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Accounting for fascicle curvature affects muscle architecture characterization in dynamic conditions (isokinetic contractions)

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A R T I C L E I N F O

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A B S T R A C T

Investigating muscle architecture in static and dynamic conditions is essential to understand muscle function and muscle adaptations. Muscle architecture analysis, primarily through extended field-of-view ultrasound imaging, offers high reliability at rest but faces limitations during dynamic conditions. Traditional methods often involve "best fitting" straight lines to track muscle fascicles, leading to possible errors, especially with longer fascicles or those with nonlinear paths. Moreover, muscle architecture varies along the same muscle, with potential differences in curvature. This study aimed to develop and test a new software for muscle architecture characterization considering fascicle curvature during dynamic conditions. Muscle architecture data from different muscle regions using various digitalization methods were compared. Ten healthy young adults (24.1 ± 1.6 years; 177.7 ± 7.4 cm; 72.7 ± 7.7 kg; 9M/1F) performed maximal knee extension at $75^{\circ}.s^{-1}$ while B-mode ultrasound images of vastus lateralis muscle were captured in two muscle sites (at 50 % and 83 % of femur length). The analysis involved automated straight-line (ST) methods and custom manual linear extrapolation (MLE) software with segmented fascicle tracking using 2 (MLE2) and 4 (MLE4) segments inside the field of view. Results indicated significant overestimations of fascicle length, muscle belly length and thickness and underestimation of pennation angle using ST compared to MLE methods, especially in the distal region. Intra-rater repeatability for MLE4 was excellent (ICC = 0.93; 0.90; 0.93; 0.88, respectively; $P < 0.001$), while inter-rater reliability varied. This study confirms the need to consider fascicle curvature for accurate resting muscle architecture characterization, even in the middle region of the muscle, and extends these considerations to dynamic conditions.

1. Introduction

Alterations in muscle architecture cause alterations in functional output; studying these matters not only enhances our understanding of muscle mechanics but also provides a foundation for a better understanding of how muscles function and adapt.

Gold standard methods to investigate muscle architecture are based on muscle samples after dissection (ex-vivo measurements) or MRI analysis (in-vivo measurements, at rest). Ultrasound imaging also allows the obtaining of representative values of muscle architecture when using the extended field of view (EFOV) (Franchi et al., 2020) in relation to other techniques that are based on trigonometrical estimations and assumptions or that only allow the measuring of a limited portion of muscle. The EFOV technique, first proposed in the late 90′s (Lin et al., 1999; Weng et al., 1997) and then validated by (Noorkoiv et al., 2010) in vastus lateralis, allows one to track the path of fascicles and aponeuroses through the entire muscle length with high reliability, avoiding any extrapolation but cannot be applied in dynamic conditions because it requires performing multiple scans along the muscle to capture the entire fascicle path.

During movement (i.e. passive stretching or during contraction), muscle architecture characterization usually involves "best fitting" of fascicles and aponeuroses in the middle region of a muscle, using straight lines inside and outside the field of view (FOV) (Blazevich et al., 2006; Narici et al., 1996). Different methods and computational solutions proposed in the literature (i.e. Farris & Lichtwark, 2016; Verheul & Yeo, 2023; van der Zee and Kuo 2022) offer the possibility to automatically or semi-automatically analyze muscle architecture during movement, drastically reducing the time required for data analysis, while still allowing good reproducibility.

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However, as pointed out by Franchi et al. (2020), at least two sources of error could affect the "straight-line" methods. When analyzing a short fascicle (as in gastrocnemius medialis) of 50 mm with a 40 mm long probe, the extrapolated part (outside the FOV) is about 20 % (Narici et al., 1996). With longer fascicles, and the same probe size, the extrapolated portion of the fascicle increases, as does the risk of error (Freitas et al., 2018). Furthermore, when the aponeuroses do not run in parallel, measurement errors could arise when using trigonometric methods for muscle architecture characterization (Sarto et al., 2021). In addition, the fascicles could not run straight, as in vastus lateralis (Muraoka et al., 2001; Sejersted et al., 1984) or in biceps femoris long head (Franchi et al., 2020; Pimenta et al., 2018) and this could lead to further errors in the estimation of fascicle length.

Ando et al. (2014) investigated these matters by using the straight-line techniques (named "model V" in their paper) on vastus lateralis and vastus intermedius muscles in cadavers at the midpoint between the greater trochanter and the lateral epicondyle. Although not to a significant level, the authors observed shorter estimated fascicle lengths with the straight-line method than with direct measurements in the vastus lateralis but similar values between the two techniques in the vastus intermedius; they suggested that the curvilinear behavior of the vastus lateralis (VL) fascicles might be the cause of these differences.

