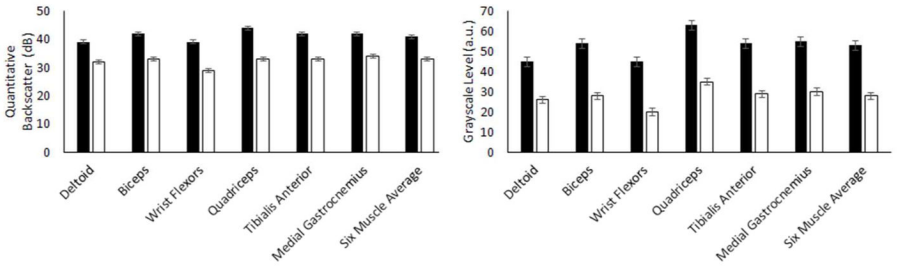

FIGURE 2. QUANTITATIVE BACKSCATTER ANALYSIS (QBA) AND GRAYSCALE LEVEL (GSL) OF MUSCLES IN DMD AND CONTROLS

QBA (a) and GSL (b) are higher (P<0.0001) in all muscles and the average of 6 muscles in DMD than control subjects. dB, decibels. a.u., arbitrary units. Black: DMD. White: Control.

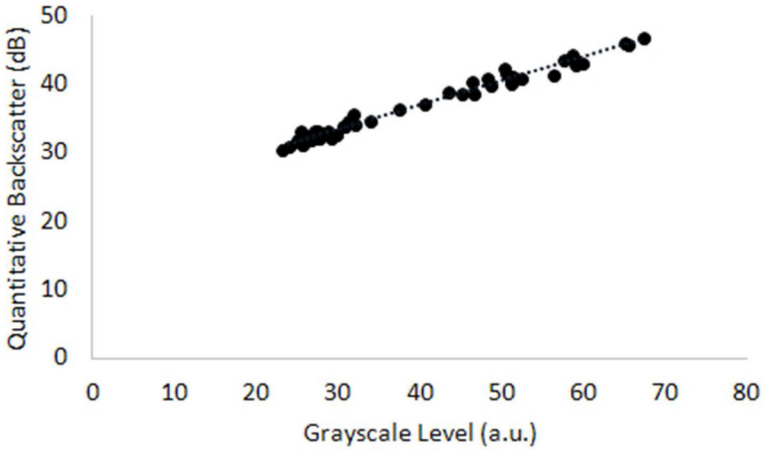

FIGURE 3. RELATIONSHIP BETWEEN QUANTITATIVE BACKSCATTER AND GRAYSCALE LEVELS

Quantitative backscatter (QBA) is compressed linearly to grayscale levels (GSL) across the range of values (rho=0.97 and $P<0.001$). dB, decibels. a.u., arbitrary units.

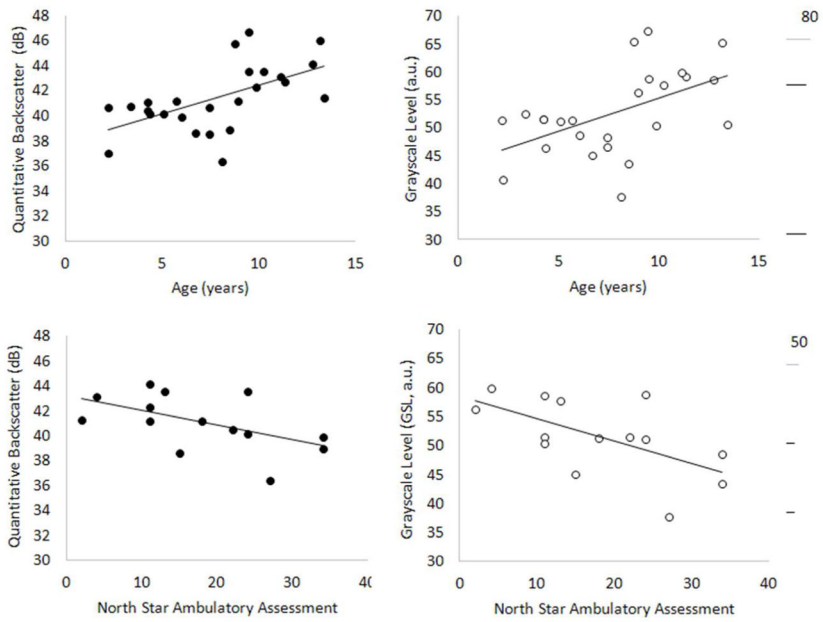

FIGURE 4. QUANTITATIVE BACKSCATTER AND GRAYSCALE LEVELS VARY WITH AGE AND FUNCTION IN DMD

The average quantitative backscatter analysis (QBA, left) and grayscale level (GSL, right) both increased with age (A and B) and lower function on the North Star Ambulatory Assessment (NSAA, C and D). dB, decibels. a.u., arbitrary units.

