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Validation of a freehand 3D ultrasound system for morphological measures of the medial gastrocnemius muscle

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ABSTRACT

Muscle volume and length are important parameters for examining the force-generating capabilities of muscle and their evaluation is necessary in studies that investigate muscle morphology and mechanical changes due to age, function, pathology, surgery and training. In this study, we assessed the validity and reliability of in vivo muscle volume and muscle belly length measurement using a multiple sweeps freehand 3D ultrasound (3DUS). The medial gastrocnemius of 10 subjects was scanned at three ankle joint angles (15°, 0° and −15° dorsiflexion) three times using the freehand 3DUS and once on the following day using magnetic resonance imaging (MRI). All freehand 3DUS and MRI images were segmented, volumes rendered and volumes and muscle belly lengths measured. The freehand 3DUS overestimated muscle volume by 1.9 ± 9.1 mL, 1.1 ± 3.8% difference and underestimated muscle belly length by 3.0 ± 5.4 mm, 1.3 ± 2.2% difference. The intra-class correlation coefficients (ICC) for repeated freehand 3DUS system measures of muscle volume and muscle belly length were greater than 0.99 and 0.98, respectively. The ICCs for the segmentation process reliability for the freehand 3DUS system and MRI for muscle volume were both greater than 0.99 and muscle belly length were 0.97 and 0.99, respectively. Freehand 3DUS is a valid and reliable method for the measurement of human muscle volume and muscle belly length in vivo. It could be used as an alternative to MRI for measuring in vivo muscle morphology and thus allowing the determination of PCSA and estimation of the force-generating capacity of individual muscles within the setting of a biomechanics laboratory.

1. Introduction

Muscle volume and muscle length are important morphological properties of muscle. Both are related to the physiological cross-sectional area (PCSA) of a muscle and provide an indication of its force-producing capacity (Fukunaga et al., 2001; Reeves et al., 2004). Direct muscle volume and muscle length measures can be used to examine muscle contracture and observe changes due to surgery or specific training interventions (Fry et al., 2007; Kawakami et al., 2008). Furthermore, accurate estimates of muscle volume and length are important in musculoskeletal modelling where variability in cadaveric muscles has made extrapolation to living, healthy individuals' problematic (Fukunaga et al., 1997).

Magnetic resonance imaging (MRI) is considered to be the "gold standard" modality for direct measurement of muscle volume and length in vivo (Mitsiopoulos et al., 1998; Holzbaur et al., 2007). However, this technique is expensive, may not be available, takes a substantial amount of time for each scan (typically >2 min) and in some cases patients require sedation. In contrast, two-dimensional (2D) B-mode ultrasound is suited to detect aspects of muscle morphology, such as muscle cross-sectional area, fascicle length and pennation angle in a safe, objective and relatively inexpensive manner (Maganaris, 2003; Lichtwark et al., 2007; Whittaker et al., 2007). Such measures have been incorporated into regular geometric models to approximate muscle volume (Miyatani et al., 2004; Albracht et al., 2008). A direct three-dimensional (3D) representation of a muscle is, however, more favourable when making morphological measurements as the variable shape of a muscle over its length will be taken into account.

Freehand 3D ultrasound (3DUS) involves combining 2D ultrasound scanning and 3D motion analysis to provide a direct in vivo measurement of tissue structure. A stack of 2D B-mode images is created by recording consecutive ultrasound scans while simultaneously tracking the position and orientation of the ultrasound transducer using 3D motion analysis. Coordinate transformations are used to map the individual 2D B-mode images into space and a 3D rendering of the tissue of interest can be constructed for morphological measurement purposes. Freehand 3DUS systems have been used to make direct volume and

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length measures of the small lower leg muscles of typically developing children and children with spastic diplegic cerebral palsy (Fry et al., 2007; Malaiya et al., 2007). This has enabled the researchers to effectively evaluate the muscle morphological differences between the two populations and assess changes due to surgery and over time. Muscle belly lengths measures have also been made in adults using freehand 3DUS and measurement on one subject has shown repeatable results (Fry et al., 2003).

Delcker et al. (1999), reports that good muscle volume accuracy can be achieved in measurements of small-sized, cadaveric muscles. However, these results cannot necessarily be applied to in vivo measurements of large human leg muscles because such scans require multiple ultrasound sweeps to capture the whole muscle volume as a result of the limited field of view of standard ultrasound transducers (40–60 mm). Recently, Weller et al. (2007), showed that a freehand 3D ultrasonography system, using multiple sweeps, provided excellent precision and accuracy in the measurement of volume of isolated dog muscles when compared with measurements based on computed tomography and a water displacement method. The system used by Weller et al. (2007), also provided repeatable measures of muscle volume measurement in live dogs.