In addition, as pointed out by Blazevich et al. (2006), fascicle orientation and curvature can differ from one region to another in the same muscle (e.g. proximal, middle or distal) and the straight-line method could over- or under-estimate fascicle length and pennation angle depending on the tendency of the fascicles (in a given region) to present a convex or concave path.

Considering that eccentric training and stretching protocols can induce different adaptations (in thickness, pennation angle and fascicle length) in the proximal, middle or distal region of a muscle (Franchi et al., 2014; Nunes et al., 2024), multi-site measurements are needed to fully characterize regional changes in muscle architecture after these interventions; to limit errors arising from the curvilinear path of the fascicles, their curvature should be taken into account (Franchi et al., 2020).

To take into account fascicle curvature, a technique called manual linear extrapolation (MLE), proposed by (Potier et al., 2009), revealed good reproducibility and showed reliable results when compared to EFOV analysis (Franchi et al., 2020; Sarto et al., 2021). However, this technique is usually utilized when analyzing data at rest on a single frame; hence, its applicability to dynamic conditions (during muscle contraction) would be extremely time-consuming.

Based on these considerations, the first aim of this study was to propose and test a new manual software for tracking curvilinear fascicles during dynamic muscle shape changes, requiring as little time as possible to analyze an entire video. The second aim was to compare, during dynamic muscle shape changes, muscle architecture data from two regions of the same muscle (middle and distal) presenting different curvatures, as determined by different methods of digitalization.

2. Materials and methods

2.1. Participants

Ten healthy young adults (24.1 ± 1.6 years of age; 177.7 ± 7.4 cm of stature; 72.7 ± 7.7 kg of body mass; 9M/1F) were recruited for the study. All participants received written and oral instructions before the study and gave their written informed consent to the experimental procedure. The experimental protocol was approved by the Ethical Committee of the University of Verona (protocol number: 2019-UNVRCLE-0193291).

2.2. Experimental procedures

Dynamometry. Maximal knee extensions were performed on an isokinetic dynamometer (Cybex, Humac Norm, division of Lumex Inc., Ronkonkoma, NY, USA). Participants were seated with the back supported and the hip joint flexed at 80°; their arms were crossed in front of the chest, and their pelvis and trunk were fixed to the dynamometer. The dynamometer was positioned so that the knee joint rotational axis was aligned with the axis of the dynamometer's arm during contraction. After a standardized warm-up based on submaximal isometric and isokinetic contractions at 75° of knee angle and at $75^{\circ}{\cdot}s^{-1}$, three maximal isokinetic contractions (angular velocity = $75^{\circ}{\cdot}s^{-1}$) were performed with the dominant limb. Participants were asked to push "as hard as possible" from 100° to 0° of knee angle (0° = fully extended leg) and were loudly encouraged. A rest period of 30s was observed between contractions. Torque, angular velocity and knee angle data were acquired at 1000 Hz with a PowerLab System, using the related software (PowerLab and LabChart v.6, ADInstruments, Dunedin, New Zealand) (see Fig. 1).

Ultrasound. B-mode images were taken from the vastus lateralis (VL) with the use of two ultrasound scanners (ArtUs EXT-1H and MicrUs EXT-1H, Telemed UAB, Vilnius, Lithuania) respectively coupled with linear transducers (40 mm, 7.5–15 MHz, L15-7H40-A5, 192 elements and 65 mm, 4–8 MHz, LV8-4L65 S3, Telemed UAB, Vilnius, Lithuania). Ultrasound settings were individually adjusted to provide the clearest possible image; the frequencies of the probes were 35 (MicrUs) and 45 (ArtUs) Hz. Femur length was measured between the great trochanter and the upper edge of the patella, as determined by palpation. The thigh was divided into three sections: proximal (0–33 % of the femur length), middle (33–66 % of the femur length), and distal (67–100 % of the femur length) (see Monte & Franchi, 2023). The probes were placed at the midpoints of the middle and distal sections, located at 50 % and 83 % of the femur length respectively, following the orientation of the fascicles, and fixed to the skin with straps with a minimum amount of pressure. In 4 participants, the MicrUs (65 mm) probe was positioned in the middle and the ArtUs (40 mm) probe in the distal portion of the muscle; in the other 6 participants it was the opposite to reduce the incidence of probe size on data analysis (Freitas et al., 2018) since it determines the size of the FOV and, thus, the proportion of the extrapolated fascicle length outside it (see Figs. S9 – S12 and Table S1 in supplementary materials.

A trigger signal output of 5 V amplitude was sent from the ultrasound systems to the PowerLab system to synchronize data.

