ORIGINAL RESEARCH

Muscle Architecture and Subcutaneous Fat Measurements of Rectus Femoris and Vastus Lateralis at Optimal Length Aided by a Novel Ultrasound Transducer Attachment

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ABSTRACT

Purpose: This cross-sectional study determines the sensitivity of muscle architecture and fat measurements of the rectus femoris (RF) and vastus lateralis (VL) muscles from ultrasound images acquired with varying transducer tilt, using a novel transducer attachment, in healthy adults. Secondary objectives were to estimate intrarater and interrater reliability of image measurement and acquisition, respectively. ***Methods:*** Thirty healthy adults participated (15 women and 15 men; 25 [SD 2.5] y). Ultrasound image acquisition was conducted by two raters at different transducer tilts relative to the skin: estimated perpendicular, and five measured angles (80°, 85°, 90°, 95°, 100°) using the transducer attachment. Muscle thickness (MT), subcutaneous fat thickness (FT), pennation angle (PA), and fascicle length (FL) were measured. Sensitivity and reliability were assessed using intra-class correlation coefficients (ICCs) and standard error of measurements (SEMs). ***Results:*** MT and FT for RF and VL were not sensitive to transducer tilt. However, PA and FL were sensitive to transducer tilt. MT and FT for both muscles showed high ICCs and low SEMs for intrarater and interrater reliability. For PA of both muscles, standardizing transducer tilt improved interrater ICCs and lowered SEMs. ***Conclusion:*** MT and FT measurements of RF and VL acquired at 60° knee flexion are robust to varying transducer tilt angles. PA measurements benefit from standardizing transducer tilt.

Key Words: anatomy, cross-sectional; muscle, skeletal; reproducibility of results; ultrasonography.

RÉSUMÉ

Objectif: étude transversale pour déterminer la sensibilité de l’architecture musculaire et des mesures lipidiques du muscle droit antérieur de la cuisse (MDAC) et du muscle vaste externe (MVE) à partir des images échographiques acquises chez des adultes en santé par diverses inclinaisons du transducteur, au moyen d’un nouveau dispositif. Les objectifs secondaires consistaient à évaluer la fiabilité intraévaluateurs et interévaluateurs des mesures et de l’acquisition des images, respectivement. ***Méthodologie:*** au total, 30 adultes en santé ont participé (15 femmes et 15 hommes de 25 [ÉT 2,5 ans]). Deux évaluateurs ont acquis des images échographiques à des inclinaisons différentes du transducteur par rapport à la peau : mesure perpendiculaire estimative et mesure à cinq angles (80°, 85°, 90°, 95°, 100°) au moyen du dispositif du transducteur. Ils ont mesuré l’épaisseur des muscles (ÉM), l’épaisseur de la graisse sous-cutanée (ÉG), l’angle de pennation (AP) et la longueur des fascicules (LF). Ils ont aussi évalué la sensibilité et la fiabilité au moyen de coefficients de corrélation intraclasse (CCI) et de l’écart-type des mesures (ÉTM). ***Résultats:*** l’ÉM et l’ÉG du MDAC et du MVE n’étaient pas sensibles à l’inclinaison du transducteur, mais l’AP et la LF l’étaient. La fiabilité intraévaluateur et interévaluateur de l’ÉM et de l’ÉG des deux muscles présentait un CCI élevé et un ÉTM faible. Pour ce qui est de l’AP des deux muscles, la standardisation de l’inclinaison du transducteur améliorait la CCI et réduisait l’ÉTM interévaluateurs. **Conclusion:** les mesures de l’ÉM et de l’ÉG du MDAC et du MVE acquises à une flexion du genou de 60° sont probantes à des angles d’inclinaison variables du transducteur. Les mesures de l’AP tirent profit d’une inclinaison du transducteur standardisée.

Mots-clés: anatomie, transversale; échographie; muscle, squelettique; reproductibilité des résultats

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The force potential of skeletal muscle is directly related to the number of available sarcomeres. Therefore, the macroscopic arrangement of muscle fascicles (i.e, muscle architecture) is foundational to force production, as well as control of movement, disease, and injury.[1] Muscle architectural features that are commonly reported include muscle thickness (MT), pennation angle (PA) (i.e., fascicle angle relative to force-generating axis of muscle), and fascicle length (FL).[1,2] Fat accumulations in and around skeletal muscle can interfere with force production.[3–5]

Ultrasonography is a relatively inexpensive, non-invasive modality that is useful for measuring muscle architecture and subcutaneous fat accumulations. Not only does this technology advance the study of muscle architecture in understanding force production, it offers an important capacity for clinical practice. For example, MT measurements from ultrasound images have tracked muscle wasting in critically ill patients,[6,7] body composition change after exercise,[8,9] and age-related sarcopenia and fall risk.[10,11] This capacity to quantify muscle and fat outcomes has the potential to advance physiotherapy (PT) practice.

Ultrasound produces reliable data on features of muscle architecture and fat,[12] including on the vastus lateralis (VL)[13,14] and rectus femoris (RF)[15] in healthy adults. To date, most of these reliability studies occur at rest, close to full knee extension.[12,16,17] In this joint position, the quadriceps are at a mechanical disadvantage because sarcomeres are shorter than their optimal length.[18] Since significant fascicle rotation occurs during muscle contraction,[19] we cannot generalize muscle architectural features near full knee extension to understand how the muscle fascicles contribute to its greatest force generation at its optimal length.

Furthermore, the quality of measurements of muscle and fat acquired from ultrasound is likely sensitive to the orientation of the transducer.[20,21] Typically, an ultrasound transducer is oriented perpendicular to the skin.[12,22] We are aware of only one study that standardized transducer tilt. Konig and colleagues used a foam cast to standardize transducer tilt to 90 degrees relative to the skin when imaging the gastrocnemius in healthy adults.[23] MT from images acquired using the cast had lower error (SEM 0.05 cm) than without (SEM 0.1 cm).[23]

We examined the sensitivity of muscle architecture and subcutaneous fat measurements from ultrasound images to variations in transducer tilt. The primary purpose of this study was to determine the sensitivity of muscle architecture (MT, PA, and FL) and subcutaneous fat thickness (FT) measurements to acquisition with five different ultrasound transducer tilt angles: 80°, 85°, 90°, 95°, and 100°, as well as at an angle estimated to be perpendicular to the skin. This work was conducted in the RF and VL while resting at the optimal length in healthy adults. To achieve this objective, we created a novel 3D-printed ultrasound transducer attachment with an affixed protractor (Figure 1). A secondary purpose was to estimate the intrarater reliability of post hoc muscle architecture measurements and interrater reliability of image acquisition by two different raters with novice imaging experience. We hypothesized that muscle architecture and FT measurements would be sensitive to large deviations of the transducer tilt angle from the perpendicular. We also expected data acquisition (interrater) and measurement analysis (intrarater) to be reliable.