Despite the continued use of 3DUS systems for the determination of muscle morphological factors in humans, no validation or reliability study has been reported in humans, in vivo. In addition, the accuracy and repeatability of large muscle volume and muscle belly length measures, requiring multiple ultrasound sweeps, in different joint positions has not been assessed. We hypothesise that 3DUS will be a valid and reliable method to determine muscle volume and length when compared to MRI. Therefore, the purpose of this study is to (1) validate and (2) assess the reliability of the measurement of medial gastrocnemius muscle volume and muscle belly length in vivo using multiple sweeps freehand 3DUS system compared to MRI at a range of ankle joint angles.

2. Methods

2.1. Subjects

Five male and five female subjects (age 26±5 years, height 174±8 cm) volunteered to participate in the study. All subjects were healthy university staff or students and provided informed consent in accordance with institutional guidelines (GU Ref No: PES/31/07/HREC). Potential subjects were excluded from the study if they had any history of lower leg injury or surgery, were pregnant or had metal implants.

2.2. Experimental design

Freehand 3DUS scans of the right lower leg were performed on the relaxed muscle to assess muscle volume and muscle belly length of the medial gastrocnemius. MRI scans of the right lower leg were performed the following day to assess medial gastrocnemius muscle volume and muscle belly length. Both the freehand 3DUS scans and the MRI scans were performed at three ankle angles, 15° dorsiflexion (DF), 0° dorsiflexion (N) and -15° dorsiflexion (PF), with a constant knee angle of 25° of knee flexion for each subject. Three freehand 3DUS scans were performed and analysed separately at each ankle joint angle to assess repeatability of measures at each angle.

2.3. 3DUS set-up and calibration

B-mode ultrasound images were recorded at 25 Hz using a PC-based ultrasound scanner with a 128-element beamformer and a 10.0 MHz linear transducer with 60 mm field of view (HL9.0/60/128Z, Telemed Echo Blaster 128 Ext-1Z system, Lithuania). Position and orientation of the transducer were recorded by tracking three reflective markers rigidly attached to the transducer (Fig. 1) using an optical motion analysis system recording at 100 Hz (8-camera MX13, Vicon Motion Systems Ltd., Oxford, UK).

A three volt square wave was produced during recording of ultrasound data that triggered synchronous collection of the motion analysis data. A 66.7 ms time delay was measured and the data adjusted accordingly. Stradwin software (v3.5, Mechanical Engineering, Cambridge University, UK) was used to integrate the ultrasound images with the transducer kinematic data for frame manipulation, 3D visualisation and reconstruction. As 3D reconstruction was not performed in real-time, customised Matlab (7.6.0 R2008a, The MathWorks, Massachusetts, USA) scripts were used to calculate the 3D position and orientation of the ultrasound transducer and to convert recorded ultrasound video and kinematic data files to Stradwin data file formats to be loaded into Stradwin.