2.3. Data analysis

Only the best of the three isokinetic contractions (highest peak torque) was analyzed. B-mode videos of both muscle regions were analyzed during the steady state of knee angular velocity (see Fig. 1): i) by using straight lines (ST) within and outside the FOV, with the automated software Ultratrack (Farris & Lichtwark, 2016); ii) by using a custom-made manual software programmed in Matlab (MLE: manual linear extrapolation). With this software, the visible part of the fascicle was divided into 2 (MLE2) or 4 (MLE4) segments to consider the visible curvature, whereas fascicles and aponeuroses outside the FOV were linearly extrapolated. To accurately select the frames of interest, the beginning and the end of the isokinetic phase were manually selected by analyzing the angular velocity profile as a function of time.

To verify the inter- and intra-rater repeatability of the MLE4 analysis in the distal region, two videos (44 and 45 frames) were analyzed 2 times by the same rater and one time by 3 different raters who were asked to track two fascicles (without specific indications as to how to select them).

2.4. Muscle architecture characterization methods

Straight-line method (ST, Fig. 2A). For this analysis, we used the modified 5.2.2 version of the Ultratrack software. Fascicle length (FL) was considered as the 2D distance between fascicle ends on the deep and

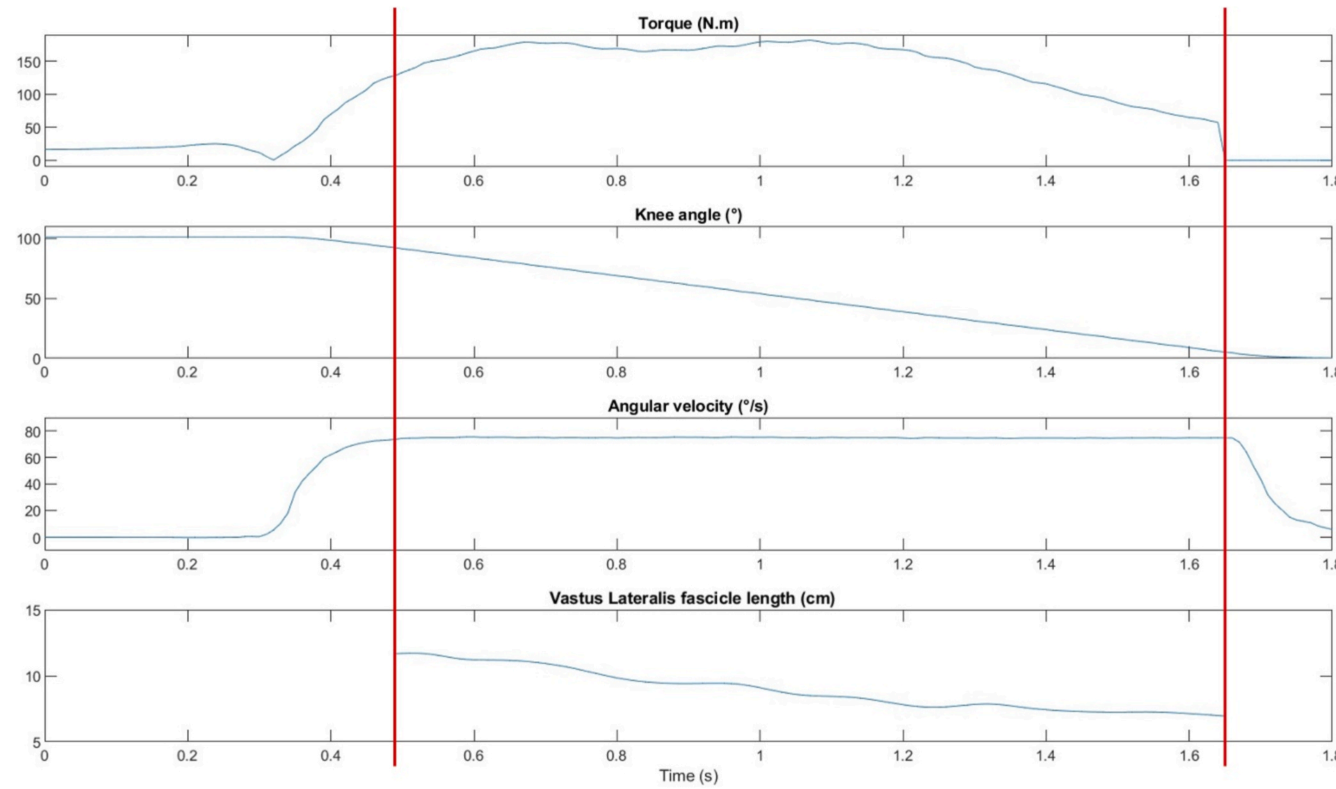

Fig. 1. Time course of torque, knee angle, angular velocity and fascicle length of vastus lateralis during a maximal isokinetic contraction at 75°·s^{-1} of angular velocity. Vertical red bars indicate the steady state of isokinetic velocity where the US data were analyzed (fascicle length data are indeed reported just in this interval). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