METHODS

Design

This cross-sectional study included ultrasound image acquisition of the RF and VL using multiple transducer tilt angles, with and without the novel transducer attachment, in healthy adults during a single visit. To answer the primary research question, sensitivity to different ultrasound transducer tilt angles was determined. The reliability of both image acquisition (interrater) and measurement analysis (intrarater) were estimated for images acquired under each tilt condition. To estimate interrater image acquisition reliability, ultrasound images (for each muscle, tilt angle) were captured by two different raters (BDB, JNCH) at the same visit. To estimate intrarater measurement reliability, muscle architecture measurements were conducted twice, separated by three weeks, by one rater (BDB).

The Hamilton Integrated Research Ethics Board approved this study, and all participants provided written, informed consent.

Participants

We recruited a convenience sample of 30 healthy adults (15 women, 15 men) aged 20–29 years using electronic advertisements to the university community and by word of mouth. Reliability studies typically require 30 participants.[24,25] We included those who self-reported a healthy status on the Get Active Questionnaire. Exclusion criteria were self-reported lower extremity pain or injury over the past year; heart, lung, kidney, gastrointestinal, or liver disease; cancer; or low back pain.

Landmarking and positioning

We asked the participants to avoid strenuous physical activity within 24hours of the visit and to wear loose-fitting shorts and athletic shoes. The study limb was selected using a random number generator to include 15 right legs and 15 left legs. Body height and mass were measured while barefoot. Participants warmed up using the six-minute walk test.[26,27] Care was taken to ensure that the scanning sites remained consistent.[28] The greater trochanter, lateral femoral condyle, anterior superior iliac spine (ASIS), and the superficial border of the patella were palpated and marked with indelible ink by a research assistant (EGW). To standardize RF measurements, the midpoint between the ASIS and the superior border of the patella was demarcated,[28–30] and the distance between the ASIS and superior border of

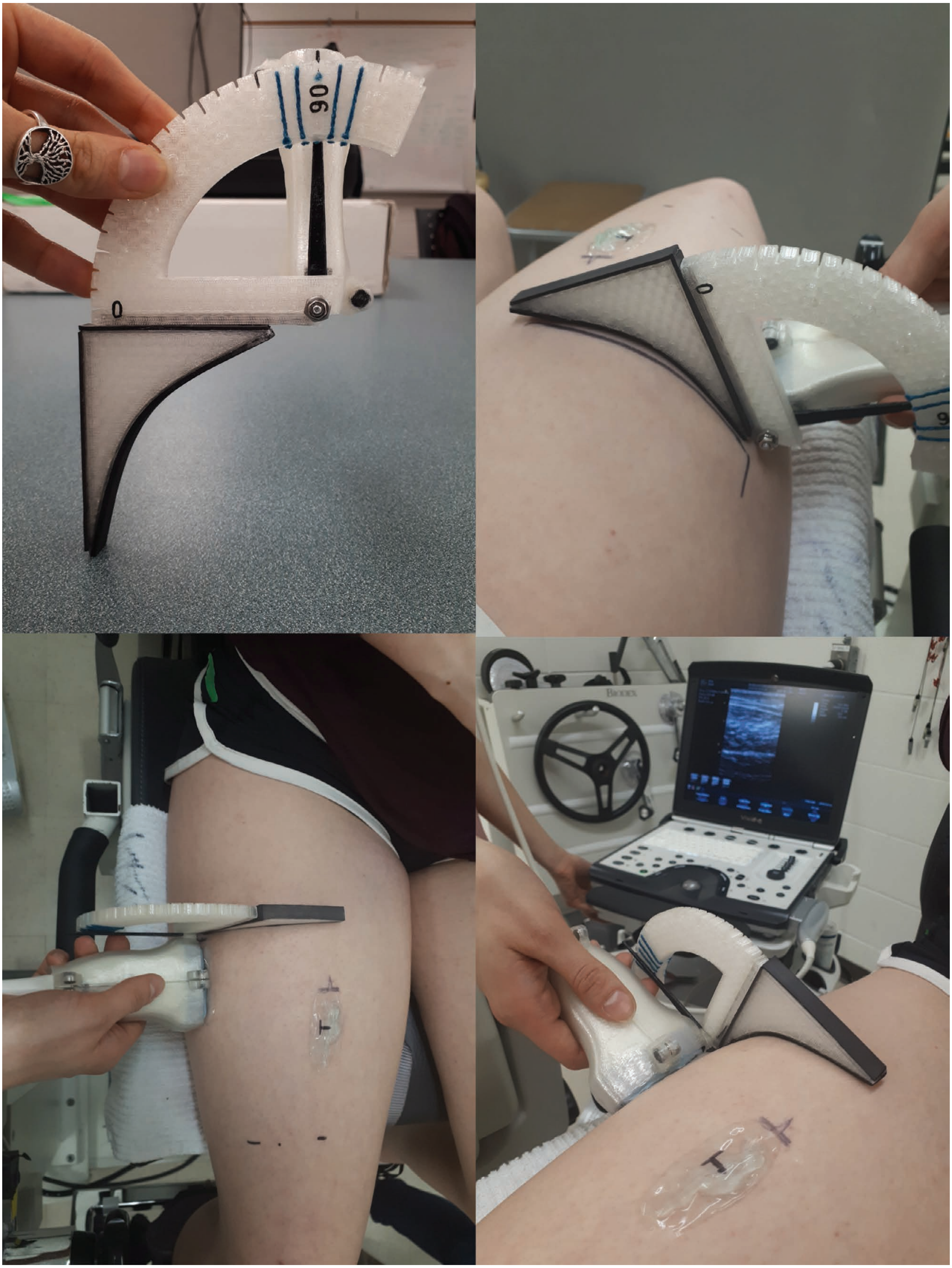

Figure 1 The novel transducer attachment is secured to the ultrasound transducer by neodymium magnets and features an affixed protractor with demarcations in five-degree increments between eighty and one-hundred degrees. The bottom of the wedge fits along the thigh. These images show the transducer with the attachment imaging the vastus lateralis.

the patella was recorded. The septum of the RF was first located in the transverse view for reference; if the septum interfered with clarity (e.g., if it was aligned through the centre of the image), the transducer was moved modestly medial at the same superior/inferior location, and this new placement was marked. To standardize VL measurements, the midpoint between the greater trochanter and lateral femoral epicondyle of the thigh was demarcated, 30–32 and the distance between the greater trochanter and lateral femoral condyle was recorded.

