Eur J Appl Physiol (2004) 91: 116–118
DOI 10.1007/s00421-003-0961-9

SHORT COMMUNICATION

**Neil D. Reeves · Constantinos N. Maganaris
Marco V. Narici**

Ultrasonographic assessment of human skeletal muscle size

Accepted: 1 July 2003 / Published online: 25 November 2003

Abstract The measurement of human muscle size is essential when assessing the effects of training, disuse and ageing. The considered 'gold standard' for cross-sectional area measurements of muscle size is magnetic resonance imaging (MRI). However, MRI is costly and often inaccessible. The aim of the present study was to test the reproducibility and validity of a more accessible alternative method using ultrasonography (ULT). We examined the cross-sectional areas in the vastus lateralis muscle of six individuals. Axial-plane ULT scans were taken at given levels along the entire muscle length. The ULT scanning was repeated on different days (reliability) and validated against MRI-based measurements. Mean intraclass correlation coefficients were 0.998 for the reliability of ULT and 0.999 for the validity of ULT against MRI. The coefficient of variation values for cross-sectional area measurements assessed by six different experimenters were 2.1% and 0.8% for images obtained with ULT and MRI, respectively. The ULT method is a valid and reliable alternative tool for assessing cross-sectional areas of large individual human muscles. The present findings justify the application of the ULT method for the detection of changes throughout large muscles in response to training, disuse or as a consequence of sarcopenia.

Keywords Ultrasound · Magnetic resonance imaging · Muscle cross-sectional area · Skeletal muscle

N. D. Reeves (✉) · C. N. Maganaris · M. V. Narici
Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University,
Alsager Campus, Hassall Road, Alsager,
Cheshire, ST7 2HL, UK
E-mail: n.reeves@mmu.ac.uk
Tel.: +44-0161-2475429
Fax: +44-0161-2476375

Introduction

The accurate measurement of muscle size is important for assessing adaptations in response to training (Fiatarone et al. 1990), disuse (Berg et al. 1991, 1997) or as a consequence of sarcopenia (Roubenoff and Hughes 2000). Magnetic resonance imaging (MRI) is widely considered to be the 'gold standard' for the assessment of muscle size due to the high contrast between tissues of different molecular properties. However, access to MRI for research purposes is often limited due to the large clinical demand and the considerable cost. Therefore, a more accessible valid and reliable alternative method is required. Ultrasonography (ULT) using a compound scanning technique has previously been applied (Sipila and Suominen 1993) to measure gross limb or muscle group cross-sectional areas (CSA). This technique, however, is not of sufficient quality to allow delineation of individual muscles. B-mode ULT produces images of high quality, but has previously only been used to scan small muscles due to the limited size of the ULT scan window (Rankin and Stokes 1998; Esformes et al. 2002). However, functionally important muscles, such as those involved in locomotion, often have large CSAs (Lieber 1992). Here, we have developed a method based on B-mode ULT that allows assessment of single, large locomotor muscles. We have examined the vastus lateralis (VL) muscle, which has one of the largest CSAs in the human body (Lieber 1992).

Methods

Subjects

Approval for this study was gained from the Ethics Committee of the Institute for Biophysical and Clinical Research into Human Movement at the Manchester Metropolitan University. Six healthy individuals (three males) [mean (SD) values for age, height and body mass were 76.8 (3.2) years, 162.9 (10.7) cm and 63.8 (15.7) kg, respectively], gave written informed consent to participate in this study.

Ultrasonography

B-mode ULT (HDI-3000, ATL, Bothell, Wash., USA), using a 7.5-MHz linear-array probe, was applied to obtain axial-plane images of the VL muscle (Fig. 1a). All measurements were performed on the right leg after subjects had been in the supine position for 20 min to allow fluid shifts to occur (Berg et al. 1993). During all measurements, subjects were instructed to relax their leg muscles. The proximal insertion of the VL muscle was identified and marked on the skin, and axial sections were then marked at intervals of 30 mm from this point. Orientated in the axial-plane, the transducer was aligned perpendicular to the VL muscle and moved from a central to lateral position along a pre-marked section over external markers fixed on the skin. Great care was taken to be consistent in applying minimal pressure during scanning to avoid compression of the muscle. Scanning was recorded on videotape, and single scans were identified for further analysis. Using the lines cast by the external markers as references, scans were fitted using contour matching. Muscle CSAs were measured using digitizing software (NIH image, National Institutes of Health, Bethesda, Md., USA). Measurements were then repeated a mean of 3 days later.