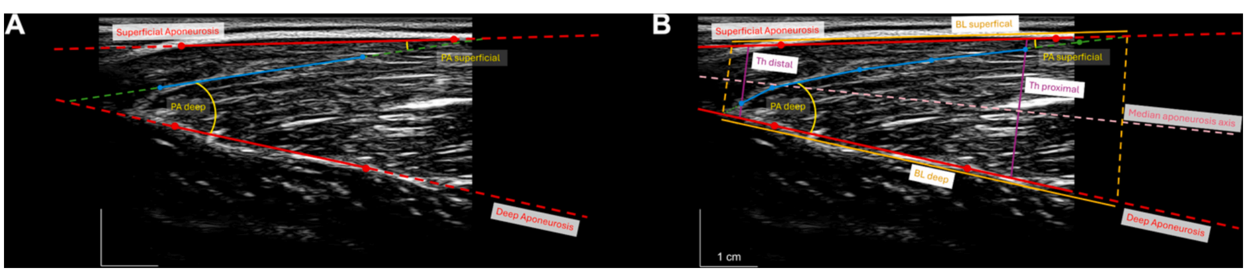

Fig. 2. Example of a muscle architecture characterization in the distal region with A. straight-line method (ST) using Ultratrak, and B. manual linear extrapolation method (MLE) using the customized software. The two horizontal red lines identify the deep and superficial aponeurosis. Yellow and blue lines refer to fascicle and pennation angle, respectively. Muscle thickness is reported in purple. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

superficial aponeuroses. Pennation angle (PA) was considered as the average angle between each fascicle and both deep and superficial aponeuroses (as suggested by Franchi et al., 2018). Thickness (Th) was considered as the average of the thickness calculated at the most proximal and distal points of each fascicle using trigonometry ($Th = FL$ * sin *(PA)*) as suggested by Farris & Lichtwark (2016). Belly length (BL) was then calculated as: $BL = FL{*}cos(\alpha)$. Note that this gives not the whole muscle length but the length of the projection of the instantaneous FL on the corresponding section of the muscle belly (Wakeling et al., 2011).

Manual linear extrapolation method (MLE, Fig. 2B). A detailed description of these procedures is reported in the supplementary materials (Figs. S2-S7) and in a video tutorial. Briefly, the superficial and deep aponeuroses were identified and assumed to remain linear inside and outside the FOV (as in the ST method). Then, FL was calculated by drawing a segmented line along the visible fascicle with 5 (MLE4) or 3 points (MLE2). Next, automated linear extrapolation was used to extend this line from the first visible point of the fascicle to the deep aponeurosis, and MLE was used to extend it from the last visible point to the superficial aponeuroses; the sum of all segments' lengths was then computed and used in further analysis.

PA was calculated at 20 % of the fascicle length from the deep aponeurosis (to avoid the very strong curvature that sometimes is visible in the distal fascicles) and at 80 % of the fascicle length for the superficial aponeurosis. BL was measured along the deep and superficial aponeuroses using the intersection points of the fascicle with both aponeuroses. Th was measured in correspondence to the first and last visible point of the fascicle within the FOV, as the shorter distance between deep and superficial aponeuroses.

For both methods, the average values of PA, FL, BL and Th (for each frame) where computed, filtered by using a 5 Hz low-pass 2nd order Butterworth filter, interpolated on 101 dots and used in further analysis.

2.5. Statistical analysis

Shapiro-Wilk tests were used to assess normality distribution of FL, BL, PA and Th in both regions. To compare the results from different techniques, we used a 1-way repeated measures analysis of variance (ANOVA) (average of the 101 points for each subject) within each region, considering all variables. Post-hoc analyses were performed using Bonferroni tests for multiple comparisons. Then, a between-region paired *t*-test was performed on the difference between the two methods (Δ=ST-MLE4) for each variable. Statistical analyses and graphics were conducted in R (version 4.3.0). The significance level for all statistical comparisons was set to $p < 0.05$.

To assess intra- and inter-rater reproducibility of the MLE4 technique in the distal region we used the two-way mixed-model, absolute definition, average rater-type intra-class correlation (ICC3k) in all variables and standard error of measurement (SEM), calculated as the standard deviation of the difference between test and retest, divided by $\sqrt{(2)}$ (as in Freitas et al., 2018). Statistical analyses and graphics were conducted in R (version 4.3.0). We used the classification of a previous study (Koo & Li, 2016) to characterize ICC as poor, moderate, good and excellent (<0.5; 0.5 – 0.75; 0.75 – 0.9; >0.9 respectively).

3. Results

Excellent intra-operator concordance was found for all measured variables ($P < 0.001$) for a rater who analyzed twice two videos in the distal portion of the VL (Table 1). Good inter-operator concordance was found between three raters analyzing the same two videos for FL and BL ($P < 0.001$), but no concordance was found for PA and Th (Table 1).

