Published in final edited form as:

Ultrasound Med Biol. 2012 September ; 38(9): 1618–1625. doi:10.1016/j.ultrasmedbio.2012.04.020.

QUANTITATIVE ULTRASOUND OF SKELETAL MUSCLE: RELIABLE MEASUREMENTS OF CALIBRATED MUSCLE BACKSCATTER FROM DIFFERENT ULTRASOUND SYSTEMS

Craig M. Zaidman*, Mark R. Holland†, and Michael S. Hughes‡

*Department of Neurology, Neuromuscular Division, Washington University School of Medicine, St. Louis, MO, USA

†Department of Physics, Washington University, St. Louis, MO, USA

‡Department of Medicine, Cardiovascular Division, Washington University School of Medicine, St. Louis, MO, USA

Abstract

Widespread implementation of quantitative muscle ultrasonography in assessing skeletal muscle pathology is limited by an inability to replicate results between different ultrasound systems. We have developed a measurement of skeletal muscle pathology, calibrated muscle backscatter (cMB), which should be reproducible between different ultrasound systems. We compared the reliability of grayscale and cMB measurements between different ultrasound systems, configurations and region-of-interest (ROI) sizes. cMB of skeletal muscle was reliably measured (intraclass correlation coefficient [ICC] 0.98) despite very dissimilar grayscale levels (ICC 0.54). cMB reliability was highest between systems using similar settings (ICC: 0.82–0.98) and was lowest when transducer type varied (ICC: 0.47–0.71). Reliability was better from ROIs spanning a narrow range of depths compared with larger ranges. cMB measurements are more reliable than grayscale between different ultrasound systems and configurations. Measuring cMB could improve widespread implementation of quantitative ultrasound in assessments of skeletal muscle pathology.

Keywords

Ultrasound; Muscle; Myopathy; Backscatter; Reliability; Quantitative; Children; Adults

INTRODUCTION

Muscle ultrasound is a useful tool in the evaluation of patients with suspected neuromuscular diseases. Increased amounts of intramuscular fat and fibrosis result in increasing echo-reflection and a brighter ultrasound image (Heckmatt et al. 1989; Pillen et al. 2008). While both qualitative and quantitative ultrasound assessments of skeletal muscle are effective in identifying neuromuscular pathology, quantitative ultrasonography offers superior inter-rater reliability and greater sensitivity for detecting pathology (Heckmatt et al. 1989; Zuberi et al. 1999; Maurits et al. 2003; Pillen et al. 2006; Brockmann et al. 2007). Quantification of the amount of muscle pathology using ultrasound provides a sensitive, noninvasive measure of disease severity from varied pathologies including Duchenne

muscular dystrophy (Zaidman et al. 2010; Jansen et al. 2011), late-onset acid maltase deficiency (Zaidman et al. 2011), spinal muscular atrophy (Wu et al. 2010) and nondystrophic myotonias (Trip et al. 2009) and is of sufficient sensitivity to detect differences between steroid treated and untreated dystrophic mice (Wallace et al. 2007). Although quantitative ultrasound offers advantages over qualitative assessments, widespread implementation of quantitative ultrasonography in the clinical setting is limited by an inability to replicate results between different ultrasound systems. This is because the characteristics of the ultrasound image are highly dependent on the ultrasound device and settings as well as physical characteristics of the acoustic array such as frequency and beam profile.

Several different approaches for improving the reliability of quantitative ultrasound have been described. Computer-assisted measurement of the grayscale pixel levels (Heckmatt et al. 1989; Scholten et al. 2003) is easily performed and is sensitive to the presence of neuromuscular disease (Pillen et al. 2007). Good reliability of gray-scale levels between different ultrasound systems can be achieved by developing a conversion algorithm relating one device to the other (Pillen et al. 2009). However, this technique requires the presence of a shared, heterogeneous phantom representing the full range of tissue pathologies and is, therefore, currently impractical for use in physically disparate settings. An alternative approach for measuring echo intensity is to digitize the raw backscattered radio-frequency (RF) time-domain signal (Hughes et al. 2007; Wallace et al. 2007). Digital RF results are independent of the postprocessing by the ultrasound device but access to the RF is often restricted by the manufacturer and calculation of the echo intensity requires signal processing not typically available in the clinical setting.

