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## Measurement of human muscle volume using ultrasonography

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**Abstract** The volume of the human tibialis anterior (TA) muscle was estimated in vivo by ultrasonography (ULT) and by magnetic resonance imaging (MRI) in six subjects. In both methods, 11 axial scans were taken along the muscle belly, and the cross-sectional area of the muscle in each scan was digitised. Muscle volume was calculated by treating the muscle as a series of truncated cones. To assess the reproducibility of the ULT method, all subjects were scanned twice. A high test-retest reliability was found ( $R^2 = 0.99$ ), with the two ULT measurements being significantly correlated with each other ( $P < 0.05$ ). The ULT and MRI methods gave similar results [mean (SD) ULT: 133.2 (20) cm<sup>3</sup>; MRI: 131.8 (18) cm<sup>3</sup>]. Nevertheless, a systematic bias of 3.33 cm<sup>3</sup> and a random error of 3.53 cm<sup>3</sup> were found when using the ULT method compared with the MRI method, which results in an error of -0.15% to 5.17%. We conclude that the ULT method is a reproducible and valid method for the estimation of human muscle volume.

**Keywords** Muscle volume · In vivo · Ultrasonography · MRI

### Introduction

The volume of a muscle reflects its power-producing potential. Magnetic resonance imaging (MRI) has often been used to estimate human muscle volume in vivo (e.g. Fukunaga et al. 1992; Maganaris et al. 2001; Narici et al. 1992). This method has been validated through comparison with phantoms of known volume, and it is now

considered as the “gold standard” for assessing the validity of other methods (Mitsiopoulos et al. 1998; Miyatani et al. 2000). Another imaging tool often used for muscle morphometry is ultrasonography (ULT). Like MRI, ULT allows the distinction between muscle and fat and does not involve exposure to ionising radiation; it is also cheaper than MRI, portable and more widely available.

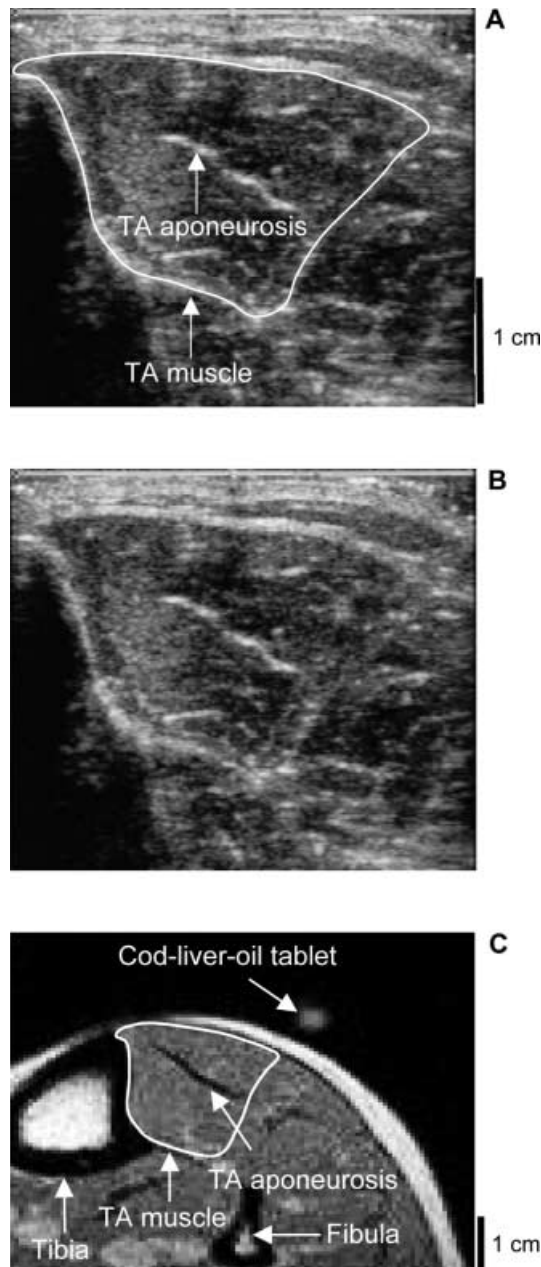
In the past, ULT has been used to estimate the dimensions of large muscle groups, using mathematical formulae for incorporating pre-determined levels of error (Walton et al. 1997). The aims of the present study were to (1) use ULT to measure the volume of a single human muscle, the tibialis anterior (TA) muscle, (2) examine the reproducibility of the ULT method and (3) examine the validity of the ULT method by comparison with results obtained using MRI.