Comparisons between techniques are reported in Fig. 3. One-way repeated measures ANOVA analysis revealed significant differences among techniques in FL and BL in both middle and distal regions ($P < 0.05$). For pennation angle, significant differences were observed only in the proximal region ($P < 0.05$) and for muscle thickness, significant differences were observed only in the distal region ($P < 0.01$). Post-hoc analysis revealed significant differences between MLE4 and ST and between MLE2 and ST for FL, BL in both regions, for PA in middle region and for Th in distal region. ST analysis gave higher FL, BL and Th and lower PA values than MLE4 and MLE2. No difference emerged between MLE4 and MLE2 analysis in both regions. The results of the post hoc analyses are reported in the figures' legends.

Comparisons between regions are reported in Fig. 4. Significant differences (paired *t*-test, $P < 0.01$) were observed regarding the values of FL, BL and Th as determined by different methods, with wider gaps (Δ=ST-MLE4) in the distal region than in the middle one. The ST method overestimates all variables but PA.

4. Discussion

The present study aimed to compare the results of muscle architecture characterization with different techniques in active curvilinear fascicles and at different muscle sites (middle/distal). Data were collected on VL, a pennate muscle with regional differences in architecture (Blazevich et al., 2006), during maximal isokinetic contractions, by using the straight-line method (ST) and two customized methods of manual linear interpolation (MLE2 and MLE4). Data reported in this study indicate that, compared to MLE, ST overestimates fascicle and muscle belly length, as well as muscle thickness, and underestimates PA, the more so in the distal region. These results confirm and extend to dynamic conditions previous data collected at rest and highlight the need to consider fascicle curvature in muscle architecture characterization.

4.1. Test – retest repeatability

Test-retest repeatability was determined for MLE4 in the distal region only. Indeed, repeatability data are already reported in the literature for the ST and the MLE method in the middle region of a muscle and are generally from good to excellent (Franchi et al., 2020; Sarto et al., 2021).

Excellent intra-rater repeatability in the distal region was also observed with the MLE4 technique for all measured variables ($0.91 < ICC > 0.99$, see Table 1) for an experienced rater who analyzed twice two videos. The inter-rater repeatability (i.e., three raters, one measurement each on the same two videos) was good for FL and BL (ICC of about 0.81) and poor for PA and Th (ICC of about 0.34, see Table 1). The inter-operator difference in the measurements was, however, rather low, especially in the case of Th (about 0.1 cm).

The poor inter-rater repeatability for PA and Th could be attributed to the relative position of the superficial and deep aponeuroses that do not run parallel in the distal region (they become closer and closer approaching the muscle–tendon junction). The determination of Th is thus more dependent (compared to the middle region) on the selection of the fascicles starting and ending points. Moreover, in the distal region, the aponeuroses often present a convex/concave curvature (even a 'S' shape, in some cases); thus, to improve repeatability (of PA and Th) also aponeuroses should be tracked with multiple points inside the FOV, as for the fascicle length. This was not implemented in our custom-made software, but others already offer this possibility in a fully automated way (Caresio et al., 2017). Also using two probes in series, like in previous studies (e.g. Brennan et al., 2017), would allow improving the assessment of the entire fascicle behaviour.

4.2. MLE4 vs. MLE2

Previous studies that used the MLE method to digitize muscle architecture did not mention the number of segments used to draw the visible part of the fascicle (e.g; Franchi et al., 2020; Potier et al., 2009; Sarto et al., 2021). We repeated the analysis by dividing the visible fascicles in 4 or 2 segments, but no significant differences were observed between MLE2 and MLE4 (and we observed similar differences between MLE4 and ST and between MLE2 and ST). Thus, identifying just two segments in the FOV seems enough to track fascicle curvature for muscle architecture characterization in the middle and distal region of the VL

Table 1
Intra- and inter-operator concordance. Data are means ± SD.

	Parameter	Measure 1	Measure 2	Measure 3	ICC 3 k (95 % CI)	P	SEM
Intra-op.	FL (cm)	10.92 ± 2.83	10.78 ± 2.53		0.99 (0.97–0.99)	**<0.001**	0.36
	BL (cm)	10.68 ± 2.84	10.54 ± 2.52		0.99 (0.97–0.99)	**<0.001**	0.37
	PA (°)	12.23 ± 3.36	12.37 ± 2.57		0.92 (0.85–0.96)	**<0.001**	0.93
	Th (cm)	1.89 ± 0.30	1.91 ± 0.30		0.96 (0.92–0.98)	**<0.001**	0.03
Inter-op.	FL (cm)	9.92 ± 2.08	9.29 ± 3.87	9.77 ± 2.63	0.81 (0.64–0.90)	**<0.001**	1.60
	BL (cm)	9.62 ± 2.03	8.99 ± 3.91	9.31 ± 2.99	0.79 (0.60–0.88)	**<0.001**	1.70
	PA (°)	14.09 ± 2.21	15.65 ± 4.84	14.39 ± 3.01	0.34 (−0.24–0.54)	**0.11**	2.81
	Th (cm)	1.88 ± 0.30	1.89 ± 0.30	1.89 ± 0.29	0.10 (−0.78–0.40)	**0.42**	0.12

FL: fiber length; BL: muscle-belly length; PA: pennation angle; Th: muscle thickness; ICC: interclass correlation coefficients, SEM: standard error of measurement.