We have developed a reliable and sensitive measurement of skeletal muscle pathology, calibrated muscle backscatter (cMB), that is a compromise between gray-scale level and digitized RF signal analysis (Zaidman et al. 2008, 2010). cMB is an estimate of the level of acoustic energy scattered by tissue back to the transducer. Unlike RF analysis, it is derived from computer assisted measurement of grayscale pixel levels in a region-of-interest (ROI) in the muscle. Unlike simple grayscale level measurements, measurement of cMB uses calibration of the ultrasound system to ensure that changes in the grayscale levels uniformly reflect changes in the estimated backscatter levels and reference to a phantom to control for some system dependent parameters such as gain and signal compression (Knipp et al. 1997; Sosnovik et al. 2001; Holland et al. 2006). cMB levels of skeletal muscle should, therefore, be independently reproducible between different ultrasound systems and settings without the need for direct reference between the devices, providing a common phantom is referenced. In this study, we assessed the two steps for calculating cMB—calibration of the estimated backscatter from grayscale levels and reference to an external phantom—on the reliability of echo intensity measurements of skeletal muscle. We also analyze the effects of changes in ultrasound systems and settings such as alteration in gain, calibration curves, transducer frequency and variations in ROI size of either fixed or varying depth.

METHODS

Subjects and ultrasound image acquisition

This study was approved by the Washington University Institutional Review Board. We analyzed ultrasound images of the elbow flexors (biceps brachii and brachialis) of 60 subjects, ages 5 weeks to 76 years, including healthy controls ($n = 23$) and subjects with neuromuscular disorders including Duchenne or Becker muscular dystrophy ($n = 15$), other myopathies ($n = 11$), neuropathy or motor neuron disease ($n = 6$) or hypotonia, cramps or weakness ($n = 5$). Each subject had images obtained of the elbow flexors using at least two different ultrasound settings. Subjects were positioned and images of the elbow flexors were

obtained as previously described (Zaidman et al. 2008). Subjects were seated with the arm extended as supported by a pillow on a table at midthoracic height with the arm and hand relaxed. Ultrasound images were obtained in the longitudinal plane with the center of the transducer placed at 2/3 the distance from the acromion to the lateral epicondyle. The ultrasound transducer was oriented perpendicular to the bone by adjusting it to yield the brightest and narrow reflection of the bone and was repositioned between each image acquisition. A single image was obtained in each subject using two or more ultrasound configurations.

Configuration of the ultrasound systems

We compared ultrasound images of the elbow flexors that were obtained using two different ultrasound systems configured with four different ultrasound system settings (Table 1). Ultrasound configurations were chosen to compare different ultrasound systems, gain and compression settings and transducers. Configuration 1 (HD11xe, high gain) employed a Philips HD11xe ultrasonic imaging system (Koninklijke Philips Electronics N.V., Amsterdam, The Netherlands), the L12-5 transducer (nominal band width 5–12 MHz), gain of 95 and compression setting 1. Configuration 2 (iU22, high gain) employed the Philips iU22 ultrasonic imaging system (Koninklijke Philips Electronics N.V.), the L12-5 transducer, gain of 95 and a compression setting based on the “contrast” factory preset. Configuration 3 (iU22, low gain) employed the iU22 ultrasonic imaging system, the L12-5 transducer, gain of 81 and a compression setting based on the “musculoskeletal-small parts” factory preset. Configuration 4 (iU22, Transducer L9-3) employed the iU22 ultrasonic imaging system, the L9-3 transducer (nominal band width 3–9 MHz), gain of 80 and a compression setting based on the “musculoskeletal-general” factory preset. For all settings, the time-gain compensation settings were held fixed in the midline and a single focal point was placed 90% deep within the image and held fixed. Depth was held at 5 cm. All settings were saved as presets and were uniform for all subjects.

