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Quantitative Ultrasound Using Backscatter Analysis in Duchenne and Becker Muscular Dystrophy

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Abstract

Evaluation of ultrasound images of muscle with calibrated muscle backscatter (cMB) provides reproducible quantitative measurements of muscle pathology. Increased cMB is associated with greater muscle pathology. We used cMB to evaluate the severity of muscle pathology in 55 patients with Duchenne and Becker Muscular Dystrophy (D/BMD) compared to 77 controls. cMB was also compared to measurements of strength and function. cMB in DMD and BMD increased linearly with age and was higher than in controls when groups are compared. cMB increased twice as fast with age in DMD than in BMD. In DMD, cMB was higher with reduced function and strength. Ultrasound measurement of muscle pathology using cMB is a sensitive and objective quantitative technique for determining the severity of muscle pathology in dystrophinopathies. Longitudinal studies are required to determine the sensitivity of this measure to changes in pathology over time.

Keywords

Ultrasound; Backscatter; Myopathy; Muscle; Children; Duchenne Muscular Dystrophy

Introduction

Duchenne (DMD) and Becker (BMD) muscular dystrophies are progressive myopathies caused by mutations in the dystrophin gene [1]. Necrosis and regeneration of muscle fibers is associated with progressive replacement of muscle with fat and connective tissue. The course of D/BMD is generally measured by serial clinical assessments of quantitative strength, pulmonary function, and functional rating scales [2]. These measures rely on patient effort or on subjective rating of function. There are no validated objective measures of disease progression or muscle pathology in very young or severely weak boys. Objective, reliable measures of disease pathology, that are independent of patient effort, are needed to improve the inclusion and monitoring of patients in clinical trials.

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Ultrasound and MRI imaging have been explored as adjuncts to the physical examination in the assessment of patients with neuromuscular disorders [3-6]. Ultrasound offers some advantages to MRI as it is rapid, inexpensive, and can be performed in the outpatient clinic without need for sedation. Qualitative and quantitative ultrasound methods have been used to visualize skeletal muscle pathology in DMD [3,7-10] and experimental mouse models [11]. We have developed a reproducible, clinically accessible, quantitative technique for measuring skeletal muscle pathology using backscatter analysis of calibrated ultrasound images (*calibrated muscle backscatter, cMB*) [12].

cMB is an estimate of the level of acoustic energy reflected by tissue back to the transducer and is derived from the measurement of the average grayscale level in a region of interest in the muscle. The ultrasound system is configured so that the grayscale measurement in tissue provides an estimate of the level of backscattered energy, expressed as the amount of gain required to produce an equivalent average grayscale level in a specific phantom (with all other scanner settings kept unaltered) [13]. This configuration identifies the range of the linear relationship between the displayed grayscale values and the change in gain, so that changes in the grayscale intensity within this range uniformly reflect changes in the estimated backscatter levels. The amplitudes of the ultrasound echoes reflected by the tissue in the region of interest constitute one of the factors influencing the final grayscale values, and therefore cMB, in the region of interest. cMB levels of skeletal muscle are reliably obtained, increase in patients with muscle pathology, and can be measured independently of patient ability or effort [12]. The purpose of this study was to determine whether cMB measurements in DMD and BMD patients can detect differences in the degree of skeletal muscle pathology and correlate with clinical measures of strength and disease severity.

METHODS

This protocol was approved by the Washington University institutional review board. All subjects/guardians gave written consent/assent. We enrolled 55 males with dystrophinopathies and a clinical presentation consistent with DMD or BMD. 39 patients, aged 9 months to 19 years, had been diagnosed with Duchenne muscular dystrophy. 16 patients, aged 1 to 47 years, had been diagnosed with Becker muscular dystrophy. All had either a muscle biopsy with absent (DMD) or reduced (BMD) dystrophin, genetic confirmation of D/BMD (n=41), or a maternal male family member with clinical and pathologic or genetic diagnosis of D/BMD. BMD in young subjects was additionally confirmed by the presence of an older family member with clinical features of BMD (late onset of presentation or ability to ambulate into adulthood). 74% (29/39) of DMD, and 25% (4/16) of BMD, patients were taking corticosteroids at the time of enrollment. Of the 10 DMD boys not taking corticosteroids, 6 were under age 5 years. Controls included 40 children (26 males), aged 1 to 18 years, and 37 adults (17 male) aged 22 to 47. Controls had no history of weakness or neuromuscular disorders involving the studied limb.