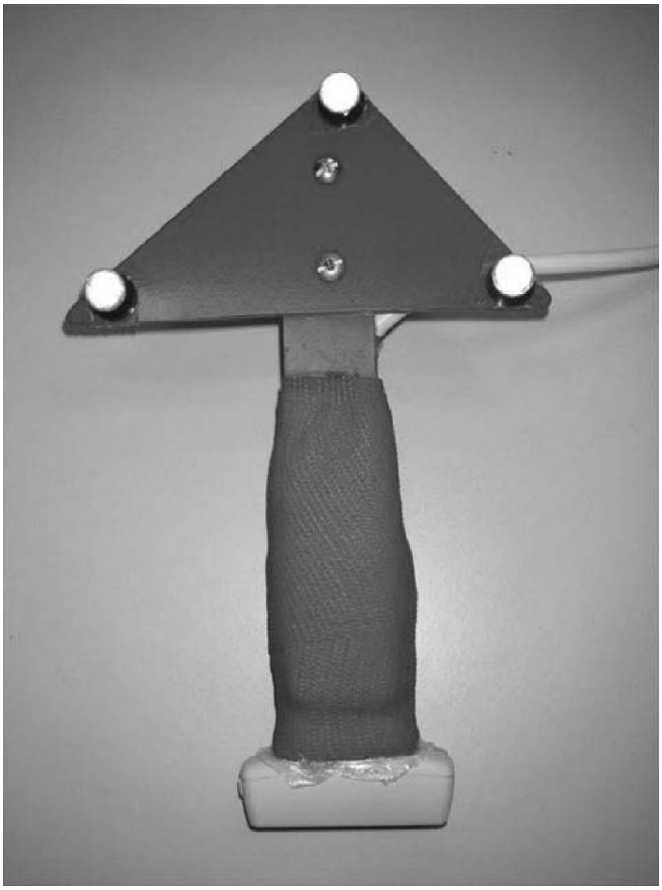

Fig. 1. 3DUS transducer setup. A Perspex frame with three retro-reflective markers was rigidly attached to the ultrasound transducer using casting material.

Prior to scanning, the system was spatially calibrated following the single-wall phantom calibration protocol provided in the Stradwin software (Prager et al., 1998). Briefly, this involves scanning a planar surface (the floor of a flat-bottomed water bath that is clearly definable) and performing a least-square fit to estimate the best three translation and three rotations of the line data that fit a plane, which is then used as the spatial calibration. The three translation and rotation offsets were added to the sensor measurements to calculate the 3D position of the B-mode scans during reconstruction.

2.4. 3DUS measurements

To eliminate tissue compression and enhance visualisation, the right medial gastrocnemius was scanned using the ultrasound transducer, while the subjects were kneeling in a water bath. Water covered the entire lower leg. The foot was rigidly stabilised at each ankle joint angle using solid blocks (Fig. 2). Knee angle was maintained at 25° of knee flexion by having the leg supported at the end of the water bath and the torso resting on an adjustable bench. Knee and ankle angles were measured using a plastic goniometer.

A stack of 2D B-mode ultrasound images was acquired by manually moving the ultrasound transducer over the length of the medial gastrocnemius muscle in a transverse orientation at a steady speed. Due to the size of the adult medial gastrocnemius muscle and the limited size of the field of view of the ultrasound transducer, two or three overlapping parallel sweeps were necessary to cover the muscle. Ultrasound settings such as power, gain, image depth and focal depth were optimised to allow ease of identification of the collagenous tissue that defines the outer border of the muscle.

All post-scanning processing was performed in Stradwin. Because multiple sweeps of the medial gastrocnemius were required, dividing planes were placed between overlapping ultrasound images (Fig. 3A). Segmentation was performed

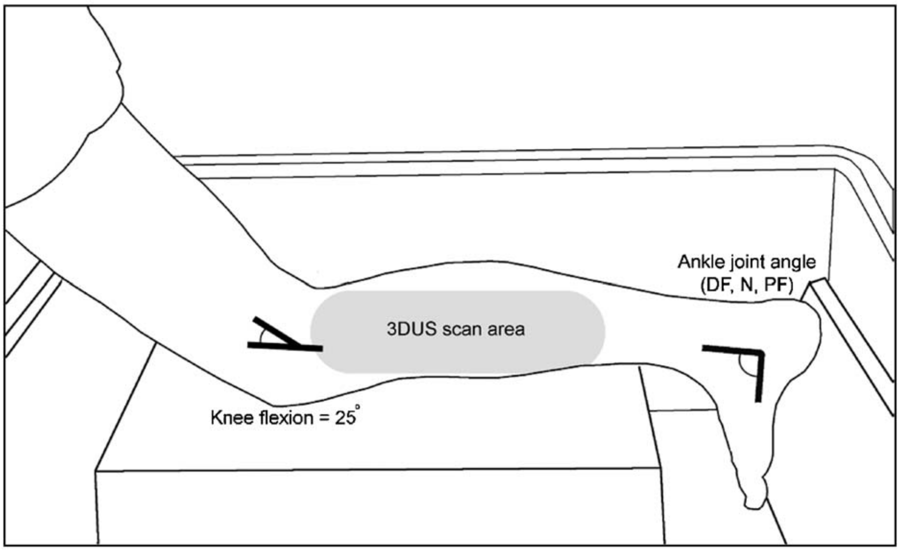

Fig. 2. Subject position for 3DUS scanning in the water bath (cut-away). The subjects were kneeling and water covered the entire lower leg. The foot was rigidly stabilised at DF, N and PF using solid blocks. Knee angle was maintained at 25° of knee flexion by having the leg supported at the end of the water bath and the torso resting on an adjustable bench. Ultrasound images were acquired by performing two or three overlapping parallel sweeps over the length of the medial gastrocnemius muscle (highlighted 3DUS scan area).

manually by outlining the perimeter of the medial gastrocnemius in each 2D ultrasound image (Weller et al., 2007). Once segmentation was complete, surface interpolation through the segmentation contours created a rendered 3D image of the muscle belly (Fig. 3B).