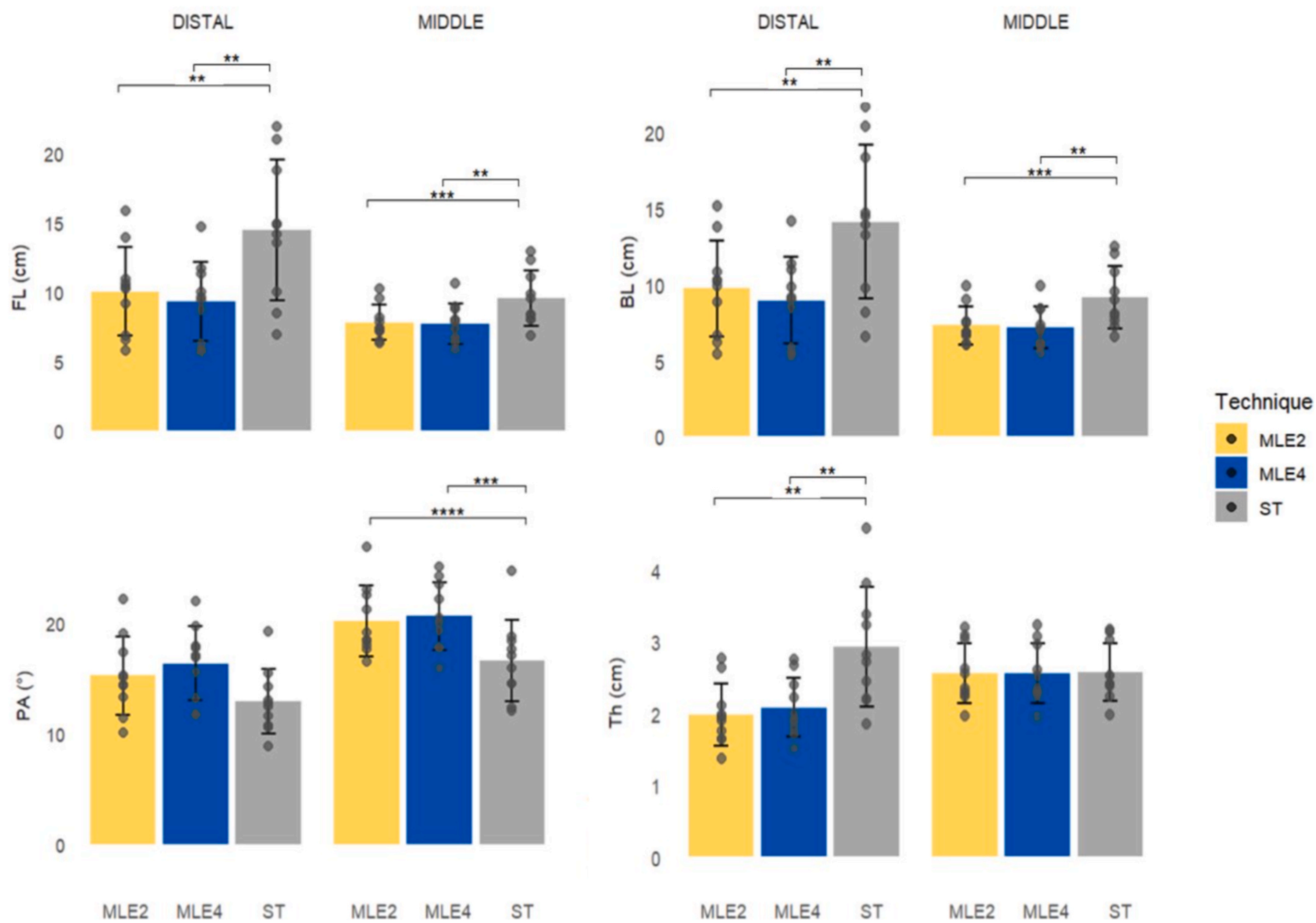

Fig. 3. Values of fascicle length (FL), muscle-belly length (BL), pennation angle (PA) and muscle thickness (Th) as determined in the distal and middle regions of VL during maximal isokinetic contractions by using different techniques: ST: straight-line method (in grey): MLE2: manual linear extrapolation with 2 segments (in yellow) and MLE4: manual linear extrapolation with 4 segments (in blue). Data refer to the average values during the entire isokinetic phase and are reported as mean and standard deviation. Results from ANOVAs: Distal region: FL ($F(2, 27) = 5.31$; $P = 0.011$; $\eta_g^2 = 0.28$); BL ($F(2, 27) = 5.37$; $P = 0.011$; $\eta_g^2 = 0.28$); PA ($F(2, 27) = 2.89$; $P = 0.073$; $\eta_g^2 = 0.18$); Th ($F(2, 27) = 7.61$; $P = 0.002$; $\eta_g^2 = 0.36$). Middle region: FL ($F(2, 27) = 4.11$; $P = 0.029$; $\eta_g^2 = 0.23$); BL ($F(2, 27) = 4.70$; $P = 0.018$; $\eta_g^2 = 0.26$); PA ($F(2, 27) = 4.43$; $P = 0.022$; $\eta_g^2 = 0.25$); Th ($F(2, 27) = 0.01$; $P = 0.990$; $\eta_g^2 = 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

muscle. In this muscle, the fascicles show just a concave fascicle path, but in a muscle featuring a concave and then a convex fascicle path, more than two segments might be necessary.

4.3. Regional differences and methodological considerations

Since the more accurate the fascicle path digitalization is, the more precise the FL measurement is, for the between regions comparisons we decided to focus on MLE4 and ST data only. The difference between the values assessed using the two techniques (Δ=ST-MLE4) was smaller in middle compared to distal region for FL, BL and Th. As an example, in the distal region, the difference in FL between the two techniques was of 5.15 ± 3.05 cm, whereas in the middle region it was less than half of it (1.82 ± 1.10 cm). This underlines the importance of considering the curvature of the fascicle, even more so in the distal region. However, some methodological aspects could also be held responsible for these differences, and these are discussed in detail below.

