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In vivo assessment of muscle fascicle length by extended field-of-view ultrasonography

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Noorkoiv M, Stavnsbo A, Aagaard P, Blazevich AJ. In vivo assessment of muscle fascicle length by extended field-of-view ultrasonography. *J Appl Physiol* 109: 1974–1979, 2010. First published September 30, 2010; doi:10.1152/japplphysiol.00657.2010.—The present study examined the reliability and validity of in vivo vastus lateralis (VL) fascicle length (L_f) assessment by extended field-of-view ultrasonography (EFOV US). Intraexperimenter and intersession reliability of EFOV US were tested. Further, L_f measured from EFOV US images were compared to L_f measured from static US images (6-cm FOV) where out-of-field fascicle portions were trigonometrically estimated (linear extrapolation). Finally, spatial accuracy of the EFOV technique was assessed by comparing L_f measured on swine VL by EFOV US to actual measurements from digital photographs. The difference between repeated VL L_f measurements by the same experimenter was 2.1 ± 1.7% with an intraclass correlation (ICC) of 0.99 [95% confidence interval (CI) = 0.95–1.00]. In terms of intersession reliability, no difference ($P = 0.48$) was observed between L_f measured on two different occasions, with ICC = 0.95 (CI = 0.80–0.99). The average absolute difference between L_f measured by EFOV US and using linear extrapolation was 12.6 ± 8.1% [ICC = 0.76 (CI = −0.20–0.94)]; EFOV L_f was always longer than extrapolated L_f. The relative error of measurement between L_f measured by EFOV US and by dissective assessment (digital photographs) in isolated swine VL was 0.84% ± 2.6% with an ICC of 0.99 (CI = 0.94–1.00). These results show that EFOV US is a reliable and valid method for the measurement of long muscle fascicle in vivo. Thus EFOV US analysis was proven more accurate for the assessment of skeletal muscle fascicle length than conventional extrapolation methods.

fiber length; vastus lateralis; muscle architecture; reliability; validity

THE LENGTH of a skeletal muscle's fibers is an important determinant of its contractile properties; longer muscle fibers tend to work over greater length ranges and possess a higher absolute shortening speed, the latter leading to a greater contractile power production (8, 14, 34). While muscle fibers are too small to be imaged in vivo, the fascicles they form can be clearly visualized using magnetic resonance (MR) or ultrasound imaging. Although it is known that some muscle fibers terminate midfascicularly, muscle fibers within a fascicle are typically connected serially to make one functional unit (33). Thus the fascicles are the most important functional unit within skeletal muscles, and changes in resting fascicle length have been detected after strength training (5, 26, 28, 31), short-term limb immobilization (7, 24), or long-term disuse (31) and have been closely linked to changes in muscle force production (1, 6, 19). Particularly, ultrasound imaging can be used to visualize the connective and adipose tissues that surround the fascicles and is commonly used to measure both fascicle length and fascicle pennation angle in human skeletal muscle.

Short fascicles that are completely visible within the ultrasound imaging field of view can be directly and accurately measured using digitizing software. However, the measurement of longer fascicles [e.g., 8–12 cm in vastus lateralis (VL)] either requires multiple scans along the muscle length to be fitted together (16), or for trigonometric estimations (linear extrapolations) to be performed to estimate the length of the part of the fascicle that cannot be imaged directly due to the limited field of view of static US imaging (4, 9, 17, 23, 27). Alternatively, more complex and expensive diffusion tensor imaging (DTI) using MR scanning may be performed (20). With respect to the more commonly used ultrasound measurements, comparing estimated (linearly extrapolated) fascicle length (L_f) with the actual L_f measured by fitting multiple images has been shown to yield an error of 2–7% (10, 27). However it is not known whether the error associated with image fitting is high so its validity remains unproven. Importantly, trigonometric estimation of L_f assumes that the nonimaged fascicle portion follows a linear path, which is rarely true in skeletal muscle (4, 23). Consequently, measuring fascicles that are curved, or those that are in muscle regions where the aponeuroses are curved, by means of trigonometric extrapolation is problematic. Thus either direct measurement (i.e., using full field-of-view imaging) or reliable image fitting procedures are required for highly accurate measurement of L_f, when DTI MR scanning is not available.