Magnetic resonance imaging

The validity of the ULT method was tested against MRI-based measurements. A 0.2 T MRI scanner (E-Scan, Esaote Biomedica, Genoa, Italy) was used to image the right thigh (Fig. 1b), after following the same procedures as with ULT to allow fluid shifts to occur. It was only possible to scan the lower portion of the thigh due to constraints in the size of the available coil. Axial-plane images of the VL muscle were acquired using a T1-weighted, spin-echo sequence with the following scanning parameters: time to echo (TE), 28 ms; time of repetition (TR), 850 ms; 1 acquisition; field of view (FOV), 200×200 mm; matrix, 256×192; 5-mm slice thickness; and 1-mm inter-slice gap. Oil-filled capsules used as external markers were secured onto the skin and used to identify the corresponding sections measured with ULT. The MRI and ULT measurements were taken on the same day, approximately 1 h apart. Muscle CSAs were measured using the digitizing software described above. The same investigator performed all measurements using MRI and ULT.

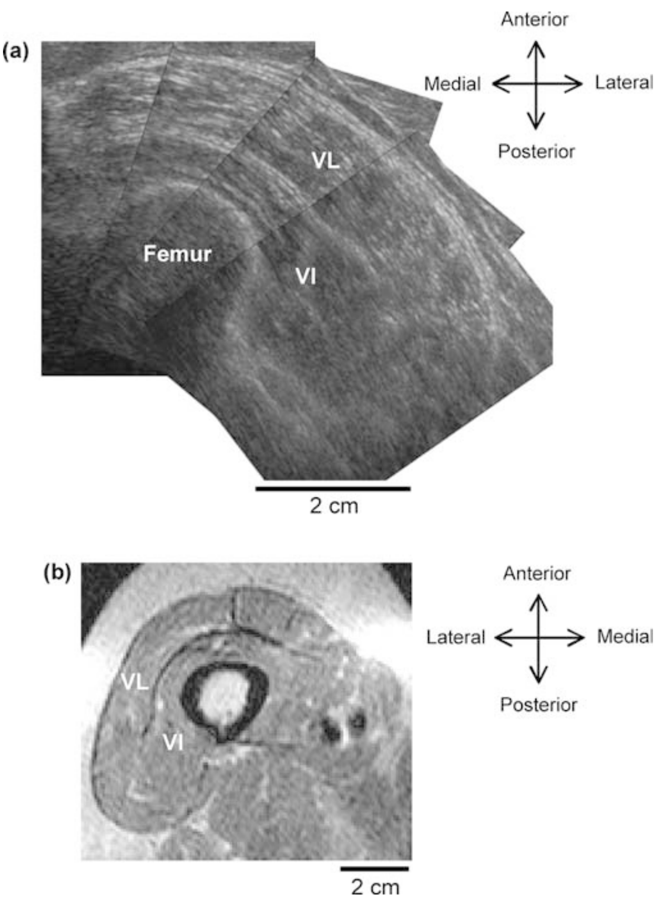

Fig. 1 Typical axial-plane scans taken at the level of scan 6, using **a** ultrasonography (ULT) and **b** MRI, showing vastus lateralis (*VL*) and vastus intermedius (*VI*) muscles in each image

Inter-experimenter reliability

Six different experimenters measured the CSA of the VL muscle at a given anatomical level in scans obtained with ULT and MRI.