The proximal insertion of the medial gastrocnemius was difficult to visualise in the B-mode images so muscle volume (mL) and muscle belly length (mm) measures were made proximally from the most superficial aspect of the medial femoral condyle to the distal musculotendinous junction. To assess the reliability of the segmentation method used for the determination of the medial gastrocnemius volume and length, ten randomly selected freehand 3DUS scans were re-analysed. One operator (LB) performed all of the ultrasound image processing.

2.5. Phantom volume validation

The accuracy of the freehand 3DUS using single and multiple sweeps was also assessed using 20 water-filled latex condom phantoms containing various volumes of water (26–296 mL). The water-filled condoms were imaged and the volumes estimated using the methods defined above. Each reconstructed volume was compared to the known volume of water within the condom. Water volume was calculated using the measured water mass (g)/0.9978 g.cm^{-3} (the density of water at 22° C).

2.6. MRI set-up and measurements

Subjects lay supine on the MRI gantry. Knee and ankle angles were reproduced from measurements in the water bath and maintained using foam bolsters and adjustable straps. Axial MRI scans were recorded, such that the right medial gastrocnemius of each subject was scanned from the proximal insertion on the femur to the distal musculotendinous junction where the gastrocnemius connects to the Achilles tendon. All subjects were scanned using a General Electric Signa HDx 1.5 T MRI scanner (Milwaukee, WI, USA.). Adequate anatomical coverage was achieved using a 12 Channel Body Array Coil (GE Healthcare). Images were acquired in the axial plane using a standard 2D spin echo pulse sequence—400 ms repetition time; 12 ms time to echo; 25 kHz receiver bandwidth; 320 × 288 image matrix (with zip 512 interpolation); 23 × 17.3 cm field of view and 5 mm slice thickness, with varied interslice gap (3–5 mm) to allow 40 slices for each subject's anatomical coverage.

The muscle boundaries of the medial gastrocnemius were manually segmented in all corresponding axial plane images using a piecewise linear boundary provided by the software program 3D Slicer (Version 2.6-opt, Harvard University, Boston, USA). Between 25 and 35 contour curves were segmented for each muscle (Fig. 3C) and surface rendering performed for measurement of volume and muscle belly length (Fig. 3D). Measurements were made proximally from the most superficial aspect of the medial femoral condyle to the distal musculotendinous junction. To assess the reliability of the segmentation method used for the determination of the medial gastrocnemius volume and length, ten randomly selected MRI scans were re-analysed. One individual (LB) performed all of the MRI image processing.

2.7. Statistical analysis

The limits of agreement method (Bland and Altman, 1986) was used to assess the agreement between (1) the freehand 3DUS and MRI-based measurement of muscle volume and muscle belly length for the medial gastrocnemius at three ankle joint angles (DF, N and PF) and, (2) the freehand 3DUS-based measurement of volume and the known water volume of the condom phantoms. Intra-session reliability of muscle volume and muscle belly length measurements made using freehand 3DUS over three trials was assessed using the intra-class correlation coefficient (ICC). To assess the reliability of the segmentation method used for the determination of the medial gastrocnemius volume and length, 10 randomly selected freehand 3DUS scans and 10 randomly selected MRI scans were re-analysed and the respective ICC was calculated.

3. Results

3.1. Validity

The mean muscle volume (± SD) assessed in the study was 274 ± 75 mL (Fig. 4) and the mean muscle belly length (± SD) was 247 ± 20 mm (Fig. 5). There was a tendency for the 3DUS to overestimate the muscle volume by 1.90 mL (1.1%) and to underestimate muscle belly length by 3.0 mm (1.3%) across all joint angles (Table 1). The 95% confidence intervals (CI) for the level of agreement between 3DUS and MRI were 18 mL for muscle volume and 10 mm for muscle belly length (Figs. 4 and 5). Three-dimensional US underestimated condom phantom volumes by 0.9 ± 1.7 mL, (95% CI = 3.4 mL), which corresponded to a percentage difference of 0.7 ± 2.6%.