We obtained ultrasound images of the elbow flexors (biceps brachii and brachialis) in all subjects and of the rectus femoris in 16 DMD, 5 BMD and 21 control children. Ultrasound images in the arm were acquired with the elbow extended and the hand open in all subjects. Subjects were seated with the knee bent and with the arm extended anteriorly at the shoulder and supported by a pillow or table at approximately mid-thoracic height. The probe was oriented longitudinally to the muscle length. The middle of the probe was placed at the area of maximal enlargement over the arm and rectus femoris (approximately 2/3 of the way distal from the lateral tip of the acromion to the lateral epicondyle of the humerus in the arm and from the anterior superior iliac spine and the superior tip of the patella in the leg). In each location the probe orientation was adjusted to yield an image with the most superficial bone reflection and then angled to achieve the brightest and narrowest bone reflection. Three

images were obtained with the probe reoriented between measurements. All images were obtained by CMZ using a Philips HD11XE imaging system with an L12-5 linear array probe.

The ultrasound system was configured and calibrated for estimation of backscatter intensity (expressed in decibels, dB). Images were analyzed and measured as described previously [12]. Serial images of a reference phantom object at known decibel increments were obtained to determine the conversion factor needed to relate changes in grayscale values obtained by the ultrasound machine to the equivalent change in decibels. This also established the range in which there is a linear relationship between the measured grayscale values and estimated backscatter (expressed in decibels). Imaging parameters (gain, transmit focus, and time gain compensation settings) were uniform in all subjects. Eight-bit bitmap (BMP) images were exported and the mean grayscale values over the region-of-interest, defined as the entire depth of muscle between the subcutaneous tissue and humerus in the arm or the fascia overlying the vastus intermedialis in the leg (excluding the lateral margins of the imaged muscle, Figure 1), were determined using NIH ImageJ™ v1.37 and averaged across each of the three images. This resulted in a wide and deep region of interest to obtain a mean grayscale value for each image. Averaging grayscale values across a large region of interest minimizes any effects of inhomogeneity. Each BMP image was analyzed as exported from the ultrasound system without any adjustments or manipulations. All ultrasound measurements gave mean grayscale values in the linear range of the calibration curve for the ultrasound machine. *Calibrated muscle backscatter* values (cMB) were calculated by dividing the average grayscale values by the slope of the best-fit line relating grayscale levels to backscatter (6.6 grayscale levels/dB) and then subtracting the backscatter of a reference phantom (13.7dB -obtained by imaging a CIRS Grey Scale Ultrasound Phantom, model 047, using the same imaging parameters as in human subjects). The calculation for cMB was (grayscale level/6.6) –13.7.

Assessments of strength and function were performed at Washington University, St Louis by a neuromuscular physician or physical therapist as part of clinical care or natural history studies. Results were obtained by record review. Upper extremity function was measured using the Brooke upper extremity functional rating scale [14] in 17 boys with DMD, aged 1 to 17 years, and five boys with BMD aged 9 to 18 years. Elbow flexor strength was rated using the modified Medical Research Council manual muscle testing scale [15] in 26 boys with DMD, aged 4 to 19 years, and seven boys with BMD, aged 9 to 18 years. Ultrasound evaluation was performed in the same arm and, on average, within 2 months (range: 0 to 7 months) of the strength and functional assessments.

Statistics were performed using SPSS graduate pack version 14.0 and expressed as mean (standard deviation). Tests include independent and paired t-tests, Mann-Whitney, Pearson's partial correlation (r), Spearman's rank correlation (r_s), univariate analysis, and linear regression modeling. Because of the high inter-correlation among height, weight, and age, only one of these measures could be included in the linear regression model. As cMB and muscle echo intensity increase with age [7,12,16] and DMD and BMD are known to worsen with age, we included age in the linear model as the measure of most clinical relevance. For univariate analysis comparing cMB in DMD, BMD, and controls, we included diagnosis, age, an interaction between age and diagnosis, and a Bonferroni correction in the analysis. Parametric distributions were confirmed using the Kolmogorov-Smirnoff test.

Correlation analysis between cMB, strength, and function was performed on DMD children only. Normal strength was defined as an MRC rating of 10. Normal function was defined as a Brooke functional rating of 1. Normal cMB of the elbow flexors was defined for these subjects as equal to or below the highest cMB obtained in the child control group.