Statistical analysis

Inter-day reliability of the ULT method in measuring the VL muscle CSAs was tested with an intra-class correlation coefficient (ICC), using a one-way random effects model. Validity of the ULT measurements against measurements obtained using MRI were analysed with an ICC, using a two-way random effects model (absolute agreement definition). The typical error (standard error of measurement) was assessed for both inter-day reliability and validity of the ULT method. Typical error was calculated using the equation $SD_{diff}/\sqrt{2}$, where SD_{diff} is the standard deviation of the difference scores between tests 1 and 2. Inter-experimenter reliability was assessed using the coefficient of variation (CV) from the equation (SD×1.96)/mean×100. Values presented are means (SD).

Results

Scans 1–10 of the VL muscle CSAs assessed on different days using ULT are displayed in Fig. 2a. The ULT method yielded ICCs between 0.997 and 0.999 for CSAs assessed in scans 1–10 when applied on different days. Typical error for ULT reliability ranged from 0.15 cm^2 (1% in scan 3) to 0.40 cm^2 (3.3% in scan 2). The reproducibility of the ULT method involved a mean typical error of 0.29 cm^2 (2.6%).

Figure 2b displays the agreement between the ULT and MRI methods in measuring the VL muscle CSAs in scans 6–10. Validity of the ULT method against MRI in assessing CSAs in scans 6–10 produced ICCs between 0.998 and 0.999. Typical error for scans 6–10 ranged from 0.07 cm^2 (0.5% in scan 6) to 0.20 cm^2 (1.9% in scan 7). The ULT method involved a mean typical error of 0.15 cm^2 (1.7%) when compared to MRI.

The CV values for CSA measurements assessed by different experimenters were 2.1% and 0.8% for images obtained with ULT and MRI, respectively.

Discussion

Our results show that the ULT method applied is valid and reliable for assessing the size of a large, individual, human locomotor muscle (Fig. 2a and 2b). This method

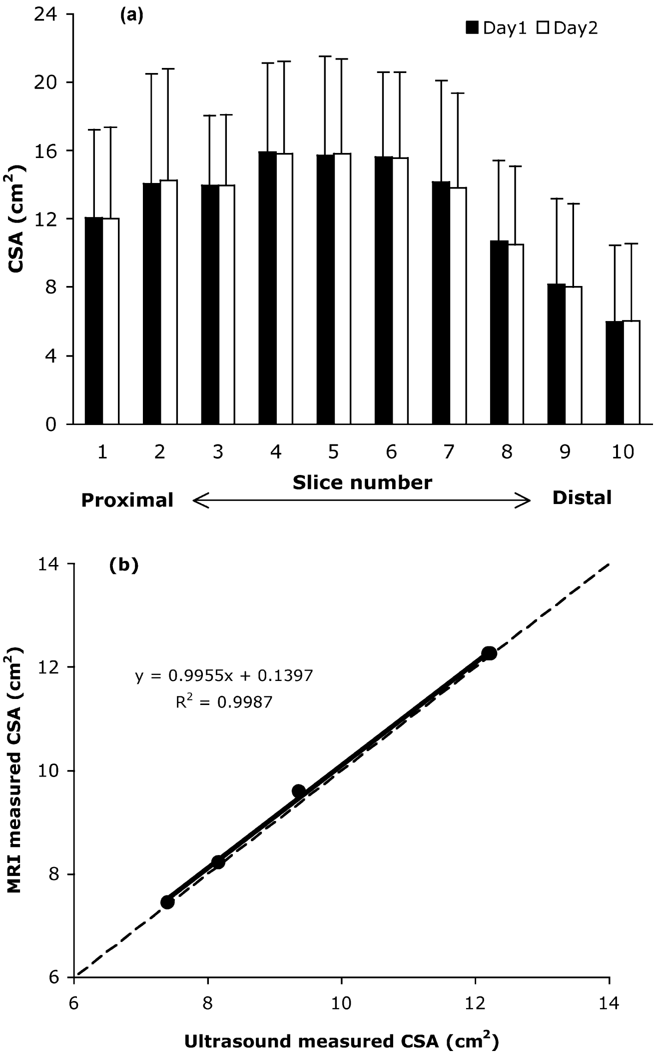

Fig. 2 **a** Inter-day reliability of ULT-based cross-sectional area (CSA) measurements in scans 1–10. For scans 1–9 $n=6$ and scan 10 $n=4$. Values are mean (SD). **b** Validity of ULT against MRI in measuring CSAs in scans 6–10. For scan 6 $n=3$, scan 7 $n=5$, scan 8 $n=5$, scan 9 $n=6$ and scan 10 $n=3$. The *dashed line* represents the line of identity. Values are means