To standardize positioning, participants were seated on a dynamometer (Biodex System 4, Biodex Medical Systems, 49 Natcon Drive, Shirley, NY). The lateral femoral condyle was centred to the axis of rotation. The leg was secured using a Velcro strap just proximal to the lateral malleolus. The knee was positioned at 60 degrees of flexion, coinciding with the optimal quadriceps length for adults.[33–35] Isokinetic contractions confirmed that 60.0 (SD 4.8) degrees of flexion corresponded with peak torque production in this sample.

Image acquisition

A high frequency linear probe (12L-RS) and ultrasound were used for imaging (Vivid Q, GE Healthcare, 9900 Innovation Drive, Wauwatosa, WI). Water-soluble gel was used for acoustic coupling.[36] Raters applied minimal pressure during imaging. Acquisition parameters included a signal depth of 5.5 cm, frequency of 11 MHz, and 2 focus points.

Two raters acquired sets of three images of the RF and VL at every transducer tilt angle (80°, 85°, 90°, 95°, and 100°) plus one image at an estimated 90° angle. All images were acquired in the longitudinal plane for each muscle.[37–39] To standardize this acquisition, one region of interest for each muscle was traced on the skin. For each rater, the first image acquired for each muscle was at an angle they estimated to be 90 degrees to the skin. The actual angle was measured by a research assistant (EGW). The raters were blinded to the actual angle until after study completion. Both raters acquired sets of 3 images for each measured transducer tilt angles of 80°, 85°, 90°, 95°, and 100° to the skin, where 80° and 85° were lateral to perpendicular, and 95° and 100° were medial to perpendicular. The order of muscles, transducer angle, and rater was block randomized. The transducer was removed from the skin between every acquisition. Before conducting analyses, images with poor clarity (of the whole image, aponeuroses, and/or fascicles) were removed. All image acquisitions (one set by each rater, BDB and JNCH) were conducted on the same day.

Muscle architecture measurements

Muscle architecture measurements were retrieved using definitions described in previous literature for MT,[40] FT,[41] PA,[42] and FL[43] (online Supplemental Figure 1). A custom, semi-automated Python programme calculated these measurements from user-identified points on the images. Rater 1 (BDB) analyzed all images twice in random order, separated by three weeks, and was blinded to participant, muscle, and transducer tilt angle. The average of the three acquired images for each angle, muscle, and rater was used for analysis.

Statistical analyses

To address the primary purpose, sensitivity was assessed between 90 degrees and every other tilt condition for MT, subcutaneous FT, PA, and FL for RF and VL. The standard error of measurement (SEM) was the standard deviation of differences divided by the square root of two. Two-way random, intra-class correlation coefficients (ICCs) were used.[44] Next were the secondary analyses. For intrarater reliability of measurements, SEMs and ICCs were calculated between repeated muscle measurements from the same underlying images by one rater (BDB). For the interrater reliability of the acquisition of images, SEMs and ICCs were calculated between the images acquired by Rater 1 and the images acquired by Rater 2; all images were analyzed by the same rater (BDB). Statistical analysis was completed with IBM SPSS Statistics, version 25.0 (IBM Corporation, Armonk, NY), and a p-value < 0.05 was considered significant.

RESULTS

The demographics of the participants are summarized in Table 1. Of the 1,920 images acquired, 1,732 images (90%) could facilitate all measurements and were included in the analyses. Of the 1,920 images, there were 99 images with unclear aponeuroses and 89 with unclear fascicles,

Table 1 Demographics of Participants

	All participants (*N* = 30)		Women (*n* = 15)		Men (*n* = 15)	
	Mean (SD)	Range, min-max	Mean (SD)	Range, min-max	Mean (SD)	Range, min-max
Age (y)	25 (2.5)	20–29	25 (2.6)	22–29	24 (2.2)	20–29
BMI (kg/m^2)	22.6 (3.0)	17.2–29.4	22.2 (2.5)	19.4–27.5	23.0 (13.0)	17.2–29.4
RF length (cm)	44.2 (2.9)	36.4–49.3	42.9 (2.4)	36.4–46	45.6 (2.6)	40.4–49.3
VL length (cm)	46.1 (3.0)	40.3–55.5	45.1 (2.6)	40.3–48.7	47.1 (3.1)	40.8–55.5
6MWT (m)	679 (91)	453–940	662 (67)	537–762	697 (110)	453–940

RF = rectus femoris; VL = vastus lateralis; 6MWT = six-minute walk test.

eliminating the measurement of PA and FL. For only RF, there were 155 images with extrapolated FLs that exceeded the actual distance measured from origin to insertion. These erroneous FLs were observed most frequently at 80- and 85-degree transducer tilt angles.

Varying transducer tilt angles

Mean muscle architecture measurements for the RF and VL are summarized in online Supplemental Table 1. When the raters estimated a perpendicular transducer tilt position, the actual transducer angles were near 90° for the RF (mean 90.0° [SD 4.8°] by Rater 1; mean 91.5° [SD 4.0°] by Rater 2) and the VL (mean 86.5 [SD 3.0]° by Rater 1; mean 85.5° [SD 3.0°] by Rater 2).

Table 2 displays the SEMs and ICCs that evaluate the sensitivity of measurements at each of the five tilt angles relative to a measured 90° transducer tilt angle for RF (online Supplemental Figure 2). SEM for RF MT and FT were < 0.16 cm for all tilt angles for both Raters 1 and 2. MT and FT displayed ICCs between 0.82 and 0.99 for all transducer tilt angles and both raters, compared to the measured 90° position. The lower limits of the 95% CIs for these ICCs were 0.87–0.98 for FT, and 0.58–0.92 for MT. However, PAs and FLs were sensitive to variations in transducer tilt, showing poor ICCs and high SEMs when compared to the measured 90-degree transducer tilt angle. Less sensitivity was observed with smaller deviations in transducer tilt from 90 degrees, where images of the RF acquired at 85- and 95-degree transducer tilt angles generally displayed better reliability for PA and FL compared to other angles.