The extended field-of-view ultrasonography (EFOV US) technique, which uses an algorithm to automatically fit series of images, allows scanning of entire fascicles within one continuous scan [see MATERIALS AND METHODS, and Tan and Liu (32) and Hedrick (13) for details]. The high spatial accuracy of this technique has been demonstrated on custom-designed flat- and curved-surfaced phantoms (11), and the technique was shown to provide valid and reliable cross-sectional area measurements in the human VL (2, 25). Nonetheless, despite EFOV US being spatially accurate, other methodological issues may affect its use for L_f measurement. As is also the case for conventional static US imaging, L_f can be distorted by the orientation of the ultrasound probe (3, 18), so the probe must be aligned with the orientation of the fascicles to minimize perspective and parallax measurement errors. Further, misalignment of the ultrasound probe in relation to the fascicular plane results in overestimation of fascicle length (16) and underestimation of fascicle angle (12, 16). This might be especially problematic when using the EFOV US technique since the probe has to be moved over a longer distance within one continuous scan. Also the probe must be kept perpendic-

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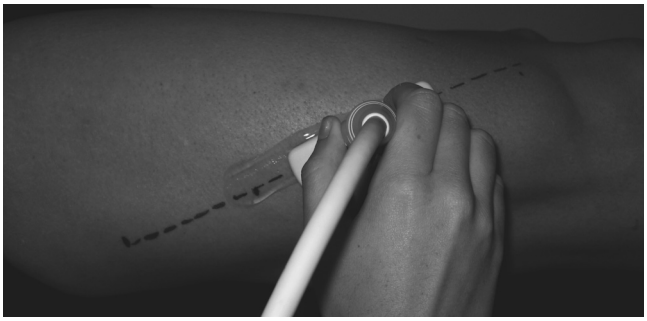

Fig. 1. Measurement of vastus lateralis (VL) fascicle length (L_f). The black line marked on the skin is the line of orientation of VL fascicles, imaged by ultrasound.

ular to the fascicular plane (i.e., when the fascicles are best visible) along the whole imaging path, which is especially problematic in muscles with curved deep aponeuroses (12). Despite the possible limitations of this technology, EFOV imaging may offer an ideal method for accurate and reliable measurement of long muscle fascicle lengths in vivo.

The aim of the present study was to test the reliability and validity of the EFOV US technique for human VL L_f measurements. For this purpose we tested inter- and intraexperimenter reliability, as well as intersession reliability. Also, L_f measured using EFOV US analysis was compared with L_f estimated from static 6-cm images (i.e., standard, single-image ultrasound technique) using trigonometric extrapolation methods. Finally, L_f was assessed in isolated swine VL by EFOV US and compared with the results obtained by dissection of the muscle (digital muscle slice photographs).

MATERIALS AND METHODS

Participants. Ten men between 19 and 40 yr of age (mean 28 ± 5 yr old) volunteered to participate in the study. Participants reported no recent history of thigh muscle injury or inflammatory disease. The study was approved by the Edith Cowan University Human Research Ethics Committee. All subjects provided their written informed consent before participating in the experiments.

Ultrasonography assessments. The EFOV technique creates a continuous composite image from photographs that are continuously captured while the ultrasound probe is moved across the surface of an object (e.g., see Fig. 2). The system used in the present study utilized software-based algorithms to perform the reconstruction so no probe-based mechanical position sensors were needed. The programmed reconstruction algorithm recognized the overlapping regions within the real-time images obtained in series when the probe was moved along the imaged surface. In generating the EFOV image in real-time, the pattern-matching technology was applied to the region of interest (ROI), which was fixed in the system. Matching of image features in successive frames was accomplished by detecting the brightness level in a B-mode image and then searching the prior frame (i.e., the reference frame) for regions in the ROI with similar properties. The different parts of the detected images were subsequently attached to the previous image. This allowed determination of overall probe motion by detecting the movement direction. The current frame was subsequently rotated and translated to be properly positioned within the panoramic image (3). While the specific algorithms used to perform this process may vary between systems of other different manufacturers, the overall process described will be essentially the same; algorithms that can be used to perform similar image reconstructions have been published previously (13).

