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Reproducibility of ultrasound-derived muscle thickness and echo-intensity for the entire quadriceps femoris muscle

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A B S T R A C T

Introduction: Muscle thickness (MT) and muscle echo-intensity (EI) allow the study of skeletal muscle adaptive changes with ultrasound. This study investigates the intra- and inter-session reliability and agreement of MT and EI measurements for each of the four heads of the quadriceps femoris in transverse and longitudinal scans, using two sizes for the region of interest (ROI); EI measurements only.
Methods: Three B-mode images from two views were acquired from each head of quadriceps femoris from twenty participants (10 females) in two sessions, 7 days apart. EI was measured using a large and a small ROI. Reliability was examined with the mixed two-way intra-class correlation coefficient (ICC), the standard error of mean (SEM) and the smallest detectable change (SDC). Bland–Altman's plots were used to study agreement.
Results: High to very high inter-session ICC values were found for MT for all muscle heads, particularly for measurements from transverse scans. For EI measurement, ICC values ranged from low to high, with higher ICC values seen with the largest ROI. SDC values ranged between 0.19 and 0.53 cm for MT and between 3.73 and 18.56 arbitrary units (a.u.) for two ROIs. Good agreement existed between MT measurements made in both scans. A small bias and larger 95% limits of agreement were seen for EI measurements collected with the two ROI sizes.
Conclusion: Ultrasound measures of MT and EI show moderate to very high reliability. The reliability and agreement of MT and EI measurements are improved in transverse scans and with larger ROIs.
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Introduction

Imaging modalities are being increasingly employed to study skeletal muscles changes occurring due to disuse, ageing, training, or disease.[1] Compared with magnetic resonance imaging, ultrasound is less expensive and more accessible.[2] Furthermore, ultrasound equipment is portable and allows dynamic assessments to be performed in real time, which is useful in assessing physiological changes[3–5] and in diagnosing muscle injury and dysfunction.[6,7] Modern ultrasound technology has also greatly improved the quality of the ultrasound images and has widened the number of ultrasound imaging applications. The development of linear transducers with frequencies in the 7–15 MHz range has largely improved the scanning of more superficial structures and the visualization and delineation of the muscles and of their fascia and tendons, allowing fast and economical measurements of muscle architecture and composition to be made.

Muscle strength and function correlates with muscle mass and composition.[8] Changes in muscle mass happen relatively fast in response to strength training,[9,10] immobilization,[11] malnutrition,[12] aging,[13,14] and disease.[15–17] Muscle thickness (MT) is a simple measure gathered from B-mode ultrasound images of muscles that is highly correlated with muscle cross sectional area. The reproducibility of ultrasound MT measurements is usually reported to be high or very high. This has been demonstrated for trunk,[18–20]

Abbreviations: EI, echo-intensity; MT, muscle thickness; VM, vastus medialis; VL, vastus lateralis; RF, rectus femoris; VI, vastus intermedius; ROI, region of interest; ICC, intraclass correlation coefficient; SD, standard deviation; SEM, standard error of measurement; SDC, smaller detectable change; LoA, limits of agreement.

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respiratory,[21] and upper and lower limb muscles,[22,23] and for inter-session[19,23,24] and inter-rater measures.[21,22,25] Regarding muscle size assessment, the major disadvantage of ultrasound is that it only scans a rather limited area of the whole muscle. Also, slight changes in the orientation of the ultrasound probe might seriously affect MT measures. These drawbacks have hitherto been solved by standardizing the scanning region or by fixing the probe over the body segment, when this is feasible. Yet, MT measures precision is similar in novices and experienced examiners.[19] In addition, the well-defined orientation of muscle fascicles aids in ultrasound probe placement when the muscle is scanned longitudinally.

Besides muscle mass, muscle composition also affects muscle function. More recently, muscle echo-intensity (EI) has been explored as a potential marker of muscle tissue status. The normal muscle appears in the ultrasound image (brightness mode) as a relatively hypoechoic structure, due to the rather low reflection of the ultrasound wave beam (low EI). In a transverse scan, muscles have a speckled appearance, which is explained by the higher EI of the perimysium surrounding muscle fiber bundles compared to that of the proper muscle tissue. The contrast in EI between muscle fascicles and the connective tissue of the perimysium is clearer in longitudinal scans and is very useful for further characterization of the muscle architecture, as well as for defining the muscle boundaries, taking advantage of the hyperechoic epimysium and overlying fascia.[1,25–27]