Table 3 shows the SEMs and ICCs that evaluate the sensitivity of measurements at each of the 5 tilt angles relative to a measured 90° transducer tilt angle for VL (online Supplemental Figure 3). Transducer tilt angles of 80° produced the highest error for VL MT (highest SEM, lowest ICC). However, FT measurements appeared consistent across angles, with ICCs of 0.97–0.99 and SEMs < 0.09 cm. PAs and FLs were sensitive to variations in transducer tilt, showing poor ICCs and high error (SEMs) for PA compared to the measured 90° transducer tilt angle (100° and estimated 90° the worst).

Secondary purposes

Intrarater reliability of measurements

SEM and ICCs for intrarater reliability of RF and VL measurements are displayed in online Supplemental Table 2. Excellent reliability was demonstrated with ICCs > 0.87 and low error (particularly for MT and FT).

Interrater reliability of image acquisition

SEMs and ICCs assessing interrater reliability of RF and VL images are displayed in online Supplemental Table 3. High interrater reliability was demonstrated for MT and FT

Table 2 Sensitivity of RF Measurements from Images Acquired at Varying Transducer Tilts

	Rater		EP vs. 90°	80° vs. 90°	85° vs. 90°	95° vs. 90°	100° vs. 90°
MT (cm)	1	SEM	0.15 (0.08–0.21)	0.10 (0.07–0.13)	0.07 (0.04–0.10)	0.16 (0.04–0.26)	0.09 (0.06–0.11)
		ICC	0.82 (0.65–0.91)	0.90 (0.83–0.98)	0.96 (0.89–0.98)	0.82 (0.65–0.91)	0.91 (0.78–0.96)
		n	28	26	29	29	28
	2	SEM	0.16 (0.08–0.22)	0.09 (0.06–0.11)	0.06 (0.04–0.07)	0.10 (0.05–0.14)	0.12 (0.06–0.16)
		ICC	0.83 (0.68–0.92)	0.92 (0.77–0.97)	0.97 (0.92–0.99)	0.92 (0.84–0.96)	0.85 (0.58–0.94)
		n	30	29	29	30	30
FT (cm)	1	SEM	0.06 (0.04–0.07)	0.07 (0.03–0.10)	0.05 (0.03–0.07)	0.06 (0.03–0.08)	0.05 (0.02–0.07)
		ICC	0.98 (0.96–0.99)	0.97 (0.94–0.99)	0.99 (0.98–1.0)	0.98 (0.95–0.99)	0.99 (0.97–0.99)
		n	29	26	29	29	28
	2	SEM	0.09 (0.04–0.13)	0.07 (0.03–0.10)	0.10 (0.03–0.14)	0.07 (0.02–0.11)	0.05 (0.03–0.06)
		ICC	0.95 (0.90–0.98)	0.97 (0.94–0.99)	0.94 (0.87–0.97)	0.97 (0.93–0.98)	0.98 (0.97–0.99)
		n	30	29	29	30	30
PA (°)	1	SEM	3.03 (1.84–4.23)	2.56 (1.85–3.14)	1.84 (1.34–2.25)	2.18 (1.56–2.68)	2.85 (2.17–3.37)
		ICC	0.25 (−0.09–0.55)	0.10 (−0.16–0.41)	0.55 (0.18–0.77)	0.43 (−0.01–0.71)	0.35 (0–0.63)
		n	26	24	28	28	27
	2	SEM	3.31 (2.32–4.13)	2.80 (1.99–3.42)	2.14 (1.24–2.98)	1.81 (1.33–2.21)	2.56 (1.88–3.06)
		ICC	0.11 (−0.21–0.43)	0.09 (−0.22–0.41)	0.42 (0.08–0.67)	0.50 (0.15–0.74)	0.44 (0.08–0.70)
		n	29	29	29	28	27
FL (cm)	1	SEM	8.32 (5.03–10.77)	6.38 (2.72–7.78)	5.98 (2.33–9.07)	4.96 (3.06–6.16)	9.00 (5.90–11.00)
		ICC	0.21 (−0.29–0.60)	0.19 (−0.16–0.68)	0.44 (−0.02–0.76)	0.65 (0.20–0.85)	0.17 (−0.25–0.56)
		n	19	8	15	18	19
	2	SEM	6.09 (3.88–7.73)	8.29 (3.83–10.33)	4.09 (3.01–4.83)	4.76 (3.15–6.05)	4.90 (3.42–5.97)
		ICC	0.51 (0.10–0.78)	0.26 (−0.30–0.73)	0.75 (0.43–0.89)	0.45 (0.04–0.74)	0.51 (−0.04–0.80)
		n	30	30	30	30	26

RF = rectus femoris; EP = estimated perpendicular; SEM = mean (95% confidence interval) of standard error of measurement; ICC = intra-class correlation coefficient; *n* = sample size; MT = muscle thickness; FT = fat thickness; PA = pennation angle; FL = fascicle length.

Table 3 Sensitivity of VL Measurements from Images Acquired at Varying Transducer Tilts