Measurement of grayscale and estimation of backscatter values

We calibrated each ultrasonic imaging system configuration by determining the compression curve describing the relationship between the displayed grayscale and the received backscatter value. This was done by measuring the grayscale of multiple images across a spectrum of dark to bright echoes. We obtained images of a phantom using ultrasound configurations 1–4 except the overall system gain was adjusted from low to high (0–100). This yielded a sample of images of the phantom for each ultrasound configuration with echoes ranging from very dark to very bright. For configurations 2–4 (the iU22 ultrasound system), QLAB[®] (Koninklijke Philips Electronics N.V.) was used to measure both the mean grayscale and backscatter within a ROI in the phantom. By measuring backscatter and grayscale levels within different areas of the phantom and from images obtained at different gain settings, we obtained a wide range of dark to bright measurements. A similar technique was performed for calibration of configuration 1 (the HD11xe system) except that NIH ImageJ (v. 1.37, Bethesda, MD, USA) image-analysis software was used to measure mean grayscale levels in the phantom associated with different receive gain settings (in decibels) representing changes in the level of backscatter (Zaidman et al. 2008). Calibration of the ultrasound system for measurement of estimated backscatter from grayscale measurements is performed by plotting the grayscale levels against the backscatter values measured from the same ROI and image. We then determined (1) the range of grayscale levels within which there is a linear relationship with the measured backscatter (the “linear range”) and (2) the slope relating the grayscale to backscatter measurements. The slope can then be used to convert changes in measured grayscale to changes in backscatter values (expressed in dB), so long as system settings are held constant and the grayscale levels are within the linear

range (Fig. 1). As each ultrasound configuration is different, each configuration 1–4 has a different calibration curve.

To determine how the size of the ROI may affect measurements using different ultrasound configurations, two ROIs were drawn within each muscle image. A “fixed height” rectangular ROI spanning a depth of 0.5 cm and a “full muscle” polygon ROI spanning variable depth extending from the superficial fascia to the bone were drawn within the muscle (Fig. 2). The lateral borders of each ROI were drawn as wide as possible within the muscle excluding artifact along the lateral edge of each image. Each ROI drawn within the muscle image was paired with an exactly reproduced (same size, depth and position) ROI within an image of a phantom (Model 047; Computerized Imaging Reference Systems, Inc., Norfolk, VA, USA). Mean grayscale levels of the ROI in the muscle image and its corresponding ROI in the phantom image were determined using ImageJ (National Institutes of Health) and converted to backscatter using the calibration slope for the appropriate ultrasound setting (Fig. 3). For each pair of measurements (the muscle and the corresponding ROI in the phantom), the backscatter of the phantom was subtracted from the backscatter of the muscle. The formula to determine cMB (Zaidman et al. 2008) is:

$$\text{cMB} = \frac{\text{mean grayscale ROI in muscle}}{\text{slope relating grayscale to backscatter}} - \frac{\text{mean grayscale of same ROI in phantom}}{\text{slope relating grayscale to backscatter}}$$

In configuration 3, some ROIs in the phantom fell in the near field and resulted in grayscale levels that were near zero and outside the linear range of the calibration curve. When this occurred, ROIs were chosen from a portion of the muscle in a deeper field. In four cases, the muscle was too small to move the region deeper. As the grayscale levels for the phantom reference for these images was not within the linear range required to calibrate the grayscale to backscatter, these images were excluded from analysis.

Statistics were performed using SPSS v. 13.1 (SPSS Inc., Chicago, IL, USA) Intraclass correlation coefficients (ICC) were determined using the 2-way random effects model for exact match. Comparisons were made between different ultrasound systems with similar gain settings (configurations 1 and 2), between different ultrasound systems with different gains (configurations 1 and 3), between the same ultrasound system but using different gain and compression settings (configurations 2 and 3) and between the same ultrasound system but using different transducers, gain, and compression settings (configurations 3 and 4). Comparisons between ultrasound configurations were made for both the “fixed height” and “full muscle” ROIs. Comparisons were treated as independent.

RESULTS

We measured a wide range of grayscale levels of skeletal muscle (from 3–205), spanning nearly the entire available 0 (black) to 255 (white) spectrum. cMB measurements ranged over 25 decibels, from –3 to 22 dB. Each ultrasound configuration yielded a calibration curve with a wide linear range (Fig. 1). Calibration slopes for converting grayscale levels (GSL) to backscatter (dB) were 6.60 GSL/dB for configuration 1, 5.93 GSL/dB for configuration 2, 4.54 GSL/dB for configuration 3 and 6.32 GSL/dB for configuration 4.

cMB of skeletal muscle was reliably measured between different ultrasonic imaging systems and configurations despite very dissimilar grayscale levels (Table 2). The two steps for calculating cMB—calibration of the estimated backscatter from grayscale levels and reference to an external phantom—both improved reliability over measurement of grayscale levels alone. Reference to a common phantom improved reliability most when different gain

settings were compared (comparisons between configurations 2 and 3). Calibrating the grayscale levels to backscatter values (expressed in dB) further improved the reliability and was most effective when comparing ultrasound configurations with very different compression curves (comparisons between configurations 1 and 3).