3.2. Reliability and repeatability

The ICCs for repeated freehand 3DUS measures of muscle volume and muscle belly length were greater than 0.99 and 0.98, respectively (Table 2). The mean muscle volumes (± SD) for each trial of the segmentation process reliability were 276 ± 76 and 274 ± 77 mL (ICC = 0.99) for freehand 3DUS, and, 273 ± 81 and 271 ± 81 mL (ICC = 0.99) for MRI. The mean muscle belly lengths (± SD) for the freehand 3DUS trials were 243 ± 16 and 245 ± 15 mm (ICC = 0.97), and MRI trials 251 ± 21 and 252 ± 22 mm (ICC = 0.99).

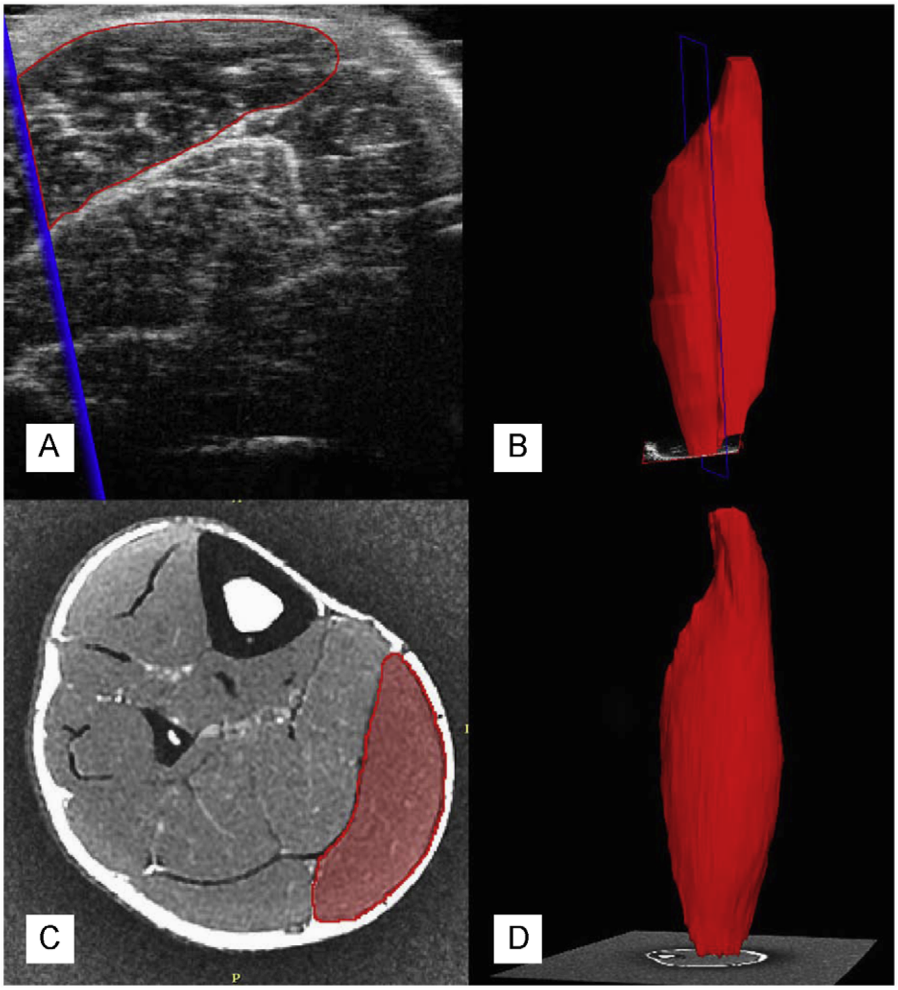

Fig. 3. (A) Typical 2D B-mode ultrasound scan with segmented medial gastrocnemius muscle (MG) and dividing plane for multiple sweeps (blue line). (B) Three dimensional volume rendering of 3DUS scan with dividing plane for multiple sweeps (blue plane). (C) Typical MRI axial scan with segmented medial gastrocnemius muscle (MG). (D) 3D volume rendering of MRI scan.