In the present study, the B-mode axial-plane US (Aloka SSD-a10, software number 6.1.0, Aloka,Tokyo, Japan) images were taken of the vastus lateralis muscle (VL) at 50% of the distance from the greater trochanter to the tibial tuberosity using a 10-MHz linear-array probe (60-mm width) in EFOV mode (sampling frequency = 90 frames/s). The participants were lying in a supine position with knee and hip joint angles at 0° (i.e., full extension). To ensure an identical sampling site for intersession reliability, in addition to the line from greater trochanter to the tibial tuberosity, a line from the central point of the border of patella to the medial aspect of anterior superior iliac spine was drawn, and the distance from the midpoint of this line to the actual scanning point (i.e., midpoint between the GT and the tibial tuberosity) was also measured (Fig. 1). The mid-part of the US probe was placed on the measurement point and the plane of the fascicles was identified as the probe angle giving the largest, continuous fascicle visualization. Then the fascicle path was drawn on the skin according to the fascicle path seen from the real-time ultrasound image. In all scans, a consistent minimal pressure was applied with the probe on the skin to avoid compression of the muscle, aided by the application of a transmission gel to improve acoustic coupling. The participants were instructed to relax their thigh muscles throughout the measurements (the foot was rested against a support to prevent lateral hip rotation). To obtain the muscle image, a continuous single view was taken by moving the probe along the marked line (length ~18 cm) in 3–4 s. The mediolateral angle of the probe was changed throughout the experiment so that it remained perpendicular to the skin, which was shown in pilot testing to yield the most reliable L_f image acquisition. In the first testing session, one experimenter performed two scans. Markings were then removed from the thigh and a second experimenter repeated the procedure. Since changes in probe angle or scanning path result in qualitative changes in adipose and connective tissue markings, the US images of each participant were printed and these markings were compared to aid image-acquisition reliability. Before the data collection phase, both experimenters practiced the image acquisition with the EFOV US technique on eight individuals; ~150 scans were taken until the images were found to be acceptable for analysis (i.e., identical images could be regularly obtained from a participant) (see Fig. 2 for example EFOV US image).

Swine VL L_f measurements. Two anterior thigh portions from a swine were obtained, and the skin, underlying subcutaneous tissue, and fascia were removed to allow clear imaging of muscle fascicles. The VL muscle was cut transversely to expose whole muscle fascicles (see Fig. 3*A*). Three needles were then positioned in the visible

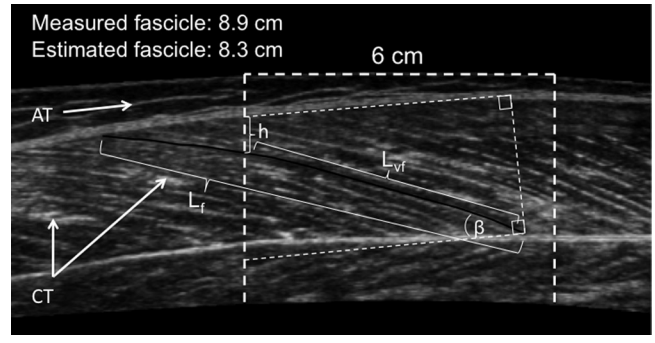

Fig. 2. Example VL extended field-of-view ultrasonography (EFOV US) image. The white dashed line represents the 6-cm-wide single scan used for comparison; the black continuous line is the analyzed fascicle (L_f), L_{vf} is visible part of the fascicle on the 6 cm scan, β is the angle between the fascicle and a line in parallel with the superficial aponeurosis, and *h* is the vertical distance from the superficial aponeurosis to the crossing point with the fascicle. Adipose (AT) and connective tissue (CT) markings, as shown, were used to ascertain that the imaging plane was similar in repeated scans (see MATERIALS AND METHODS). In this image, the fascicle length difference between directly measured and estimated fascicle lengths was 4.8%.