To our knowledge, this is the first study to assess the distal muscle region with two different digitalization methods (MLE and ST) in dynamic conditions. At rest, in the mid part of the biceps femoris long head, Franchi et al. (2020) observed an 18.8 % overestimation in FL when comparing the trigonometric (straight-line) method (named Equation A in their article) with MLE; this value is comparable with the 23.51 % difference we observed in the middle region of the VL between ST and MLE4 (55.38 % in the distal region).

In the study of Franchi et al., (2020) on the long head of the biceps femoris, FL was overestimated in some subjects and underestimated in others (see also Sarto et al., 2021). We did not observe such a different behavior in the vastus lateralis distal region. In this area, the fascicle curvature near the deep aponeurosis is pronounced, so that the ST technique always overestimates FL compared to the MLE techniques, the more so the higher the fascicle curvature. On the other hand, we observed differences in individual responses in measured FL between MLE4 and MLE2, in both the middle and distal regions. However, the FL overestimation of the ST technique in the distal region is more significant than the individual variations in the MLE techniques.

Regarding the factors responsible for an under or overestimation of FL (in different subjects and by means of ST method) the behavior of convex and concave fascicles is not the only factor to be considered (as suggested, as an example by Franchi et al. 2020). Indeed, if a straight line is drawn starting from the deep part of a concave fascicle, it will result in an underestimation of FL. Conversely, if the line is drawn starting from the superficial part of the same fascicle, it will result in an overestimation of FL, as illustrated by (Muramatsu et al., 2002). The same rationale applies to convex fascicles. Thus, it is crucial to consider not only the concave or convex nature of the fascicle but also the part of