4. Discussion

This study has demonstrated good agreement between multiple sweeps freehand 3DUS and MRI measures across a range of medial gastrocnemius volumes and varying ankle joint angles. The mean percentage difference between the two methods was minimal with freehand 3DUS overestimating the volume measured using MRI by 1.1%. In further support, only 0.7% variability was calculated when comparing the freehand 3DUS system to known volume water-filled condom phantoms in measurements over a range of volumes from 26 to 296 mL. The results of this validation study support those of previous studies on smaller muscles. Weller et al. (2007) found that freehand 3DUS underestimated the in vitro dog muscle volume by 3.33 mL compared to CT volume measures and overestimated by 1.38 mL compared to the water displacement method. Delcker et al. (1999) reported a 10% difference between freehand 3DUS and the water displacement method in cadaveric human hand muscles. The current study had a tendency for the 3DUS to overestimate the muscle volume by 1.90 mL. Comparing the accuracy of our study to the previous validation studies is problematic considering the dissimilarities in the methods used; however, the multiple sweeps 3DUS method is valid for measuring large in vivo muscle volumes.

Our muscle belly length measurements compare favourably with the values made from adult cadavers (Wickiewicz et al., 1983) and are consistent with the expected relationship between length and joint angle (Fry et al., 2003). Agreement of measurement of muscle belly length between MRI and freehand 3DUS at each ankle joint angle was also good. The freehand 3DUS system underestimated the muscle belly length by 1.3% as compared to the MRI measures. Measurements at DF are almost identical but at N and PF there appears to be an underestimation of muscle belly length by the freehand 3DUS. This may be due to numerous confounding factors including background muscle activation, variable connective tissue image quality due to tissue stretch and/or depth, difficulty in imaging the most prominent posterior aspect of the medial femoral condyle with B-mode ultrasound and subject position (supine versus prone) effecting passive forces acting on the muscle bulk despite the same joint configuration. Furthermore, MRI length measurements may be inaccurate due to the axial slice widths, which in this study were 5 mm. This limits the accuracy of the length measures using the MRI technique to distance between axial slice planes. This may also account for the lack of difference in length measurement that was observed between the DF and N positions.

The intra-session reliability of the freehand 3DUS system to measure muscle volume and muscle belly length was very high with all ICCs greater than 0.997 and 0.988, respectively. A 0.9% underestimation in volume and 0.6% overestimation difference in muscle belly length between re-analysed scans indicated that the manual segmentation process is also a major source of measurement error. To note, repeated MRI scans were not performed but a