can provide information on CSA changes along the entire muscle length in response to training, disuse or as a consequence of sarcopenia. The absence of any systematic bias and the minimal typical error found (~2%) indicates that the ULT method is valid in comparison to MRI and does not under- or overestimate CSAs within the investigated range, which comprised between 7.45 cm^2 and 12.27 cm^2 (Fig 2b). Assessment of muscle size using MRI has previously shown that older individuals typically display increases in quadriceps CSA of ~10% after a strength-training programme (Fiatarone et al. 1990). Also, in response to unloading/disuse of various durations, muscle atrophy assessed by MRI has been reported to be between 7% and 23% (Berg et al. 1991, 1997; Narici et al. 1998). Changes of such levels are much larger than the typical error associated with using the ULT method in the present study. This suggests that the ULT method can detect changes similar to those expected in response to increased and decreased use with a comparable degree of precision to MRI.

B-mode ULT allows excellent differentiation between the hyperechoic tendon and connective tissue and the hypoechoic muscle. This enables accurate delineation of the borders of individual muscles. The low CV for inter-experimenter measurements indicates that both scanning methods produce images with sufficient clarity and contrast between tissues to allow accurate CSA measurement. An advantage of ULT is that it provides a greater magnification of superficial structures and therefore might reduce the potential for errors as compared to peripheral and whole-body MRI scanners. Nevertheless, ULT has the disadvantage that it demands a greater scanning time as compared to MRI (ULT: ~20 min; MRI: ~6 min, in the present study). A source of potential error with ULT is applying too much pressure on the skin with the transducer and compressing the underlying muscle, especially in lean individuals. Therefore, it is important to be consistent in applying minimal pressure, to use a stand-off, or immerse the limb in a water-bath (Esformes et al. 2002).

In summary, it has been shown that the ULT method applied to measure CSAs of a large, individual locomotor muscle is valid and reproducible. These characteristics make ULT a useful, alternative tool to MRI for assessing alterations in human skeletal muscle size typically observed in response to training, disuse and as a consequence of sarcopenia.

Acknowledgements This work has been partly supported by Italian Space Agency funds.

References

Berg HE, Dudley GA, Haggmark T, Ohlsen H, Tesch PA (1991) Effects of lower limb unloading on skeletal muscle mass and function in humans. J Appl Physiol 70:1882–1885

Berg HE, Tedner B, Tesch PA (1993) Changes in lower limb muscle cross-sectional area and tissue fluid volume after transition from standing to supine. Acta Physiol Scand 148:379–385

Berg HE, Larsson L, Tesch PA (1997) Lower limb skeletal muscle function after 6 wk of bed rest. J Appl Physiol 82:182–188

Esformes JI, Narici MV, Maganaris CN (2002) Measurement of human muscle volume using ultrasonography. Eur J Appl Physiol 87:90–92

Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ (1990) High-intensity strength training in nonagenarians. Effects on skeletal muscle. J Am Med Assoc 263:3029–3034

Lieber RL (1992) Skeletal muscle: structure and function. Williams and Wilkins, Baltimore, Md.

Narici MV, Capodaglio P, Minetti AE, Ferrari-Bardile A, Maini M, Cerretelli P (1998) Changes in human skeletal muscle architecture induced by disuse-atrophy. J Physiol (Lond) 506P:59

Rankin G, Stokes M (1998) Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. Clin Rehabil 12:187–199

Roubenoff R, Hughes VA (2000) Sarcopenia: current concepts. J Gerontol A Biol Sci Med Sci 55:M716–724

Sipila S, Suominen H (1993) Muscle ultrasonography and computed tomography in elderly trained and untrained women. Muscle Nerve 16:294–300