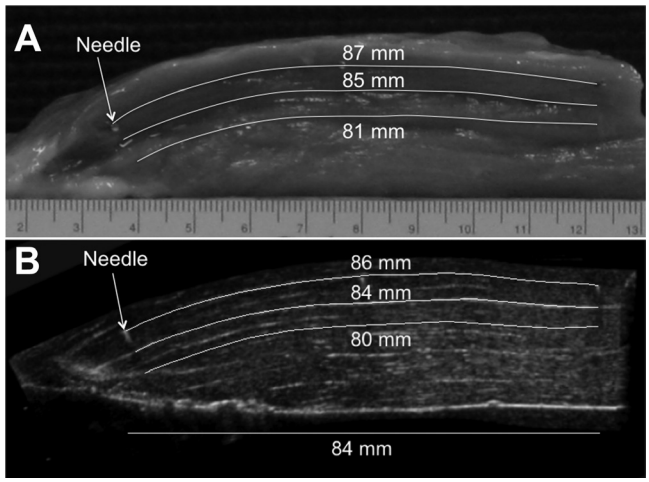

Fig. 3. Swine thigh VL muscle (*A*) and the ultrasound scan (*B*) of the same muscle. The measured fascicles are marked in white.

proximal and distal ends and the middle part of the fascicles as markers, guided by the real-time ultrasound (Fig. 3, *A* and *B*). Three EFOV US scans were obtained with at least three visible fascicles. The probe was moved in parallel with the edge of the muscle slice to visualize superficial fascicles. A high-resolution digital camera was then used to obtain digital photographs of the muscle. The camera was placed 2 m from the muscle, oriented perpendicular to the muscle surface. Care was taken during the US imaging that the fascicles of the whole muscle ran parallel, so it was assumed that measuring the length of the well-visible superficial fascicles gave an appropriate representation of the fascicles. A total of nine fascicles was analyzed from various muscle sections.

Digital analyses and calculation of L_f. Both human and swine L_f were measured using publically available digitizing software (ImageJ 1.41, Wayne Rasband, National Institutes of Health, Bethesda, MD). Since L_f varies along the length of the human VL, and two fascicles were analyzed from each image, it was important to choose the fascicles closest to each other from the middle of the image. For the comparison of measured and estimated L_f, two fascicles from 10 images were measured. For the trigonometric extrapolation method, a region (6-cm width) was drawn perpendicular to the superficial edge of the image (see Fig. 2), which gave a scanning region that would normally be acquired using standard, static ultrasound procedures. From that image, L_f was calculated using the method reported by Finni and Komi (10) as $L_{vf} + (h/\sin\beta)$, where L_{vf} is the visible length of the curved fascicle measured directly on the 6-cm square, h is the vertical distance from the visible fascicle end point to the superficial aponeurosis, and β is the angle between the fascicle and deeper aponeurosis. In cases where the deeper aponeurosis was not parallel with the superficial aponeurosis a line parallel to the upper aponeurosis was drawn and β was calculated as an angle between that line and the fascicle (Fig. 2).