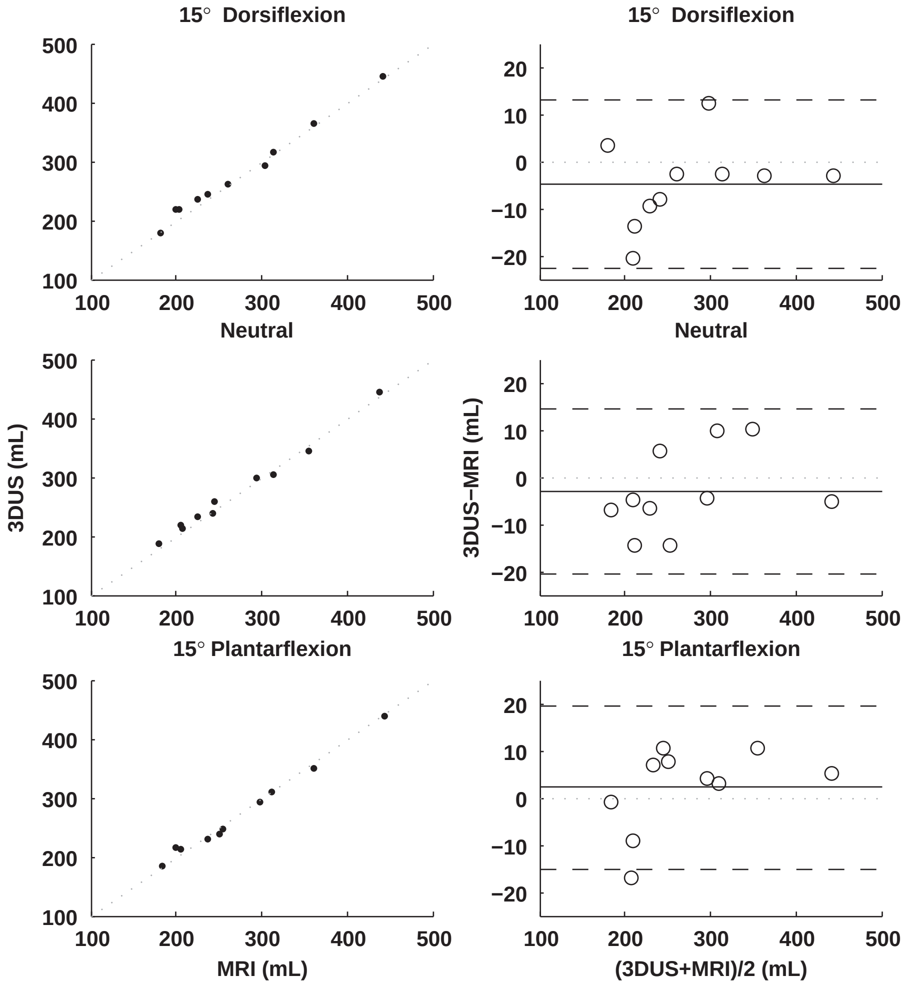

Fig. 4. Scatter plots, MRI versus mean 3DUS, and Bland–Altman plots, difference (MRI-3DUS) versus average of values measured by MRI and 3DUS, of the medial gastrocnemius volume in three ankle positions (15°, 0° and −15° dorsiflexion). The diagonal line in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent the mean difference and the upper and lower 95% limits of agreement.

Table 1
Comparison of muscle volume and muscle belly length measurements of the medial gastrocnemius between freehand 3DUS and MRI.

Ankle joint position	Muscle volume		Mean difference (mL)	Mean difference (%)
	MRI (mL)	3DUS (mL)		
DF	273±83	278±80	−4.8±9.1	−2.1±4.0
N	271±80	274±77	−3.1±8.9	−1.6±3.5
PF	274±81	272±77	2.2±8.9	0.4±3.9
Mean	273±79	275±75	−1.9±9.1	−1.1±3.8
Ankle joint position	Muscle belly length		Mean difference (mm)	Mean difference (%)
	MRI (mm)	3DUS (mm)		
DF	255±20	255±20	−0.6±5.0	−0.1±2.0
N	251±21	247±20	4.2±5.1	1.7±2.0
PF	245±19	240±18	5.4±4.7	2.2±1.9
Mean	250±20	247±20	3.0±5.4	1.3±2.2

Data are presented as mean±1 SD.

repeated segmentation process produced a difference of 0.7% for volume and −0.5% for muscle belly length indicating one source of error in our current ‘gold standard’. Unlike techniques for examining bone, there is currently no image processing technique that is able to automatically threshold and segment muscles from MRI to minimize the manual processing error. Considering the