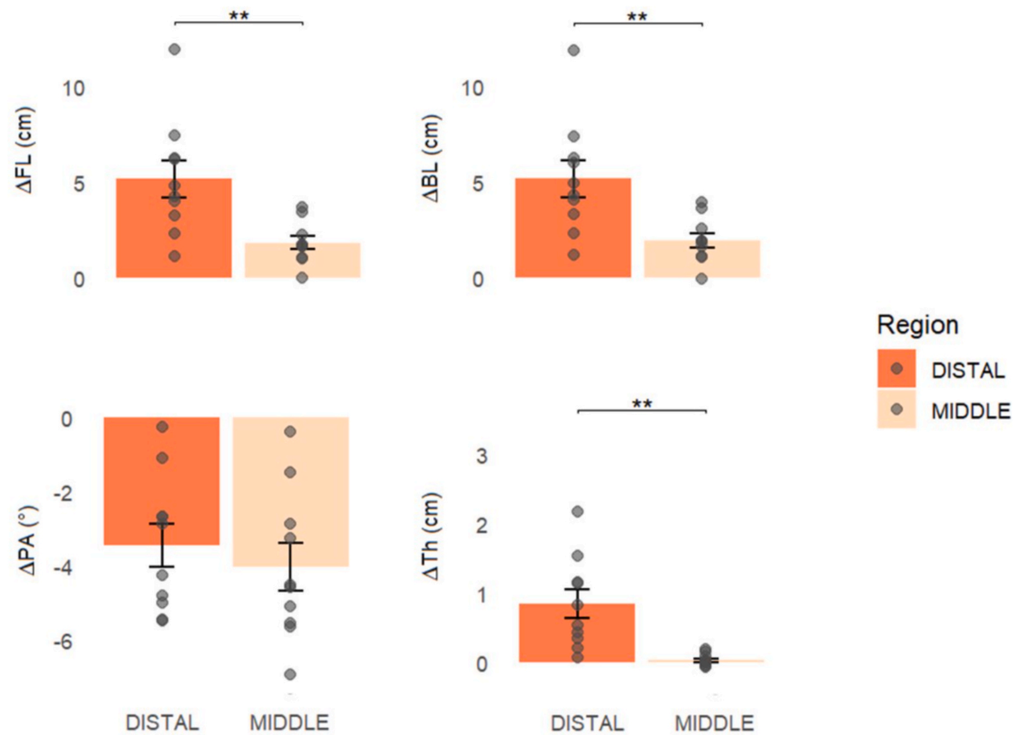

Fig. 4. Differences in the values of fascicle length (FL), muscle-belly length (BL), pennation angle (PA) and muscle thickness (Th) as determined with the ST and MLE4 methods (Δ=ST-MLE4) in the distal (in orange) and middle (in pink) regions of VL during maximal isokinetic contractions. Data refer to the average values during the entire isokinetic phase and are reported as mean and standard deviation; individual values (dots) are reported as well. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the fascicle used for extrapolation.

The differences between techniques in both regions could be explained by other factors than fascicle curvature. First, the selection of the fascicle to be analyzed influences the results since this could affect the FL, Th or PA values. The intra-rater variability was excellent (and this indicates that the same operator tends to repeat the same choices in fascicle selection), but the inter-rater variability was not (at least for PA and Th). Data reported in this study were analyzed by a single operator, eliminating operator variability as a confounding factor. However, this variable should be considered in future studies.

Second, using probes of different sizes (65 vs 40 mm in our case) could influence muscle architecture characterization, as highlighted by (Freitas et al., 2018), since it determines the size of the FOV and, thus, the proportion of the extrapolated fascicle length outside it (and the risk of error in the measure). At first, we tried to use two probes of the same size (with just one ultrasound device), but with this set-up the sample frequency was too low (~20–25 Hz). We then decided to test about half subjects with the 40 mm probe in the distal region and with the 65 mm probe in the middle region, and the other half with the opposite set-up. This should have reduced the probe size influence on the aggregated data, but it is recommended, in future studies, to use similar probes in the two regions. As reported in the supplementary materials, a simple model indicates that, whatever the field of view, the ST technique overestimates fascicle length in the case of strongly curved fascicles (Supplementary materials: Figs. S9 – S12 and Table S1).

4.4. Further considerations

The time needed to analyze ultrasound images with the MLE4 and MLE2 techniques is notably higher than with the ST technique, and this constitutes a limitation when investigating muscle architecture in dynamic conditions. Our video clips were composed of about 45 frames, and to an expert rater, the analysis took about 10 min with MLE4 or MLE2, whereas it required less than 5 min with ST. Semi-automated methods allowing the consideration of fascicle (and aponeuroses) curvature should then be developed. We believe that, by cross-referencing the results of ST analysis of FL with the results of a curvature vector field analysis in the same muscle region, it would be possible to modulate the reliability of ST results or even more, to recalculate the curved fascicle length.

Finally, it must be pointed out that the muscle fascicle is a 3D entity and its dynamics can appear not only in the sagittal but also in the other planes (Takahashi et al., 2023). For this reason, the present results could not tell the whole story.

5. Conclusion

The MLE methods offer the opportunity to better assess muscle architecture in different muscle regions and in dynamic conditions (compared to the ST one), allowing for a better understanding of local muscle adaptations and behavior.

Our results confirm previous data (collected at rest) that point out an overestimation of the fascicle length, and underestimation of pennation angle, in the VL when digitized with the straight-line method (especially in the distal region), underlining the need to better track fascicle curvature within the FOV. Furthermore, our results extend these considerations to dynamic conditions. The MLE4 method presented in this study appears to be highly reliable within the same rater (in the distal region), more work is still needed to increase reliability between raters for the muscle thickness and the pennation angle in the distal region.

CRediT authorship contribution statement

Baptiste Bizet: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Michele Trinchi:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Riccardo Magris:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Andrea Monte:** Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization. **Paola Zamparo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiomech.2025.112520.

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