Statistical analysis. Reliability of the digitizing process (i.e., intraexperimenter digitizing reliability) was evaluated by calculating the coefficient of variation [$CV\% = (L_{f,mean}/SD) \times 100\%$, where $L_{f,mean}$ is the mean L_f and SD is the standard deviation] for a measurement of a single image 10 times. Interexperimenter digitizing reliability was assessed by intraclass correlation (ICC) by comparing L_f measurements of two experimenters each digitizing 10 different images (i.e., the same 10 images were digitized by both experimenters). Intraexperimenter reliability of the combined image acquisition and digitizing process was determined by calculating mean L_f from two repeated images taken on each subject. "Intersession reliability" was determined by comparing the mean L_f of two repeated images obtained in each subject when skin markings from acquisition of the first image were completely removed. ICCs with 95% CI (1-way random effects model) were calculated for intrameasurer and intersession reliability. The measured and calculated L_f were compared using ICC (2-way random effects model, absolute agreement definition) with 95% CI. To examine the validity of the EFOV US technique for measuring the length of whole fascicles in vivo, swine muscle L_f measured by EFOV US and from dissections (digital sagittal plane photographs) were compared using ICC (2-way random effects model, absolute agreement definition) with 95% CI. An ICC with 95% CI in the range of 0.8–1.0 was considered to represent "good" reliability. The average difference was calculated using the formula $\Delta = [(L_{f1} - L_{f2})/L_{f1}] \times 100$, where L_{f1} and L_{f2} are the mean L_f of the first and second measurements, respectively. Relative differences (%) with SD are reported. The typical error of measurement (TEM) was calculated using the equation: $TEM = SD/\sqrt{2}$, where SD is the standard deviation of the difference between measurements. The relative error of measurement (%) was calculated for the two methods (EFOV US vs. dissected digital images) for swine VL L_f measurements using the formula $[(L_{fe} - L_{fp})/L_{fe}] \times 100$, where L_{fe} and L_{fp} were L_f measured from the EFOV US image and digital photographs, respectively. Significance for all tests was set at $P < 0.05$.

RESULTS

Intra- and interexperimenter digitizing reliability. Intraexperimenter digitizing reliability (CV% for the same fascicle digitized 10 times) was 1.0% (1.1% and 0.9% for the first and second experimenters, respectively). The difference in mean L_f between two experimenters digitizing the same 10 fascicles was 2.6 ± 1.9 mm, corresponding to 3.1 ± 2.8% with an ICC of 0.97 (95% CI: 0.88–0.99).

Intra- and intersession reliability. The average L_f for all participants was 80 ± 9 mm (Table 1). The mean difference in L_f measured from two images taken during the same measurement session with the probe path lines being left on the subjects was 1.6 ± 1.1 mm corresponding to 2.1 ± 1.7% [ICC = 0.99 (95% CI = 0.95–1.00)]; the typical error of measurement was 8 mm, and there was no statistical difference between these two measurements ($P = 0.48$). The average difference in L_f measured when markings were removed between scans (i.e., assessing "intersession" reliability) was 3.0 ± 2.5 mm corresponding to 3.8 ± 3.2% with an ICC of 0.95 (95% CI = 0.80–0.99). The absolute intersession measurement error was 1.8 mm. The L_f measured during the first session is plotted against those measured during the second session in Fig. 4.

Comparison of trigonometric estimation and EFOV US methods. The average difference in L_f between the measured (EFOV) and extrapolation (from a 6-cm-wide static image)

Table 1. *Intra- and intersession reliability of first and second measurements of VL muscle fascicle length*

	First Measurement, mm	Second Measurement, mm	Absolute Difference, mm	ICC (95% CI)
Intrasession reliability	79.4 ± 7.8	80.3 ± 8.7	1.6 ± 1.1	0.99 (0.95–1.00)
Intersession reliability	79.9 ± 8.2	83.1 ± 7.4	3.0 ± 2.5	0.95 (0.80–0.99)

VL, vastus lateralis; ICC, intraclass correlation; CI, confidence interval.