Reliability between ultrasound configurations was better for the “fixed height” ROI spanning a fixed and narrow depth (0.5 cm) than the “full muscle” ROI, which incorporated the entire muscle area and therefore spanned a larger depth within the image. Differences in the ROI size affected measurement reliability only when comparing different ultrasound systems or transducer designs but not when comparing ultrasound configurations of the same system and transducer (configurations 2 and 3, Table 2). Reliability was optimal when comparing two ultrasound systems using similar gain settings and transducers but deteriorated somewhat when comparing two different transducers. In most cases, cMB can be measured reliably between systems within 3 decibels with 95% confidence. This amount of variation is only 12% of the range of cMB represented in this sample of healthy and pathologic skeletal muscle. There was no systematic difference in cMB between different ultrasound systems and configurations when the same transducer was used. When different transducers were compared, however, cMB was slightly higher with the lower frequency transducer (Table 3). No other trend was seen for systematic measurement error across the range of measured cMB levels (Fig. 4).

DISCUSSION

Two steps are required to quantify reliably the ultrasound backscatter of skeletal muscle from grayscale levels. First, the calibration curve between the estimated backscatter and grayscale levels must be determined for each ultrasound configuration in order to convert grayscale levels to backscatter (expressed in dB). This step corrects for variations between systems because of the compression of the received backscatter into the displayed gray-scale levels and identifies the range through which the relationship between changes in backscatter and the displayed grayscale is linear. Second, the estimated backscatter values must be referenced to a common external image- in this case, we chose a commercially available phantom. This step accounts for differences in the gain settings between systems. Reliable quantitation of gray-scale levels and cMB requires that the ultrasound settings remain constant for each image. We accomplish this by setting all time-gain compensation levels to the midlevel and using a programmed preset to ensure the ultrasound settings remain constant. Using these techniques, we were able to reliably measure backscatter of skeletal muscle between different ultrasound configurations within 3 decibels in most cases. This measurement variability in cMB may obscure small effects and can be minimized by using the same or similar ultrasound system configuration and transducer and by selecting a ROI spanning a narrow (0.5 cm) depth.

Reliability of cMB was reduced between different transducers, widely different gain settings or when the depth spanned by the ROI varied for each image and was larger than 0.5 cm. There are several reasons why cMB could not entirely compensate for these factors. For example, some individual pixel grayscale levels in the ROI may not fall within the linear range identified during calibration of the ultrasound system. The number of these outlying pixel values and their effect on the reliability of cMB are likely to vary with differences in gain, transducer and compression settings. Furthermore, characteristics that vary with transducer properties and system settings that are unique to each ultrasound system (such as transmit focal points, beam width, center frequency and differential gain amplification that are independent of the manual time-gain compensation settings) as well as the physical properties of the tissue imaged (*e.g.*, frequency dependent attenuation properties, variations in the speed of sound) can affect ultrasonic beam properties and spectral content (Wagner et

al. 1983, 1987; Lizzi et al. 1997; Shankar 2000) that could reduce reliability of cMB, especially when the size of the ROI is variable and large.

Our technique for measuring cMB requires that the reference phantom is homogenous and yields values that are within the linear range of the calibration curve. Variations between and within phantoms could affect the reliability of cMB. Previous studies using cMB referenced images of the muscle to a single, static ROI of the entire imaged area of the phantom (Zaidman et al. 2008, 2010, 2011). In our preliminary studies, we found that selecting an identical ROI in the phantom as in the muscle, rather than a ROI including the entire imaged area of the phantom, was required to reliably measure cMB between systems. Another approach for standardizing ultrasound measurements of muscle is to reference measurements from a ROI in the muscle to a ROI in the subcutaneous fat, which is in the near field relative to the muscle (Wu et al. 2010). Additional studies are required to determine the reliability of this approach in different ultrasound systems, as our results suggest that variability between systems is affected by variations in the depth of the ROI.

One advantage of measuring cMB rather than gray-scale levels is that grayscale levels have no inherent physical reference and are generated from proprietary processing algorithms of the ultrasound system. Therefore, changes in grayscale levels have limited external validity regarding the magnitude of energy required to generate an effect. cMB instead estimates the amount of energy received by the ultrasound system in a manner intended to compensate for variations between systems. Measurement of the actual amount of energy received by the ultrasound transducer requires analysis of the digitized backscattered RF. This is not feasible for most ultrasound practitioners because access to the RF is often restricted and advanced mathematics are required to decipher it. Improved access to the RF and the tools required to interpret it could improve quantitative analysis of ultrasonic signal.