The EI in an ultrasound scan can be measured simply as the average intensity of the pixels inside the muscle of interest, usually using a scale of levels of gray within a given region of interest (ROI). Although a few studies confirm the good inter-session reliability of EI measures for muscles, there are still important questions about what would be the most desirable method for collecting such measures. One of the doubts regards ROI size that for some authors should include as much of the muscle as possible, avoiding bones and surrounding fascia. Imaging a whole section of the muscle would probably be important since internal fascia and non-homogenous distribution of EI might affect the measures. The orientation of the muscle bundles might also affect the reliability of EI measures, particularly in longitudinal scans.[27–29]

Some studies have investigated reliability of MT and EI using the quadriceps muscle,[26,27,29] although using generally only one of its four heads. However, the quadriceps femoris is anatomically and functionally complex and its different heads may adapt differently to training.[28] Due to their anatomy, different ultrasound examination techniques are required to image each of the four heads of the quadriceps femoris, thus potentially affecting the reliability of ultrasound measures.[28]

Therefore, this study assesses the intra and inter-session reliability (one week apart) of ultrasound measures of MT and EI in each of the four quadriceps femoris heads both in transverse and longitudinal scans and employing a rectangular ROI or the entire scanned section of the muscle.

Methods

Participants

Twenty healthy participants (10 females, mean ± standard deviation; age = 20.0 ± 2.3 years; height = 1.7 ± 0.1 m; mass = 64.2 ± 10.9 kg; right thigh perimeter = 52.0 ± 3.8 cm; left thigh perimeter = 51.7 ± 4.1 cm) not engaged in sports or intense physical activities were informed about the study's protocol and procedures and gave written informed consent. Participants were excluded from the study if they sustained an injury in the lower extremity in the past six months, suffered from an orthopedic condition or had surgery involving the lower extremities. Participants were also excluded if they have resistance trained their legs anytime during the past 12 months.

Procedures

To assess intra- and inter-session reliability of ultrasound measurements of MT and EI, three different ultrasound B-mode images were acquired bilaterally in transverse and longitudinal views from the four heads of the quadriceps muscle in two sessions, with an interval of 7 days between them. All participants were right-side dominant. To avoid possible effects related with daily routine, participants were evaluated at the same time of day in the two sessions and by the same examiner, a certified musculoskeletal ultrasound sonographer. Between each scan, the transducer was moved away from the thigh and then placed back again over the same region of the thigh for the next scan.

Each head of the quadriceps muscle [vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF) and vastus intermedius (VI)] was imaged with participants lying in supine, with the legs extended and relaxed. The ultrasound probe was placed over the midbelly region of each head of the quadriceps femoris, away from the patella at the following percentage of the distance between the upper edge of the patella and the superior iliac spine: 22% for VM, 39% for VL, and 56% for RF and VI.[26]

A portable ultrasound machine (LOGIQe, General Electric Healthcare, GE Ultraschall, Deutschland GmbH & Co, Germany) equipped with a linear-array transducer with band frequency 7–12 MHz was used for collecting the images. Gain was set at 48% of the range, dynamic range was maintained at 93 dB, and time compensation was kept at the same (neutral) position for all imaged depths. The depth setting was adjusted for each muscle in order to visualize their superior and inferior margins. Images were recorded as DICOM files and stored in a personal computer for later processing.

MT and EI were obtained using ImageJ (National Institutes of Health, Bethesda, MD, USA) by the same examiner. The three images of each muscle for the two sides and from both data-collecting sessions and for transverse and longitudinal views from the twenty participants were analyzed, in a total of 1824 images.

MT was measured as the largest distance between the superficial and deep fasciae, identified by their hyperechoic appearance. Two different ROIs were selected to measure EI: (1) maximum ROI, draw for each scan to include as much of the muscle as possible, avoiding bone and surrounding fasciae (Fig. 1); (2) small ROI, a 70 mm^2 ROI positioned over the central region of the muscle image (Fig. 1). EI was then defined as the mean level of gray within the ROI in 8-bit resolution images (gray levels from 0 to 255, where black = 0 and white = 255).

Statistical analysis

Intra- and inter-session reliability for MT and EI were assessed using intra-class correlation coefficient (ICC $_{3,1}$; method: alpha, two-way mixed, consistency). For inter-session reliability, the average of the three measures obtained in each session was used.

Standard error of measurement (SEM) and smallest detectable change (SDC) were also calculated. SEM indicates the precision of the measurement and was calculated based on the ICC and the standard deviation of the mean of the differences between the two measurements (i.e., $SEM = SD\sqrt{1 - ICC}$). The SDC was based on the SEM, using the formula: $SDC = 1.96 \times \sqrt{2} \times SEM$.

The level of agreement between transverse and longitudinal scans and between the two ROI sizes was evaluated by Bland–Altman's analysis and respective 95% limits of agreement (LoA), using the data collected in the first session. In the Bland

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