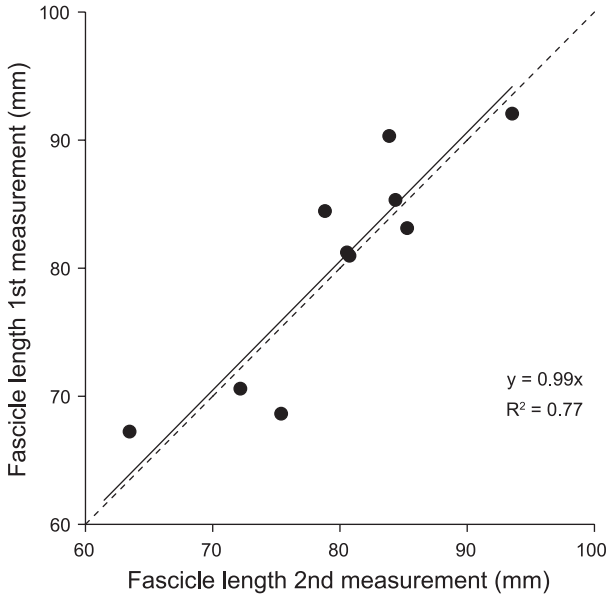

Fig. 4. Fascicle length measured in the first scan session plotted against that measured in a second scan performed after the removal of the probe and skin markings (i.e., intersession reliability). The dashed line is the line of identity, and the solid line is the line of best fit (Pearson's $r = 0.88$).

methods was 9.8 ± 6.1 mm corresponding to 12.6 ± 8.1%, with an ICC of 0.76 (CI = −0.20 to 0.94) (Table 2). Measured L_f was always longer than the estimated length, and the Pearson's correlation coefficient between measured and estimated L_f was 0.58 ($r^2 = 0.34$). However, the intradigitizer reliability of L_f measurement using trigonometric extrapolation was good (CV = 0.9%) when six fascicles were digitized twice.

Animal muscle measured by EFOV US and from dissections. The average difference between the swine VL fascicle length measured by EFOV US and directly from the dissected planar cross sections (digital photographs) was 2.1 ± 1.3 mm corresponding to 2.4 ± 1.2% with an ICC of 0.99 (95% CI = 0.94–1.00) (Table 2). The relative error of the measurement was 0.8% ± 2.6%.

DISCUSSION

Fascicle length is a key determinant of muscle function and, because it varies with growth, aging, physical training, detraining, and disuse, is an important target of scientific study. Using the scanning techniques described herein, we have demonstrated a very high reliability for the assessment of VL L_f using the extended field-of-view ultrasonography (EFOV US) technique. Both within- and between-experimenter digitizing reliability was very high, showing not only that experienced researchers can obtain highly reproducible results when digitizing the images but that the results differ little between experimenters (the CV for repeated measurements was ~1%). Thus it is likely that studies performed by different researchers with a demonstrated high reliability can be directly compared. Also, a high intersession reliability was observed (intersession ICC of 0.95 with narrow CIs), and there was no significant difference in L_f measurements between the repeated measurements ($P = 0.48$). Therefore, the EFOV US technique should be useful for detecting even small changes in L_f over time. For example, previous studies have reported changes of ~3–20% (5, 21, 28–30) after periods of resistance training, which are equal to or much greater than the 3.8% absolute error for repeated measurements that were observed in the present study.

Although intersession error was small using the methodology described in the present study, the inability to achieve negligible error might have resulted from the difficulty in ensuring an identical US probe angle in relation to the muscle between scans. It is known that errors in L_f can be induced by tilting the ultrasound probe away from the orientation of the fascicular plane (3, 18). In the present study, all scans were printed and the characteristic patterns of subcutaneous adipose tissue and interfascicular connective tissue were compared to maximize the likelihood that the scanning plane was similar; small changes in scanning plane resulted in noticeable changes in the echo-density pattern of the various tissues. Yet, small errors might have still resulted from between-scan discrepancies in probe orientation, especially since the probe had to be moved over a curved thigh surface. Consequently, considerable practice is required by the examiner to minimize errors associated with scanning plane variations when using the EFOV US technique.

It has been shown previously that EFOV US is spatially accurate (11); the relative error of EFOV US measurement of 10-cm curved phantoms in the study of Fornage et al. (11) was 1.3% ± 0.9%. In the present study, we compared fascicle lengths measured by EFOV US to those measured in planar dissections of swine VL. We were able to identify the same connective tissue regions using both methods and found a relative measurement error of ~1%. The fact that the difference between animal thigh measurements measured using EFOV US and direct measurements in dissected sections (digital photographs) was smaller than the within-measurer and between-session differences demonstrates that the in vivo assessment of L_f using the EFOV US technique provides highly valid results.