### Methods

Three males and three females [mean (SD) age, height and body mass: 23 (3) years, 175 (8) cm and 70 (6) kg, respectively] volunteered to participate in this study, with approval from the Manchester Metropolitan University Ethics Committee. All subjects were healthy and physically active. The left leg of the subjects was imaged while they were in the supine position at rest, with the ankle positioned at 10° of plantarflexion on a footplate (where 0° is with the sole of the foot at a right angle to the lower-leg axis) and the knee fully extended. All images were taken after 20–30 min of rest to avoid fluid shifts that might induce interstitial and/or intracellular changes (Berg et al. 1993). During the measurements, the subjects were asked to keep their leg muscles relaxed. A 7.5-MHz, linear, B-mode, ULT probe (HDI 3000, ATL Ultrasound, Bothell, USA) was placed in the mid-sagittal plane of the TA muscle over its proximal and distal regions to identify the myotendinous and osteotendinous junctions. The length of the muscle was taken as the distance between these two reference points over a straight line on the skin. Eleven axial-plane scans were taken along the length of the TA muscle, including its myotendinous and osteotendinous junctions, using both MRI and ULT. Scans were taken with guidance from an orthogonal ruler oriented along the tibial axis. The inter-scan distance was kept constant for each subject, but it was altered between subjects according to the length of the muscle. Examples of MRI and ULT axial-plane scans are shown in Fig. 1.

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The ULT method was applied first. To avoid tissue compression in the ULT measurements, all sonographs were taken without touching the skin, with the lower leg immersed in a water-filled container. All ULT images were recorded twice, 5 min apart, stored on videotape and then analysed. The MRI method was applied on a second visit, which took place 1–3 days later. MRI scans were recorded in a 0.2-T magnet (E-type, ESAOTE S.p.A, Genoa, Italy) using a T<sub>1</sub> spin-echo sequence with a 940-ms repetition time, 26-ms echo time, two excitations, a 260×192 matrix and 180×180-mm field of view. The MRIs were taken at the same positions were the ULT scans had previously been taken, with guidance from cod-liver-oil tablets attached on the skin.



**Fig. 1.** **A** A typical axial-plane sonograph 4 cm below the osteotendinous junction of the tibialis anterior (TA) muscle. **B** The sonograph shown in **A** without the superimposed border. **C** The corresponding axial-plane magnetic resonance image (MRI)

In all scans recorded, the anatomical cross-sectional area (ACSA) of the muscle was digitised using computerised image analysis (NIH Image, National Institute of Health, Bethesda, USA). All morphometrics were performed by the same investigator three times, and average values were used for further analysis. The volume  $V$  of the muscular portion between every two consecutive scans was calculated from the equation:

$$V = 1/3(a + \sqrt{ab} + b) \quad (1)$$

where  $a$  and  $b$  are the ACSAs of the muscle in the two scans. The volume of the entire muscle was calculated by summing up all of the inter-scan muscular volumes.

#### Statistics

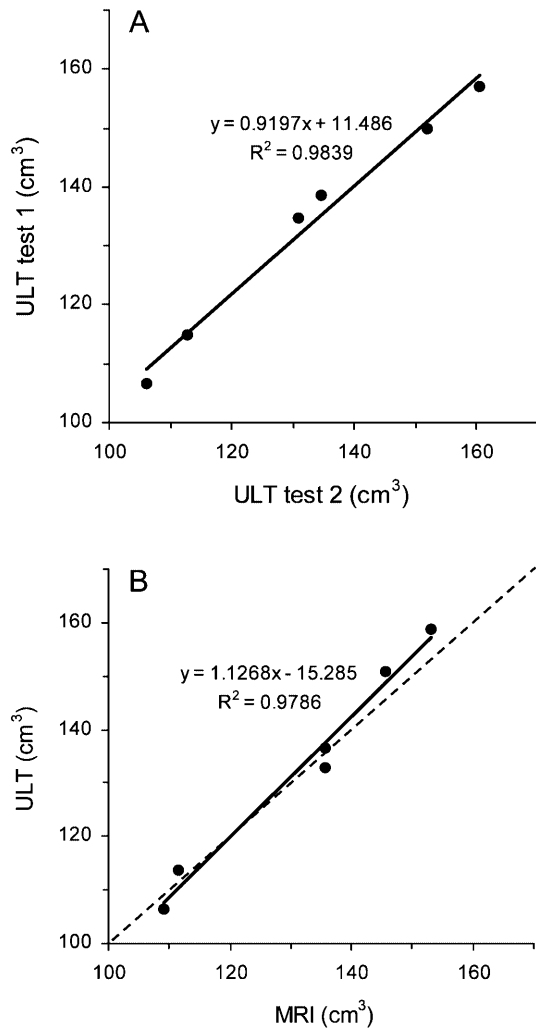
Values are presented as means (SD). An intra-class correlation coefficient (ICC) based on a two-way random-effect model was used to examine the test-retest reliability between the two ULT measurements. The 95% limits of agreement (LOA) was used to assess the degree of agreement between the MRI and ULT methods. Statistical significance was set at a level of  $P < 0.05$ .