Results

cMB in controls

There was no difference in cMB between male and female controls ($p=0.9$). For controls who had images obtained of both the arm and leg, cMB was higher in the elbow flexors (1.6(2.7) dB) than in the rectus femoris ($-0.2(2.2)$ dB, paired $t=3.4$, $p=0.003$).

cMB is higher in DMD than BMD and Control Subjects

cMB in the elbow flexors and rectus femoris was higher in DMD than BMD subject and control groups (Table 1). cMB in the elbow flexors was higher in BMD subjects than controls. In children aged five years or less, cMB was greater than 1.6 dB in the elbow flexors in eight of 10 boys with DMD but in only three of 17 controls (80% sensitivity and 82% specificity). cMB increased linearly with age without an additional polynomial (age^2) effect in DMD ($r=0.8$, $p<0.001$) and BMD ($r=0.7$, $p=0.001$) (Figures 1 and 2). cMB of the elbow flexors increased twice as much per year in DMD than BMD patients (Figure 3). Unlike in controls, D/BMD boys who had images obtained of both the arm and leg had similar cMB in the elbow flexors (3.8(2.7) dB) and rectus femoris (4.1(4.2) dB, paired $t=-0.4$, $p=0.7$).

cMB in elbow flexors is increased with reduced function and strength in DMD patients

In DMD patients cMB in the elbow flexors was greater with worse upper extremity function ($r_s=0.6$, $p=0.02$) and weaker elbow flexor strength (MRC) ($r_s=-0.4$, $p=0.03$, Figure 4). Age was also greater with worse upper extremity function ($r_s=0.6$, $p=0.006$) and less strength ($r_s=0.5$, $p=0.01$). cMB values were abnormal (higher than the maximal value seen in child controls: 7.3 db) in 21 of 26 boys (ages 6 to 19 years) with abnormal strength or function. cMB levels were also abnormal in five of 10 boys (ages 6, 6, 7, 8, and 8 years) with normal function. Only four of 25 boys (ages 4, 5, 5, and 8 years) with abnormal strength had normal cMB levels. The three DMD boys with abnormal function but normal cMB levels were ages 5, 6, and 8 years.

There is a wide range of cMB levels in DMD patients with similar levels of strength or function (Figure 4). For example, in eight boys (ages 5-12 years) with an MRC score of seven, cMB levels ranged from 5.4 to 15 dB. In the 10 boys (ages 1-8 years) with normal function, cMB levels ranged from 0.8 to 10.1 dB. cMB levels also varied in BMD patients with normal strength or function. Of the six patients with BMD and normal strength or function, cMB was abnormal in two (ages 15 and 18 years) and ranged from 0 to 9.1 dB. All patients with BMD had normal function or strength except one (age 12 years, MRC score: 8, cMB elbow flexor: 3.5 dB).

Discussion

cMB differentiates DMD, BMD, and control subject groups

We found that cMB in the elbow flexors (and rectus femoris in DMD) differentiates among subjects with DMD, BMD, and controls. A difference from controls is detectable in some but not all DMD boys younger than five years old and increases with age, becoming most prominent in the teenage years. Strength and functional outcomes are difficult to measure in boys with DMD at these ages. As cMB can be measured objectively and without patient effort, it could theoretically be used as a clinical outcome marker for children who are too young, or too weak, for reliable strength measurements or functional rating scores [2]. The use of cMB to measure different degrees of pathology could be optimized by defining and controlling for effects of attenuation, tissue heterogeneity, and variations between ultrasound systems.

cMB increases linearly with age in DMD and BMD

The linear relationship between cMB and age differs from absolute strength measurements in DMD, which increase until about age eight and then gradually decline [17]. cMB levels in DMD thus more accurately reflect the progressive changes in muscle pathology with age than quantitative strength measurements. The increase in cMB with age likely results from the progressive replacement of muscle with fat and connective tissue [7,18,19]. The impact by treatment with corticosteroids or other therapies remains to be determined. Such differences have been measured using ultrasound in *mdx* mice [11]. We could not adequately evaluate differences in cMB due to steroid treatment because most of our DMD patients were on steroids, while BMD patients were usually not.