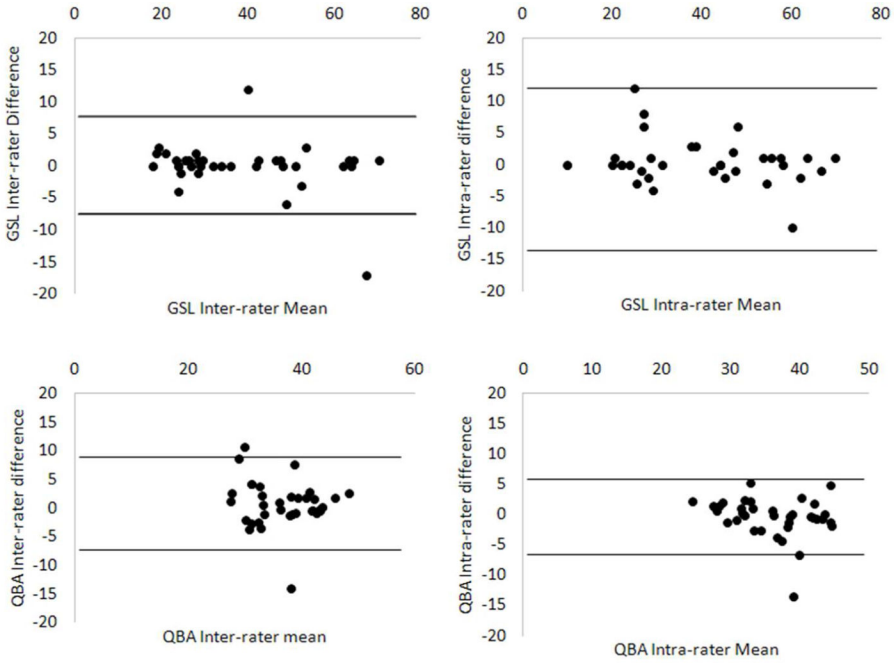

FIGURE 5. INTRA-RATER AND INTER-RATER BLAND-ALTMAN ANALYSIS

Both grayscale (GSL, top panels) and quantitative backscatter (QBA, bottom panels) show good inter-rater (a and c) and intra-rater (b and d) reliability. a. GSL inter-rater Bland-Altman plot with CR=5.23. b. GSL intra-rater Bland-Altman plot with CR=8.69. c. QBA inter-rater Bland-Altman plot with CR=5.78. d. QBA Intra-rater Bland-Altman plot with CR=4.28. CR, coefficient of repeatability.

Table 1

Locations of Ultrasound Measurements

Muscle	Location
Deltoid	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 with 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

Spearman correlations between quantitative backscatter analysis (QBA) and grayscale level (GSL) with age and NSAA measured from superficial regions of muscle in DMD and control subjects.

	Age				NSAA	
	DMD (n=25)		Control (n=25)		DMD (n=14)	
Muscle	**QBA**	**GSL**	**QBA**	**GSL**	**QBA**	**GSL**
Deltoid	0.70***	0.63***	0.097	0.030	−0.36	−0.33
Biceps Brachii	0.54**	0.38	0.17	0.050	−0.20	−0.28
Wrist/Finger Flexors	0.58**	0.53**	−0.20	−0.320	−0.19	−0.18
Quadriceps	0.014	0.20	0.0030	0.059	−0.31	−0.17
Tibialis Anterior	0.45*	0.40*	0.14	0.15	−0.76**	−0.68**
Gastrocnemius	0.11	0.058	−0.11	−0.20	−0.27	−0.44
Six Muscle Average	0.67***	0.47*	−0.0020	−0.085	−0.59*	−0.60*

* $P<0.05$,

** $P<0.01$,

*** $P<0.001$;

DMD: Duchenne Muscular Dystrophy, NSAA: North Star Ambulatory Assessment, QBA: quantitative backscatter analysis; GSL: Grayscale level