	Rater		EP vs. 90°	80° vs. 90°	85° vs. 90°	95° vs. 90°	100° vs. 90°
MT (cm)	1	SEM	0.15 (0.10–0.19)	0.21 (0.15–0.25)	0.12 (0.09–0.15)	0.12 (0.09–0.14)	0.16 (0.11–0.20)
		ICC	0.88 (0.76–0.94)	0.72 (0.49–0.86)	0.91 (0.82–0.96)	0.92 (0.85–0.96)	0.84 (0.66–0.93)
		n	30	30	30	29	24
	2	SEM	0.22 (0.14–0.29)	0.25 (0.19–0.30)	0.20 (0.13–0.26)	0.16 (0.09–0.20)	0.16 (0.10–0.20)
		ICC	0.70 (0.47–0.85)	0.49 (0.17–0.72)	0.69 (0.45–0.84)	0.82 (0.65–0.92)	0.79 (0.58–0.91)
		n	30	30	30	30	26
FT (cm)	1	SEM	0.09 (0.06–0.11)	0.07 (0.04–0.10)	0.06 (0.03–0.08)	0.07 (0.03–0.10)	0.05 (0.03–0.06)
		ICC	0.97 (0.94–0.99)	0.98 (0.94–0.99)	0.99 (0.97–0.99)	0.98 (0.95–0.99)	0.98 (0.95–0.99)
		n	30	30	30	29	24
	2	SEM	0.09 (0.06–0.12)	0.05 (0.03–0.07)	0.06 (0.03–0.08)	0.08 (0.04–0.12)	0.04 (0.03–0.05)
		ICC	0.97 (0.94–0.99)	0.99 (0.97–0.99)	0.99 (0.97–0.99)	0.98 (0.95–0.99)	0.99 (0.98–1.00)
		n	30	30	30	30	26
PA (°)	1	SEM	2.78 (2.03–3.45)	3.68 (2.40–4.66)	2.46 (1.78–2.97)	2.99 (2.05–3.68)	5.09 (3.87–6.04)
		ICC	0.56 (0.26–0.76)	0.22 (−0.13–0.53)	0.61 (0.33–0.80)	0.45 (0.12–0.69)	0 (−0.57–0.14)
		n	30	30	30	29	24
	2	SEM	3.77 (2.03–5.51)	3.51 (2.46–4.35)	3.23 (2.07–4.35)	3.10 (1.82–4.15)	4.03 (2.55–5.47)
		ICC	0.10 (−0.28–0.45)	0.25 (−0.13–0.56)	0.27 (−0.11–0.57)	0.36 (0.01–0.63)	0 (−0.38–0.35)
		n	30	30	30	30	26
FL (cm)	1	SEM	2.19 (1.40–2.74)	1.16 (0.85–1.39)	1.24 (0.80–1.61)	1.52 (1.08–1.82)	3.08 (1.55–4.07)
		ICC	0.33 (−0.03–0.61)	0.49 (0.09–0.74)	0.50 (0.18–0.72)	0.55 (0.03–0.80)	0.20 (−0.10–0.52)
		n	30	30	30	29	24
	2	SEM	3.12 (1.34–4.69)	3.00 (1.19–4.70)	2.58 (0.75–4.15)	1.74 (1.00–2.42)	3.61 (1.60–5.54)
		ICC	0.13 (−0.23–0.46)	0.08 (−0.24–0.40)	0.23 (−0.10–0.52)	0.78 (0.58–0.89)	0.17 (−0.16–0.50)
		n	30	30	30	30	26

VL = vastus lateralis; EP = estimated perpendicular; SEM = mean (95% confidence interval) of standard error of measurement; ICC = intra-class correlation coefficient; MT = muscle thickness; FT = fat thickness; PA = pennation angle; FL = fascicle length.

measurements of RF and VL (ICC > 0.88, SEM < 0.18 cm). RF PA was most reliable at the 80° transducer tilt angle, as evidenced by the highest ICC (0.82) and lowest SEM (1.18°), while VL PA was most reliable at the 85° tilt angle (ICC 0.85, SEM 1.84°). Reliability was poorest at 100 degrees and estimated perpendicular conditions. RF FL reliability was considerably lower at the 85-degree angle relative to other angles, with a low ICC (0.33) and high SEM (7.24 cm). VL FL reliability was fairly consistent across tilt angles, also showing the highest reliability for the 85-degree tilt condition.

DISCUSSION

We explored the impact of altering ultrasound transducer tilt angles on measurements of muscle architecture and subcutaneous FT, from quadriceps positioned at the optimal length in healthy adults. Our results suggest that measurements of MT and subcutaneous FT of the RF and VL are not sensitive to different transducer tilt angles during acquisition or to different raters. However, PA measurements benefit from standardizing transducer tilt angle and staying within 10 degrees of the perpendicular (i.e., acquiring images 85°–95°) to the skin surface. Beyond this range of transducer tilt (i.e., at 80° or 100°), or when raters estimated a perpendicular transducer tilt, lower ICCs and higher SEMs were observed. Importantly, estimations of FL extrapolated from the images did not appear trustworthy. Finally, interrater reliability on image acquisition and intrarater reliability (considering both the lower limit of the ICC confidence intervals and the upper limit of the SEM confidence intervals) on measurement analysis were acceptable for MT and FT measurements.

A novel transducer attachment that standardizes the ultrasound transducer tilt angle improves the acquisition of PA of RF and VL in healthy, young adults. First, estimating a transducer tilt angle during acquisition is inaccurate. In this study, low ICCs and high SEM (Tables 2 and 3) for PA (of RF and VL) were observed when comparing the estimated perpendicular angles with the measured 90-degree transducer tilt angle. This inability to reliably estimate transducer tilt is confirmed by the recent work of Ishida and colleagues who found that while intrarater reliability of estimates of transducer tilt were strong, interrater reliability between two raters was poor, with ICC of 0.40 and a SEM of 4°.[45] Second, the attachment improves data consistency for some but not all measurements. Interrater reliability of PA is improved by using the attachment compared to estimating transducer positioning (online Supplemental Table 3). Furthermore, as the angle of the transducer deviates farther from the perpendicular to the skin, the SEM and ICCs worsen. Previous work found five-degree deviations from the perpendicular produced muscle architecture values (MT, PA, FL) of the gastrocnemius with low error (4%); but deviations greater than five degrees produced high error (up to 25%).[46] Finally, the transducer attachment features a wedge to accommodate the curvature of the thigh, which may distribute contact forces and thereby lower pressure of the transducer head on the region of interest. Future work should explore this hypothesis. It is important to note that, while it is currently unconventional to capture specific muscle architecture measurements using ultrasound in PT practice, there is growing interest in using this relatively accessible, inexpensive technology in measuring sarcopenia (e.g., with aging, prolonged immobilization, clinical conditions) and fatty accumulation. Findings from this study suggest that MT and FT measurements are robust to variations in transducer tilt which has great potential for advancing PT practice. Measures of FL and PA, which appear sensitive to protocol, may be more difficult to standardize in a clinical environment.