Limitations of cMB include reliance on a common reference phantom and that construction of the calibration curve requires either identifying the decibel output associated with changes in gain or measuring the backscatter directly using proprietary software. Access to this information may not be available for all ultrasound systems. Constructing the calibration curve and imaging the phantom only needs to be performed once for each system and setting. The remaining steps involved in measuring cMB require no special equipment except access to a computer and imaging software. An alternative to cMB for reliably quantifying ultrasound signal is to calibrate grayscale levels from ultrasound systems directly to each other (Pillen et al. 2009). This method requires a specially made phantom that is not commercially available and is not practical for labs in disparate physical locations. A combined approach of calibrating devices directly to each other using measurements of cMB from a commercially available phantom requires further study.

In conclusion, cMB is more reliably measured than grayscale levels between different ultrasound systems and configurations. Determining the calibration curve between grayscale levels and backscatter and reference to a common phantom controls for differences in compression and gain between ultrasound systems. Some measurement variability of cMB persists, especially between transducers of different design, and could obscure small effects. Reliability is optimized by measuring cMB from similar ultrasound configurations and from a ROI spanning a narrow depth within the muscle.

Acknowledgments

The study was supported by the Washington University Neuromuscular Research Fund, the National Institute of Health Neurological Sciences Academic Development Award Grant Number K12 NS00169009 and the Institute of Clinical and Translational Sciences with support from the National Center for Research Resources Grant Number UL1 RR024992.

References

- Brockmann K, Becker P, Schreiber G, Neubert K, Brunner E, Bonnemann C. Sensitivity and specificity of qualitative muscle ultrasound in assessment of suspected neuromuscular disease in childhood. *Neuromuscul Disord.* 2007; 17:517–523. [PubMed: 17537637]
- Heckmatt J, Rodillo E, Doherty M, Willson K, Leeman S. Quantitative sonography of muscle. *J Child Neurol.* 1989; 4(Suppl):S101–S106. [PubMed: 2681371]
- Holland MR, Gibson AA, Peterson LR, Areces M, Schaffer JE, Perez JE, Miller JG. Measurements of the cyclic variation of myocardial backscatter from two-dimensional echocardiographic images as an approach for characterizing diabetic cardiomyopathy. *J Cardiometab Syndr.* 2006; 1:149–152. [PubMed: 17694597]
- Hughes MS, Marsh JN, Wallace KD, Donahue TA, Connolly AM, Lanza GM, Wickline SA. Sensitive ultrasonic detection of dystrophic skeletal muscle in patients with duchenne muscular dystrophy using an entropy-based signal receiver. *Ultrasound Med Biol.* 2007; 33:1236–1243. [PubMed: 17467153]
- Jansen M, van Alfen N, 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 Disord.* 2012; 22:306–317.
- Knipp BS, Zagzebski JA, Wilson TA, Dong F, Madsen EL. Attenuation and backscatter estimation using video signal analysis applied to B-mode images. *Ultrason Imaging.* 1997; 19:221–233. [PubMed: 9447670]
- Lizzi F, Feleppa E, Astor M, Kalisz A. Statistics of ultrasonic spectral parameters for prostate and liver examinations. *IEEE Trans Ultrason Ferroelectr Freq Control.* 1997; 44:935–942.
- Maurits NM, Bollen AE, Windhausen A, De Jager AE, Van Der Hoeven JH. Muscle ultrasound analysis: Normal values and differentiation between myopathies and neuropathies. *Ultrasound Med Biol.* 2003; 29:215–225. [PubMed: 12659909]
- Pillen S, Tak RO, Zwarts MJ, Lammens MM, Verrijs 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 Med Biol.* 2009; 35:443–446. [PubMed: 19081667]
- Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, Zwarts MJ. Quantitative gray-scale analysis in skeletal muscle ultrasound: A comparison study of two ultrasound devices. *Muscle Nerve.* 2009; 39:781–786. [PubMed: 19301363]
- Pillen S, van Keimpema M, Nievelstein RA, Verrips A, van Kruijsbergen-Raijmann W, Zwarts MJ. Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound Med Biol.* 2006; 32:1315–1321. [PubMed: 16965971]
- Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: Diagnostic value in childhood neuromuscular disease. *Neuromuscul Disord.* 2007; 17:509–516. [PubMed: 17537635]
- Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: Normal values. *Muscle Nerve.* 2003; 27:693–698. [PubMed: 12766980]
- Shankar P. A general statistical model for ultrasonic backscattering from tissues. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2000; 47:727–736. [PubMed: 18238602]
- Sosnovik DE, Baldwin SL, Lewis SH, Holland MR, Miller JG. Trans-mural variation of myocardial attenuation measured with a clinical imager. *Ultrasound Med Biol.* 2001; 27:1643–1650. [PubMed: 11839409]
- Trip J, Pillen S, Faber CG, van Engelen BG, Zwarts MJ, Drost G. Muscle ultrasound measurements and functional muscle parameters in non-dystrophic myotonias suggest structural muscle changes. *Neuro-muscul Disord.* 2009; 19:462–467.
- Wagner RF, Insana MF, Brown DG. Statistical properties of radio-frequency and envelope-detected signals with applications to medical ultrasound. *J Opt Soc Am A.* 1987; 4:910–922. [PubMed: 3298583]
- Wagner RF, Smith SW, Sandrik JM, Lopez H. Statistics of speckle in ultrasound b-scans. *IEEE Trans Ultrason Ferroelectr Freq Control.* 1983; 30:156–163.