### Results and discussion

The data obtained by the ULT and MRI methods are depicted in Fig. 2. The relationship between ULT muscle volume and MRI muscle volume may be described by the linear function  $ULT V = 1.1268 \times MRI V - 15.285$  (Eq. 2). The volume values obtained in the first and second ULT measurements were 133.6 (20) cm<sup>3</sup> and 132.8 (21) cm<sup>3</sup>, respectively. The MRI method yielded a volume value of 131.8 (18) cm<sup>3</sup>. The two ULT measurements were significantly correlated with each other ( $P < 0.05$ ) and the average ICC was 0.99. The 95% confidence interval for the ICC was 0.96–1.00. The LOA for the ULT method was 3.33 (3.53) cm<sup>3</sup>, indicating a bias of 3.33 cm<sup>3</sup> and a random error of 3.53 cm<sup>3</sup>. Thus, the range within which an individual's difference scores would fall in 95% of the cases would be between  $-0.20$  cm<sup>3</sup> and  $+6.86$  cm<sup>3</sup>. The above analysis indicates that the result of the ULT method has 1 chance in 20 of being greater than 5.17% or lower than 0.15% of the result obtained using the MRI method. Such differences would not be considered relevant in clinical and applied physiology applications. Moreover, the ICC for the ULT method was 0.99, indicating that the ULT method is highly reproducible.

Although our results indicate that the ULT method may be a useful tool for measuring muscle volume, several considerations need to be taken into account:

1. As shown in Fig. 2, compared with the MRI method, the ULT method tends to underestimate muscle volumes smaller than 120 cm<sup>3</sup>, and to underestimate muscle volumes larger than 120 cm<sup>3</sup>. For muscle volumes 70–400 cm<sup>3</sup> (most of the individual muscles of the lower extremity lie within this range; see Fukunaga et al. 1992; Lieber 1992), our data indicate that the ULT method would introduce a measurement error of only  $\approx 7\%$ . Clearly, the predictive power



**Fig. 2.** **A** The volume data obtained in the first series of ultrasonography (ULT) measurements (*ULT test 1*) in six subjects plotted against the corresponding volume data obtained in the second series of ULT measurements (*ULT test 2*). **B** Average volume data obtained across the two ULT measurements in the six subjects plotted against the corresponding volume data obtained by MRI. The *dotted line* represents the linear regression that would have been obtained if the ULT and MRI data coincided. Note that compared with the MRI method, the ULT method underestimates and overestimates volumes below and above 120 cm<sup>3</sup>, respectively

of the relationship between ULT and MRI volume measurements in muscles outside the above range diminishes, and therefore Eq. 2 should not be used.

2. ULT-based morphometry depends upon the orientation of the probe relative to the scanned structure. Trigonometry-based calculations indicate that

placing the probe at an angle of 10–20° to the skin instead of perpendicular would result in overestimating muscular volume by 2–6.5%. Appropriate guidance from external markers needs to be provided during scanning if such errors are to be avoided.

3. The ULT method is more time consuming than the MRI method. In our experiment, the MRI measurements lasted 20 min, whereas the ULT measurements lasted 45 min. Shorter scanning times would be achieved by obtaining fewer ULT axial-plane scans along the muscle, but this would increase the difference between the actual and measured volumes (Narici et al. 1992; Walton et al. 1997).
4. In contrast to MRI, the use of ULT is currently restricted to superficial muscles only.

To conclude, we have shown that ultrasonography can provide accurate and reproducible measurements of muscle volume. Although it has certain disadvantages compared to MRI-based measurements, ULT can be a helpful tool in studies involving superficial human muscles.

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