Interrater and intrarater reliability of image acquisition and measurement, respectively, depend on the specific outcome of interest from the ultrasound images. The protocols used in this study resulted in reliable data on thicknesses (muscle, fat), regardless of transducer tilt angle or rater. These data complement recent work. High to very-high test-retest reliability of quadriceps MT measurements were observed in 20 healthy, young par- ticipants.[47] Similarly, excellent reliability of subcutaneous FT has been shown in lean, overweight, and obese adults at eight sites, including overlying the quadriceps, in 38 adults.[48] Our intrarater ICC values for FT over the RF are the same as a previously reported.[35] The robustness of these thickness measurements enables exploration of the mechanisms underlying pathologies in which voluntary movements are either compromised or unattainable (e.g., among critically ill),[49–51] and enables applications in clinical practice. It is important to note, though, that samples that are older or with disease may not produce the same results. Unlike tissue thickness measurements, anisotropy (property of being directionally dependent) explains why PA measurements appear sensitive to transducer tilt angle.[52] Nonetheless, the relative and absolute reliabilities of PA measurements (intrarater and interrater at estimated perpendicular and 90° acquisition angles) exceeded the minimum documented in a systematic review of architectural measurements of ultrasound, which were always ICC > 0.50.[12] Strasser and colleagues also reported high reliability for MT (ICC > 0.96) and lower ICC values for FL (ICC 0.57–0.62) and PA measurements (ICC = 0.53) in RF and VL.[53] Together, these data suggest that PA measurements require great care to derive quality data.

The extrapolation technique used to quantify FL from images acquired with the knee positioned at the optimal length of the quadriceps produces data that is sensitive to transducer tilt angle and, unfortunately, is of questionable accuracy. While intrarater reliability values are excellent and interrater reliability values at measured transducer tilts are good or better, the restricted field of view of the ultrasound required estimations of FLs that likely introduced error. Extrapolation resulted in FLs that exceeded the actual length of the muscle in 10% of RF images. These erroneously long FLs were removed from the analyses; however, it is likely that overestimates of FL that did not exceed the muscle length remained in the data set. This error is likely reflected in the relatively large interrater SEMs; that is, at 90° the SEM was 22% of mean FL for VL, and 32% for RF. Average lengths exceed those reported in previous literature.[6,9,49] For example, our findings (16.7–27.7 cm) far exceeded those of Moreau and colleagues, who report mean 9.75 (SD 2.3) cm FLs in the RF.[15] This discrepancy is not as obvious in the VL as values remained within plausible ranges (range, min-max, 9.4–14.1 cm) similar to those found in the literature (range, min-max, 9.9–15.21 cm).[14,54,55] These discrepancies reflect differences in positioning; previous literature placed the quadriceps in the shortest position (at or near full knee extension), and the current study placed the quadriceps at optimal length (60° of knee flexion). Caution should be taken in interpreting the FLs reported here, particularly for RF. Future work should repeat these analyses of FL using an ultrasound with an extended field of view.

Regarding limitations, first, the pressure of the transducer was not quantified. Excessive pressure of the transducer against the skin was minimized by using a generous amount of gel.[36,56,57] Second, given the two-dimensional nature of ultrasound, there was no way to confirm that the transducer head was aligned to the fascicles. An attempt to overcome this was made by scout-scanning to find an optimal alignment. Third, participants were recreationally active university-aged students, which limits the generalizability to other populations, such as those with higher subcutaneous FTs. Fourth, the reliability of anatomical landmarking for ultrasound transducer placement was not assessed, with all landmarking performed by one rater. Future research should investigate the effect of anatomical landmark palpation on muscle architecture measurements. As well, while patients in clinical environments are typically positioned using pillows, participants in this research were seated on a dynamometer. While we expect the reliability outcomes of this study to translate to clinical practice, the impact of variations in patient positioning could be a focus of future work. Fifth, the wide confidence intervals noted for PA and FL measurements suggest that our sample size (particularly in light of removing unclear images) was likely small. Finally, the acquisition of muscle architectural features did not occur during an isometric contraction, which would have provided greater insight into muscle fascicle arrangement during the greatest peak torque production. Future work should use the novel ultrasound transducer attachment to explore the measurement properties of muscle architectural features during isometric and, potentially, dynamic contractions using ultrasound.

CONCLUSION

Measurements of RF and VL thickness and subcutaneous FT acquired at 60° knee flexion are robust to varying transducer tilt angles. PA measurements benefit from the standardization of the transducer tilt angle, which in this study was achieved using a novel ultrasound transducer attachment.

KEY MESSAGES

What is already known on this topic

Ultrasonography is a commonly used medium for quantifying muscle and fat properties.

What this study adds

We present a novel approach to standardizing MSK ultrasound image acquisition and highlight that MT and subcutaneous FT measurements are more reliable than PA and FL measurements of the quadriceps when the muscle is placed in the optimal position.