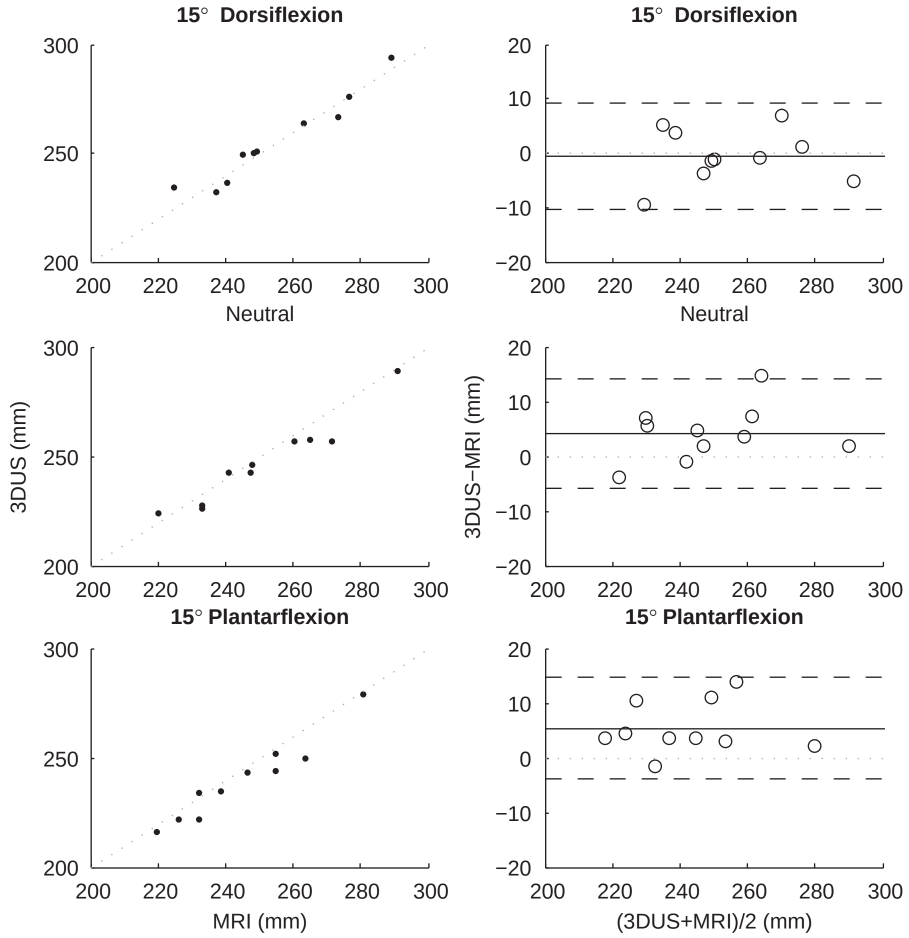

Fig. 5. Scatter plots, MRI versus mean 3DUS, and Bland–Altman plots, difference (MRI-3DUS) versus average of values measured by MRI and 3DUS, of the medial gastrocnemius muscle belly length in three ankle positions. The diagonal line in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent the mean difference and the upper and lower 95% limits of agreement.

Table 2
Reliability of intra-session repeated measures of muscle volume (mL) and muscle belly length (mm) by the freehand 3DUS system assessed using the Intra-class correlation coefficient (ICC).

Ankle joint position	Muscle volume (mL)			ICC
	Trial 1	Trial 2	Trial 3	
DF	278±83	277±81	278±76	0.998
N	274±76	271±79	276±76	0.997
PF	273±77	270±79	274±77	0.998
Ankle joint position	**Muscle belly length (mm)**			**ICC**
	Trial 1	Trial 2	Trial 3	
DF	255±20	255±20	256±20	0.991
N	245±17	248±22	248±20	0.988
PF	240±20	239±18	240±18	0.988

Experimental data are presented as mean±1 SD.

variability implicated with the segmentation process of both imaging methods, much of the calculated differences in muscle volume and muscle belly length may be explained simply by manual segmentation error. It is encouraging that only small percentage differences in accuracy and repeatability between the two techniques were found making its potential application to deriving PCSA and estimating the force-generating capacity of muscle acceptable.

Ultrasound images are subject to distortion due to tissue compression from the transducer and, in general, have poor resolution of deep muscles (Fry et al., 2004; Infantolino et al., 2007). In this study, the focus was on superficial muscle, and to enhance image quality and eliminate transducer pressure, a water bath was used. The use of a water bath also assisted the multiple sweeps scanning procedure by allowing sufficiently overlapping parallel sweeps and the maintenance of an orthogonal transducer orientation to the skin surface. Lack of overlap resulted in gaps in the 3D-rendered muscle and, hence, inaccuracies in the measurement of the muscle volume and muscle belly length. Usually echogenic gel is the coupling medium used between the skin and the ultrasound transducer during scanning. Our experience using gel for single sweep scanning was positive, but for multiple sweeps scanning was mixed in regard to recorded image quality and tissue-deformation differences between sweeps that ultimately affected the post-processing procedures. While water baths can be specifically designed for examining peripheral musculature of the upper and lower limb, other coupling medium may be required to be developed to make this technology more applicable to clinical settings. To ensure quality ultrasound images for 3D reconstruction, muscles must have clearly identifiable borders. The latter can pose a problem if muscles have been affected by pathology, are partly fused to other muscles, or insert on poorly defined aponeuroses. Further investigations of the validity of 3DUS for volume and length measurements in other specific muscles and using difference subject populations may be required.

4.1. Concluding remarks

This study has demonstrated that accurate and repeatable measurement of relatively large muscle volume and muscle belly length in vivo is possible using multiple sweeps freehand 3DUS imaging over a large range of ankle joint angles. Errors in length and volume measurement of less than 2% can be considered negligible when using morphology measures to make estimates of muscle force or a muscles length range, however, for smaller muscles, the percentage errors are likely to increase as was the case in the study by Delcker et al. (1999). Ultrasound, compared to other imaging techniques, can be performed quickly and in almost any subject position, can scan large objects using multiple sweeps, and is relatively cheaper and more portable. Also other measures of in vivo muscle morphology can be obtained during the brief time for data collection such as anatomical cross-sectional area, muscle–tendon length, fibre length and pennation angle allowing an individual subject estimation of PCSA within the setting of a biomechanics laboratory. The freehand 3DUS system lends itself to in vivo muscle morphological measurements for monitoring changes due to age, function, pathology, surgery and training and researchers may consider the use of freehand 3DUS interchangeably with MRI.

Conflict of interest statement

No financial or personal relationships were conducted with individuals or organizations that could inappropriately influence or bias this work.

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