In the vast majority of studies that have previously examined the length of long skeletal muscle fascicles, L_f has been measured by summing the length of the portion visible on the sonograph and the estimated (extrapolated) length of the fascicle portion extending off the image (Fig. 2). In the present study it was observed that L_f calculated using this estimation method systematically underestimated "true" L_f as measured

Table 2. *Extended field-of-view ultrasonography method compared with trigonometric estimate and animal dissection methods for assessment of VL muscle fascicle length*

	EFOV US, mm	Comparison Method, mm	Absolute Difference, mm	ICC (95% CI)
EFOV US vs. trigonometric	80.9 ± 9.1	71.0 ± 12.5	9.8 ± 6.1	0.76 (−0.20 to 0.94)
EFOV US vs. animal dissection	84.0 ± 9.2	84.8 ± 10.7	2.1 ± 1.3	0.99 (0.94–1.00)

EFOV US, extended field-of-view ultrasonography.

with EFOV US. Furthermore, the relationship between the estimated and measured L_f was not consistent (Pearson's $r = 0.58$) so a uniform correction factor cannot be applied to the estimated L_f. Our finding that fascicles digitized from the EFOV US images were always longer than those calculated by trigonometric estimation is not surprising. Trigonometric estimation relies on the assumption that the fascicle and superficial aponeuroses are linear, but visual inspection of the EFOV images showed that this was rarely the case. Some curvature existed in almost every visible fascicle (VL was held at its shortest length in our study), and especially at the distal ends of the fascicles close to the superficial and deep aponeuroses. This curvature also makes it difficult to accurately measure the pennation angle, which is a component of L_f trigonometric extrapolation. The pennation angle of curved fascicles has been defined differently by different researchers (12, 21, 23) and is an additional source of L_f estimation error. It has been shown previously that the error in estimating VL L_f using trigonometric extrapolation was 2–7% (10) and 5.9% (15), which was considerably lower than found in the present study (12.6 ± 8.1%). This discrepancy might be attributable to the present use of EFOV US imaging, with which it was possible to make direct comparisons (we measured the length of the same fascicle using both methods), or to comparisons in other studies being done at different muscle lengths and contraction intensities where aponeurosis and fascicle curvature was different (10, 15). Regardless, the present data and those of others have revealed substantial inaccuracies in the estimation method, which in some circumstances may be of a magnitude greater than the longitudinal changes in L_f reported previously (5, 28–31).

One limitation of the EFOV US technique is that it is not applicable during dynamic muscle contractions and image acquisition may take too long to be useable during maximal isometric contraction. Thus it is particularly useful for longitudinal studies or population assessments where measurements are made in relaxed muscle. Technical difficulties inherent in the EFOV technique when measuring L_f include the need to maintain the same probe angle in relation to the fascicles and ensuring that the probe is moved in parallel with the fascicular plane during scanning. However, the benefit of using the EFOV US technique instead of taking multiple single images is that it is less time consuming and the error arising from manual image fitting is avoided (the EFOV US frame capture rate is high and instant image fitting is ensured by computerized algorithm procedures). The analysis process itself is also less time consuming and less erroneous when using EFOV US instead of trigonometric extrapolation methods.

Conclusions. The present data show that the EFOV US technique, when done by experienced researchers after extensive practice, is a reliable and valid method with which to measure fascicle length in vivo in relaxed muscles when fascicles are too long to be visualized by conventional static ultrasound scanning. Further, the EFOV US technique showed high intra- and interexperimenter reliability, which suggests that multiple experienced researchers are able to obtain similar measurements in the same muscles. Finally, experiments in excised animal muscle indicated a high degree of validity for the EFOV US technique. The high validity and intersession reliability indicate that it is possible to detect small temporal changes in fascicle length. For these reasons, EFOV US appears to be the preferential method with which to measure the length of long muscle fascicles at rest in vivo.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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