REFERENCES

1. Lieber RL, Friden J. Functional and clinical significance of skeletal muscle architecture. Muscle Nerve. 2000;23(11):1647–66. https://doi.org/10.1002/1097-4598(200011)23:11%3C1647::aid-mus1%3E3.0.co;2-m
2. Narici M, Franchi M, Maganaris C. Muscle structural assembly and functional consequences. J Exp Biol. 2016;219(Pt 2):276–84. https://doi.org/10.1242/jeb.128017. Medline: 26792340
3. Goodpaster BH, Park SW, Harris TB, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci. 2006;61(10):1059–64. https://doi.org/10.1093/gerona/61.10.1059. Medline: 17077199
4. Kidde J, Marcus R, Dibble L, et al. Regional muscle and whole-body composition factors related to mobility in older individuals: a review. Physiother Can. 2009;61(4):197–209. https://doi.org/10.3138/physio.61.4.197. Medline: 20808481
5. Visser M, Kritchevsky S, Goodpaster B, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. J Am Geriatr Soc. 2002;50(5):897–904. https://doi.org/10.1046/j.1532-5415.2002.50217.x. Medline: 12028178
6. Sabatino A, Maggiore U, Regolisti G, et al. Ultrasound for non-invasive assessment and monitoring of quadriceps muscle thickness in critically ill patients with acute kidney injury. Front Nutr. 2021;8:622823 https://doi.org/10.3389/fnut.2021.622823. Medline: 33937303
7. Hoffmann RM, Ariagno KA, Pham IV, et al. Ultrasound assessment of quadriceps femoris muscle thickness in critically ill children. Pediatr Crit Care Med. 2021;22(10):889–897
8. Escriche-Escuder A, Trinidad-Fernández M, Pajares B, et al. Ultrasound use in metastatic breast cancer to measure body composition changes following an exercise intervention. Sci Rep. 2021;11(1):8858. https://doi.org/10.1038/s41598-021-88375-5. Medline: 33893370
9. Battaglia Y, Ullo I, Massarenti S, et al. Ultrasonography of quadriceps femoris muscle and subcutaneous fat tissue and body composition by BIVA in chronic dialysis patients. Nutrients. 2020;12(5):1388 https://doi.org/10.3390/nu12051388. Medline: 32408709
10. Sai A, Tanaka K, Ohashi Y, et al. Quantitative sonographic assessment of quadriceps muscle thickness for fall injury prediction in patients undergoing maintenance hemodialysis: an observational cohort study. BMC Nephrol. 2021;22(1):191 https://doi.org/10.1186/s12882-021-02347-5. Medline: 34022848
11. Benton E, Liteplo AS, Shokoohi H, et al. A pilot study examining the use of ultrasound to measure sarcopenia, frailty and fall in older patients. Am J Emerg Med. 2020;46:310–16. https://doi.org/10.1016/j.ajem.2020.07.081. Medline: 33041131
12. Kwah LK, Pinto RZ, Diong J, Herbert RD. Reliability and validity of ultrasound measurements of muscle fascicle length and pennation in humans: a systematic review. J Appl Physiol. 2013;114(6):761–9. https://doi.org/10.1152/japplphysiol.01430.2011. Medline: 23305989
13. Brancaccio P, Limongelli FM, D'Aponte A, et al. Changes in skeletal muscle architecture following a cycloergometer test to exhaustion in athletes. J Sci Med Sport. 2008;11(6):538–41. https://doi.org/10.1016/j.jsams.2007.05.011. Medline: 17905658
14. Chleboun GS, Busic AB, Graham KK, et al. Fascicle length change of the human tibialis anterior and vastus lateralis during walking. J Orthop Sports Phys Ther. 2007;37(7):372–9. https://doi.org/10.2519/jospt.2007.2440. Medline: 17710906
15. Moreau NG, Teefey SA, Damiano DL. In vivo muscle architecture and size of the rectus femoris and vastus lateralis in children and adolescents with cerebral palsy. Dev Med Child Neurol. 2009;51(10):800–6. https://doi.org/10.1111/j.1469-8749.2009.03307.x. Medline: 19459913
16. Benjafield AJ, Killingback A, Robertson CJ, et al. An investigation into the architecture of the vastus medialis oblique muscle in athletic and sedentary individuals: an in vivo ultrasound study. Clin Anat. 2015;28(2):262–8. https://doi.org/10.1002/ca.22457. Medline: 25244030
17. Ema R, Wakahara T, Miyamoto N, et al. Inhomogeneous architectural changes of the quadriceps femoris induced by resistance training. Eur J Appl Physiol. 2013;113(11):2691–703. https://doi.org/10.1007/s00421-013-2700-1. Medline: 23949789
18. Enoka RM, Pearson KG. The motor unit and muscle action. In Kandel ER, Schwartz JH, Jessell TM, et al. Principles of neural science. 5th edUSA: McGraw Hill; 2012; 768–89
19. Narici M. Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. J Electromyogr Kinesiol. 1999;9(2):97–103. https://doi.org/10.1016/s1050-6411(98)00041-8
20. Klimstra M, Dowling J, Durkin JL, et al. The effect of ultrasound probe orientation on muscle architecture measurement. J Electromyogr Kinesiol. 2007;17(4):504–514. https://doi.org/10.1016/j.jelekin.2006.04.011. Medline: 16919969
21. Prado CM, Heymsfield SB. Lean tissue imaging: a new era for nutritional assessment and intervention. JPEN J Parenter Enteral Nutr. 2014;38(8):940–953. https://doi.org/10.1177/0148607114550189. Medline: 27208036
22. Alegre LM, Ferri-Morales A, Rodriguez-Casares R, et al. Effects of isometric training on the knee extensor moment-angle relationship and vastus lateralis muscle architecture. Eur J Appl Physiol 2014;. 114(11):2437–2446. https://doi.org/10.1007/s00421-014-2967-x. Medline: 25099962
23. König N, Cassel M, Intziegianni K, et al. Inter-rater reliability and measurement error of sonographic muscle architecture assessments. J Ultrasound Med. 2014;33(5):769–777. https://doi.org/10.7863/ultra.33.5.769. Medline: 24764331
24. Riddle D, Stratford P. Is this change real? Interpreting patient outcomes in physical therapy. 2013; FA Davis, Philadelphia, PA:
25. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016;15(2):155–163. https://doi.org/10.1016/j.jcm.2016.02.012. Medline: 29276468