- Wallace KD, Marsh JN, Baldwin SL, Connolly AM, Keeling R, Lanza GM, Wickline SA, Hughes MS. Sensitive ultrasonic delineation of steroid treatment in living dystrophic mice with energy-based and entropy-based radio frequency signal processing. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2007; 54:2291–2299. [PubMed: 18051163]
- Wu JS, Darras BT, Rutkove SB. Assessing spinal muscular atrophy with quantitative ultrasound. *Neurology*. 2010; 75:526–531. [PubMed: 20697104]
- Zaidman CM, Connolly AM, Malkus EC, Florence JM, Pestronk A. Quantitative ultrasound using backscatter analysis in Duchenne and Becker muscular dystrophy. *Neuromuscul Disord*. 2010; 20:805–809. [PubMed: 20817454]
- 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]
- Zaidman CM, Malkus EC, Siener C, Florence J, Pestronk A, Al-Lozi M. Qualitative and quantitative skeletal muscle ultrasound in late-onset acid maltase deficiency. *Muscle Nerve*. 2011; 44:418–423. [PubMed: 21755514]
- Zuberi SM, Matta N, Nawaz S, Stephenson JB, McWilliam RC, Hollman A. Muscle ultrasound in the assessment of suspected neuromuscular disease in childhood. *Neuromuscul Disord*. 1999; 9:203–207. [PubMed: 10399745]

Fig. 1.

Calibration of grayscale to backscatter. (a) Example of the calibration curve for configuration 1, the HD11xe using high gain and an L12-5 transducer showing the linear range of the relationship between backscatter (dB) and grayscale level. (b) Calibration curves for different ultrasonic imaging systems and configurations.

Regions of Interest:

Fig. 2.

Region-of-interests (ROI)s for quantitative ultrasound analysis of skeletal muscle. A “fixed height” rectangular ROI spanning a depth of 0.5 cm (left) in a portion of the muscle and a “variable height” polygon ROI (right) spanning a variable depth incorporating the entire area of muscle between the subcutaneous fat (SC) and bone identify the area of the image selected for quantitative analysis in the elbow flexors of a 9 year old boy with Duchenne muscular dystrophy. Bar = 1 cm.

System 1: HD11xeMuscle Phantom

$$\text{cMB: } 23\text{dB} - 8\text{dB} = 15 \text{ dB}$$

System 2: iU22Muscle Phantom

$$\text{cMB: } 34.8\text{dB} - 20\text{dB} = 14.8 \text{ dB}$$

Fig. 3.

Calculation of calibrated muscle backscatter. Calibrated muscle backscatter (cMB) is calculated from longitudinal images of the elbow flexors in a 9-year-old boy with Duchenne muscular dystrophy using two ultrasound systems: the Philips HD11xe (top) and the Philips iU22 (bottom). Three steps are required to measure cMB. First, the grayscale levels (GSL) from a region-of interest (ROI) in the skeletal muscle are measured. Second, GSLs are converted to backscatter using the slope of the calibration curve relating grayscale to backscatter. The conversion for system 1 (upper) is 6.6 GSL/dB and for system 2 (lower) is 5.93 GSL/dB. Third, backscatter levels of a phantom (right) are subtracted from the backscatter of the skeletal muscle (left) from identical ROIs. For each system, images of the muscle and the phantom are obtained using identical settings. For comparison between systems, reference to a common phantom is required. Bar = 1 cm.