cMB is a measure of disease severity

To determine the relationship between cMB and disease severity, we compared cMB measurements between differentially affected muscles in the arm and leg, between DMD and less severely affected BMD subjects, and to measures of strength and function. In controls but not in D/BMD, cMB in an individual subject is higher in the elbow flexors than in the rectus femoris. This differential increase in cMB levels in the leg compared to the arm is consistent with the earlier onset and more severe degree of weakness in the quadriceps than elbow-flexors in D/BMD [20]. cMB in the elbow flexors is higher and increases twice as much with age in DMD compared to BMD subjects. This differential increase in cMB with age reflects the greater severity of disease in DMD compared to BMD.

cMB is higher in DMD subjects with reduced function and strength. There is also a wide range of cMB levels in D/BMD patients with similar levels of strength or function. These variations may be due to differences in the effect of age or pathology on cMB, strength, and function. Longitudinal studies of cMB are needed to determine how cMB levels in young and older dystrophinopathy patients change over time and in relationship to other quantitative outcomes measures. Few prior studies have assessed the relationship between ultrasound signal intensity and function in neuromuscular diseases. A previous study found no correlation comparing ultrasound signal intensity and function in children with neuromuscular disorders [18]. Our findings may differ as we measured quantitative backscatter of calibrated images, different functional outcomes, and our larger sample size focused only on DMD boys. Additional studies are needed to determine the relationship between cMB and strength and function in other neuromuscular disorders as this may vary with pathophysiology.

Conclusion

Ultrasound measurement of muscle pathology using cMB is a sensitive, objective technique for measuring the presence and severity of disease in D/BMD. cMB was higher with reduced function and strength in DMD boys and can be measured in patients who are too young or weak to participate these assessments. cMB of the elbow flexors, which are involved later and to a lesser degree than the quadriceps, is a sensitive index of disease severity over a wide range of ages in D/BMD. cMB could potentially be implemented as an outcome measure for use in the clinical setting. Prospective, longitudinal studies are needed to assess the relative sensitivity of cMB to measure changes in disease pathology as compared to strength and functional outcome measures and for detecting the effects of treatment in neuromuscular disorders.

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Figure 1. Ultrasound Images of the Elbow Flexors in DMD and Control Subjects

Longitudinal ultrasound images of the elbow flexors are shown in DMD boys, ages A: 1.5 years, cMB 3.0dB, B: 8 years, cMB 10.1 dB, and C: 15 years 15.7 dB. An 8 year old control is shown in D: cMB -1.8 dB with the region of interest outlined in the muscle (m) between the subcutaneous fat (sc) and bone (b).

Figure 2. cMB in the Elbow Flexors in DMD Boys and Controls

In DMD boys, cMB in the extended arm increases linearly with greater age. In controls, the increase with age is more gradual. When comparing groups of subjects with controls, the difference between DMD and controls is detectable even for the 5 years and younger group ($p < 0.01$) and increases with greater age.

Figure 3. cMB in the Elbow Flexors Differentiates Boys with Duchenne from Becker Muscular Dystrophy

cMB of the elbow flexors was higher in boys with DMD (solid line) than BMD (dashed line, $p < 0.001$), after accounting for age and age*diagnosis. cMB increased approximately 0.8 dB/year in DMD and 0.4 dB/year in BMD boys (age < 20 years). The mean (SD) cMB of the elbow flexors in adult men ages 35-47 years, four with BMD and seven healthy controls, is also shown.

Figure 4. Ultrasound cMB in DMD Patients: Relations to Function and Strength

In DMD patients cMB in the elbow flexors was greater with less upper extremity function ($r_s=0.6$, $p=0.02$) and weaker elbow flexor strength (MRC) ($r_s = -0.4$, $p=0.03$). There is a wide range of cMB levels in patients with similar levels of strength or function. The maximal value seen in child controls was 7.3 dB (dotted line).

Calibrated Muscle Backscatter in Controls and Duchenne and Becker Muscular Dystrophy cMB (mean(SD)) in the arm and leg was higher in DMD than controls and BMD after accounting for age and an interaction between age and diagnosis. cMB in the rectus femoris was not assessed (n/a) in adult controls.

Table 1

Muscle(s)	cMB (db)					P values		
	Control Children	Control Adults	All Controls	DMD	BMD	DMD		BMD
						vs. controls	vs. BMD	vs. controls
Biceps Brachii and Brachialis	1.6 (2.8)	4.6 (2.6)	3.0 (3.0)	7.9 (4.9)	5.8 (3.2)	<0.001	<0.001	<0.001
Rectus Femoris	-0.2 (2.3)	n/a	-0.2 (2.3)	4.6 (4.6)	2.5 (2.2)	<0.001	0.004	0.1