26. American Thoracic Society. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111–117. https://doi.org/10.1164/ajrccm.166.1.at1102. Medline: 12091180
27. Wilken JM, Darter BJ, Goffar SL, et al. Physical performance assessment in military service members. J Am Acad Orthop Surg. 2012;20(Suppl 1):S42–S47. https://doi.org/10.5435/jaaos-20-08-s42. Medline: 22865136
28. Ando R, Saito A, Umemura Y, et al. Local architecture of the vastus intermedius is a better predictor of knee extension force than that of the other quadriceps femoris muscle heads. Clin Physiol Funct Imaging. 2015;35(5):376–382. https://doi.org/10.1111/cpf.12173. Medline: 24915999
29. Arts IM, Pillen S, Schelhaas HJ, et al. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve. 2010;41(1):32–41. https://doi.org/10.1002/mus.21458. Medline: 19722256
30. Scanlon TC, Fragala MS, Stout JR, et al. Muscle architecture and strength: adaptations to short-term resistance training in older adults. Muscle Nerve. 2014;49(4):584–592. https://doi.org/10.1002/mus.23969. Medline: 23893353
31. Csapo R, Alegre LM, Baron R. Time kinetics of acute changes in muscle architecture in response to resistance exercise. J Sci Med Sport. 2011;14(3):270–274. https://doi.org/10.1016/j.jsams.2011.02.003. Medline: 21411367
32. Vaz MA, Baroni BM, Geremia JM, et al. Neuromuscular Electrical Stimulation (NMES) reduces structural and functional losses of quadriceps muscle and improves health status in patients with knee osteoarthritis. J Orthop Res. 2013;31(4):511–516. https://doi.org/10.1002/jor.22264. Medline: 23138532
33. Lindahl O, Movin A, Ringqvist I. Knee extension: measurement of the isometric force in different positions of the knee-joint. Acta Orthop Scand. 1969;40(1):79–85. https://doi.org/10.3109/17453676908989487. Medline: 5368556
34. Ng AV, Agre JC, Hanson P, et al. Influence of muscle length and force on endurance and pressor responses to isometric exercise. J Appl Physiol. 1994;76(6):2561–2569. https://doi.org/10.1152/jappl.1994.76.6.2561. Medline: 7928884
35. Welsch MA, Williams PA, Pollock ML, et al. Quantification of full-range-of-motion unilateral and bilateral knee flexion and extension torque ratios. Arch Phys Med Rehabil. 1998;79(8):971–978. https://doi.org/10.1016/s0003-9993(98)90097-1
36. Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. J Anat. 2006;209(3):289–310. https://doi.org/10.1111/j.1469-7580.2006.00619.x. Medline: 16928199
37. Narici MV, Maganaris CN, Reeves ND, et al. Effect of aging on human muscle architecture. J Appl Physiol. 2003;95(6):2229–2234. https://doi.org/10.1152/japplphysiol.00433.2003. Medline: 12844499
38. Noorkoiv M, Stavnsbo A, Aagaard P, et al. In vivo assessment of muscle fascicle length by extended field-of-view ultrasonography. J Appl Physiol. 2010;109(6):1974–1979. https://doi.org/10.1152/japplphysiol.00657.2010. Medline: 20884841
39. Rutherford OM, Jones DA. Measurement of fibre pennation using ultrasound in the human quadriceps in vivo. Eur J Appl Physiol Occup Physiol. 1992;65(5):433–437. https://doi.org/10.1007/bf00243510. Medline: 1425649
40. Blazevich AJ, Cannavan D, Coleman DR, et al. Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. J Appl Physiol. 2007;103(5):1565–1575. https://doi.org/10.1152/japplphysiol.00578.2007. Medline: 17717119
41. Núñez M, Nuñez E, Moreno JM, et al. Quadriceps muscle characteristics and subcutaneous fat assessed by ultrasound and relationship with function in patients with knee osteoarthritis awaiting knee arthroplasty. J Clin Orthop Trauma. 2019;10(1):102–106. https://doi.org/10.1016/j.jcot.2017.11.014. Medline: 30705541
42. Ema R, Wakahara T, Mogi Y, et al. In vivo measurement of human rectus femoris architecture by ultrasonography: validity and applicability. Clin Physiol Funct Imaging. 2013;33(4):267–273. https://doi.org/10.1111/cpf.12023. Medline: 23692615
43. Finni T, Komi P. Two methods for estimating tendinous tissue elongation during human movement. J Appl Biomech. 2002;18(2):180–188. https://doi.org/10.1123/jab.18.2.180
44. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979;86(2):420–428. https://doi.org/10.1037/0033-2909.86.2.420
45. Ishida H, Suehiro T, Suzuki K, et al. Muscle thickness and echo intensity measurements of the rectus femoris muscle of healthy subjects: intra and interrater reliability of transducer tilt during ultrasound. J Bodyw Mov Ther. 2018;22(3):657–660. https://doi.org/10.1016/j.jbmt.2017.12.005. Medline: 30100293
46. Bénard MR, Becher JG, Harlaar J, et al. Anatomical information is needed in ultrasound imaging of muscle to avoid potentially substantial errors in measurement of muscle geometry. Muscle Nerve. 2009;39(5):652–665. https://doi.org/10.1002/mus.21287. Medline: 19291798
47. Santos R, Armada-da-Silva PAS. Reproducibility of ultrasound-derived muscle thickness and echo-intensity for the entire quadriceps femoris muscle. Radiography. 2017;23(3):e51–61. https://doi.org/10.1016/j.radi.2017.03.011. Medline: 28687301
48. Störchle P, Müller W, Sengeis M, et al. Standardized ultrasound measurement of subcutaneous fat patterning: high reliability and accuracy in groups ranging from lean to obese. Ultrasound Med Biol. 2017;43(2):427–438. https://doi.org/10.1016/j.ultrasmedbio.2016.09.014. Medline: 27866704
49. Gellhorn AC, Stumph JM, Zikry HE, et al. Ultrasound measures of muscle thickness may be superior to strength testing in adults with knee osteoarthritis: a cross-sectional study. BMC Musculoskelet Disord. 2018;19(1):350. https://doi.org/10.1186/s12891-018-2267-4. Medline: 30261863
50. Sabatino A, Regolisti G, Bozzoli L, et al. Reliability of bedside ultrasound for measurement of quadriceps muscle thickness in critically ill patients with acute kidney injury. Clin Nutr. 2017;36(6):1710–1715. https://doi.org/10.1016/j.clnu.2016.09.029. Medline: 27743614
51. Valla FV, Young DK, Rabilloud M, et al. Thigh ultrasound monitoring identifies decreases in quadriceps femoris thickness as a frequent observation in critically ill children. Pediatr Crit Care Med. 2017;18(8):e339–e347. https://doi.org/10.1097/pcc.0000000000001235. Medline: 28650903
52. Klauser AS, Peetrons P. Developments in musculoskeletal ultrasound and clinical applications. Skelet Radiol. 2010;39(11):1061–1071. https://doi.org/10.1007/s00256-009-0782-y. Medline: 19730857
53. Strasser EM, Draskovits T, Praschak M, et al. Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. Age. 2013;35(6):2377–2388. https://doi.org/10.1007/s11357-013-9517-z. Medline: 23456136
54. Ward SR, Eng CM, Smallwood LH, et al. Are current measurements of lower extremity muscle architecture accurate? Clin Orthop Relat Res. 2009;467(4):1074–1082. https://doi.org/10.1007/s11999-008-0594-8. Medline: 18972175
55. Baroni BM, Geremia JM, Rodrigues R, et al. Muscle architecture adaptations to knee extensor eccentric training: rectus femoris vs. vastus lateralis. Muscle Nerve. 2013;48(4):498–506. https://doi.org/10.1002/mus.23785. Medline: 23852989
56. Lixandrão ME, Ugrinowitsch C, Bottaro M, et al. Vastus lateralis muscle cross-sectional area ultrasonography validity for image fitting in humans. J Strength Cond Res. 2014;28(11):3293–3297. https://doi.org/10.1519/jsc.0000000000000532. Medline: 24845210
57. Valle MS, Casabona A, Micale M, et al. Relationships between muscle architecture of rectus femoris and functional parameters of knee motion in adults with down syndrome. BioMed Res Int. 2016;2016:1–8. https://doi.org/10.1155/2016/7546179. Medline: 27896273