Fig. 4.

Bland-Altman Plots of cMB from different ultrasound configurations. The difference in cMB (y axis, device 1 – device 2) is plotted against the mean cMB (x-axis) from two ultrasound configurations. When comparing different transducers, cMB was consistently slightly higher with the lower frequency transducer (triangles). Otherwise, the difference in cMB between configurations is centered and evenly distributed around zero across the range of the mean cMB levels for a 0.5 cm high region-of interest (ROI) of a portion of the muscle (left) and for a larger, variably sized ROI that includes the entirety of the muscle image (right).

Table 1

Characteristics of different ultrasound configurations

Ultrasound configuration	Machine	Transducer	Gain	Mechanical index	Thermal index	Calibration (grayscale levels/dB)
1	HD11xe	L12-5	95	Unknown	Unknown	6.6
2	iU22	L12-5	95	0.15	<0.0	5.93
3	iU22	L12-5	81	0.4	0.1	4.54
4	iU22	L9-3	80	0.6	0.1	6.32

dB = decibel.

Table 2

Reliability of calibrated muscle backscatter measurements

N	Machine/setting 1	Machine/setting 2	ROI size	Transducer	Intraclass correlation coefficient		
					cMB (dB) (muscle-phantom)	Referenced GSL (muscle-phantom)	Raw GSL (muscle only)
15	HD11xe/high gain	iU22/high gain	0.5 cm	L12-5	0.98	0.97	0.54
15	HD11xe/high gain	iU22/high gain	Full	L12-5	0.82	0.89	0.3
9	HD11xe/high gain	iU22/low gain	0.5 cm	L12-5	0.96	0.77	0.35
9	HD11xe/high gain	iU22/low gain	Full	L12-5	0.6	0.3	0.09
28	iU22/high gain	iU22/low gain	0.5 cm	L12-5	0.82	0.7	0.12
32	iU22/high gain	iU22/low gain	Full	L12-5	0.81	0.7	0.12
14	iU22/high gain transducer L12-5	iU22/transducer L9-3	0.5 cm	L12-5 and L9-3	0.71	0.67	0.46
14	iU22/high gain transducer L12-5	iU22/transducer L9-3	Full	L12-5 and L9-3	0.47	0.42	0.1

Full = ROI spanning variable depth encompassing the full amount of muscle from superficial fascia to bone; GSL = grayscale level; cMB = calibrated muscle backscatter; ROI = region-of-interest; N = number of subjects.

Table 3

Variability in calibrated muscle backscatter measurements

N	Machine/setting 1	Machine/setting 2	ROI size	Transducer	Difference in cMB (system 1 minus system 2) (Mean (SD))	95% confidence interval of difference	Range of 95% confidence interval	p value *
15	HD11xe/high gain	iU22/high gain	0.5 cm	L12-5	-0.13 (1.4)	-0.88-0.62	1.51	0.69
15	HD11xe/high gain	iU22/high gain	Full	L12-5	-0.65 (1.72)	-1.60-0.31	1.91	0.21
9	HD11xe/high gain	iU22/low gain	0.5 cm	L12-5	0.71 (1.84)	-0.71-2.11	2.83	0.52
9	HD11xe/high gain	iU22/low gain	Full	L12-5	1.09 (2.53)	-0.85-3.03	3.88	0.21
28	iU22/high gain	iU22/low gain	0.5 cm	L12-5	0.55 (3.43)	-1.15-1.51	2.66	0.44
32	iU22/high gain	iU22/low gain	Full	L12-5	0.34 (2.49)	-0.38-1.41	1.79	0.58
14	iU22/high gain transducer L12-5	iU22/transducer L9-3	0.5 cm	L12-5 and L9-3	-3.23 (2.52)	-4.70-1.78	2.92	0.002
14	iU22/high gain transducer L12-5	iU22/transducer L9-3	Full	L12-5 and L9-3	-2.11 (3.29)	-4.01-0.22	3.79	0.048

Bold indicates $p < 0.05$.

* Wilcoxon signed rank test, comparing cMB from paired ultrasound configurations.