Muscle-Tendon Unit Length Measurement Using 3D Ultrasound in Passive Conditions: OpenSim Validation and Development of Personalized Models

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Abstract
This study investigated the validity of using OpenSim to measure muscle-tendon unit (MTU) length of the bi-articular lower limb muscles in several postures (shortened, lengthened, a combination of shortened and lengthened involving both joints, neutral and standing) using 3D freehand ultrasound (US), and to propose new personalized models. MTU length was measured on 14 participants and 6 bi-articular muscles (*semimembranosus* SM, *semitendinosus* ST, *biceps femoris* BF, *rectus femoris* RF, *gastrocnemius medialis* GM and *gastrocnemius lateralis* GL), considering 5 to 6 postures. MTU length was computed using OpenSim with three different models: OS (the generic OpenSim scaled model), OS + INSER (OS with personalized 3D US MTU insertions), OS + INSER + PATH (OS with personalized 3D US MTU insertions and path obtained from one posture). Significant differences in MTU length were found between OS and 3D US models for RF, GM and GL (from −6.3 to 10.9%). Non-significant effects were reported for the hamstrings, notably for the ST (−1.5%) and BF (−1.9%), while the SM just crossed the alpha level (−3.4%, $p = 0.049$). The OS + INSER model reduced the magnitude of bias by an average of 4% for RF, GM and GL. The OS + INSER + PATH model showed the smallest biases in length estimates, which made them negligible and non-significant for all the MTU (i.e. ≤ 2.2%). A 3D US pipeline was developed and validated to estimate the MTU length from a limited number of measurements. This opens up new perspectives for personalizing musculoskeletal models using low-cost user-friendly devices.

Keywords Inverse kinematics · Participant-specific modeling · Bi-articular muscle · Hamstrings · Rectus femoris · Gastrocnemii

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Introduction

Understanding muscle function during movement generation remains a great challenge. Tracking changes in the muscle-tendon unit (MTU) length is a useful way to improve our understanding of the contraction modalities (i.e. eccentric, concentric and/or isometric) that can take place during different tasks. It can also be very relevant for providing training recommendations, as was recently done in alpine skiing [1]. Such tracking can also be used to analyze muscle-tendon interactions, if coupled with ultrasound measurements of fascicle length during different activities, such as walking, running [18] and jumping [15, 17]. MTU length is also important for estimating muscle forces from MTUs modeled as a Hill-type model [25, 29].

Anthropometric models were developed to calculate MTU length using both joint angles and external body segment length [12, 14]. More sophisticated approaches, such as those using OpenSim or Anybody, involve scaling a generic skeleton using coordinates of body markers obtained using a 3D motion capture system [6, 7]. Both approaches are based on MTU insertions derived from cadaveric data and a linear interpolation of muscle path between insertions. However, cadaveric data are often obtained on the elderly, so the question remains of whether these models can fit a broader range of the population. While the mean pattern may be accurate, there is a high probability that these models cannot adequately describe inter-individual variability. Thus, Persad et al. [21] reported differences of up to 22% between *gracilis* MTU length assessed by dissection compared with OpenSim modeling. Although this analysis was only performed for a single muscle, it highlights the need to assess the validity of these models for measuring MTU lengths and changes in length.

As imaging techniques can be used to assess the anatomical properties of muscles, some previous studies have already incorporated personalized MTU insertion points from magnetic resonance imaging (MRI) measurements [2, 5, 19, 24], which improve the prediction of musculoskeletal parameters. However, the influence of muscle trajectory has not yet been investigated. In addition, due to the restricted number of positions possible in MRI, this technique cannot easily be used to study the influence of joint angles on MTU length. 3D ultrasound (US) can be considered an alternative to overcome these limitations, having been previously validated against MRI for the measurement of bi-articular muscle and tendon morphology [3, 11]. However, to our knowledge, this technique has not been used to measure MTU length or to assess the effect of various joint angles compared with modeling predictions.

The first aim of this study was to assess the MTU length and change in length of the main bi-articular muscles of the lower limb, using 3D US across various different joint configurations (shortened, lengthened, a combination of shortened and lengthened involving both joints, neutral and standing), and to compare these measurements with the predicted results of a commonly used generic model in OpenSim [22]. We focused these analyses on the *semimembranosus* (SM), *semitendinosus* (ST), *biceps femoris long head* (BF), *rectus femoris* (RF), *gastrocnemius medialis* (GM) and *gastrocnemius lateralis* (GL). These muscles were chosen due to their bi-articular nature, which makes it harder to predict changes in length due to the concomitant situation (i.e. rotation and position) of the adjacent segments (i.e. pelvis, femur, tibia and foot) where the muscle has its proximal and distal insertions. Considering that the OpenSim model is accurate for a 'typical' participant, we hypothesized that it would provide unbiased mean MTU length estimations. However, since individuals differ from the typical participant used to develop the model, we expected large errors for some participants and thus that inter-individual variations would not be correctly taken into account by the model.

The second aim was to develop participant-specific OpenSim models, based on 3D US measurements of insertions and paths. It was expected that the personalization process would greatly improve the inter-individual variation errors compared with the generic OpenSim model.

Materials and methods

Participants

Fourteen healthy participants (8 males and 6 females, age 26.5 ± 3.0 years, height 174.0 ± 10.0 cm, mass 72.4 ± 18.6 kg) volunteered to take part in this study and provided informed consent prior to participation, in accordance with institutional guidelines set by the Declaration of Helsinki (World Declaration of Helsinki, 2013). Inclusion criteria were to be older than 18 years old and to practice a physical activity. (ranging from daily strength training to very low weekly activity). Exclusion criteria were to have had any lower limb surgery, recent (< 1 year) injury, or any contraindication to the practice of physical activity. All procedures were approved by the local Ethics Committee (CERNI no 03122021-2).

Experimental procedure

Participants took part in one session that combined data acquisition for musculoskeletal modeling and 3D US imaging. They were equipped with 35 reflective markers, which were placed on the pelvis (8), right femur (9), right tibia (8), right foot (7) and left femur (3) [23]. An optoelectronic motion capture system (10 cameras Optitrack Flex 13, NaturalPoint, USA) was used to measure the 3D coordinates of the markers in space at 60 Hz. After the Optitrack calibration process, the mean tracking error of the markers was estimated at 0.310 mm. Participants performed functional movements (right and left hip circumduction without pelvis motions, maximal right knee and ankle flexions-extensions, as well as describing circles with the knee during a static forward lunge) to assess joint centers using the SCoRE method [9]. They also performed a standing static trial designed to scale their anatomical posture to the generic musculoskeletal model. Afterwards, they were placed in 16 different static positions inducing different hip, knee and ankle angles (Fig. 1). These positions were chosen to allow the ultrasound probe to scan the entire MTU lengths of the hamstrings, RF and *gastrocnemii*, while keeping each of the markers of interest visible and allowing the participant to hold the joint angles without having to apply any force. For each MTU,

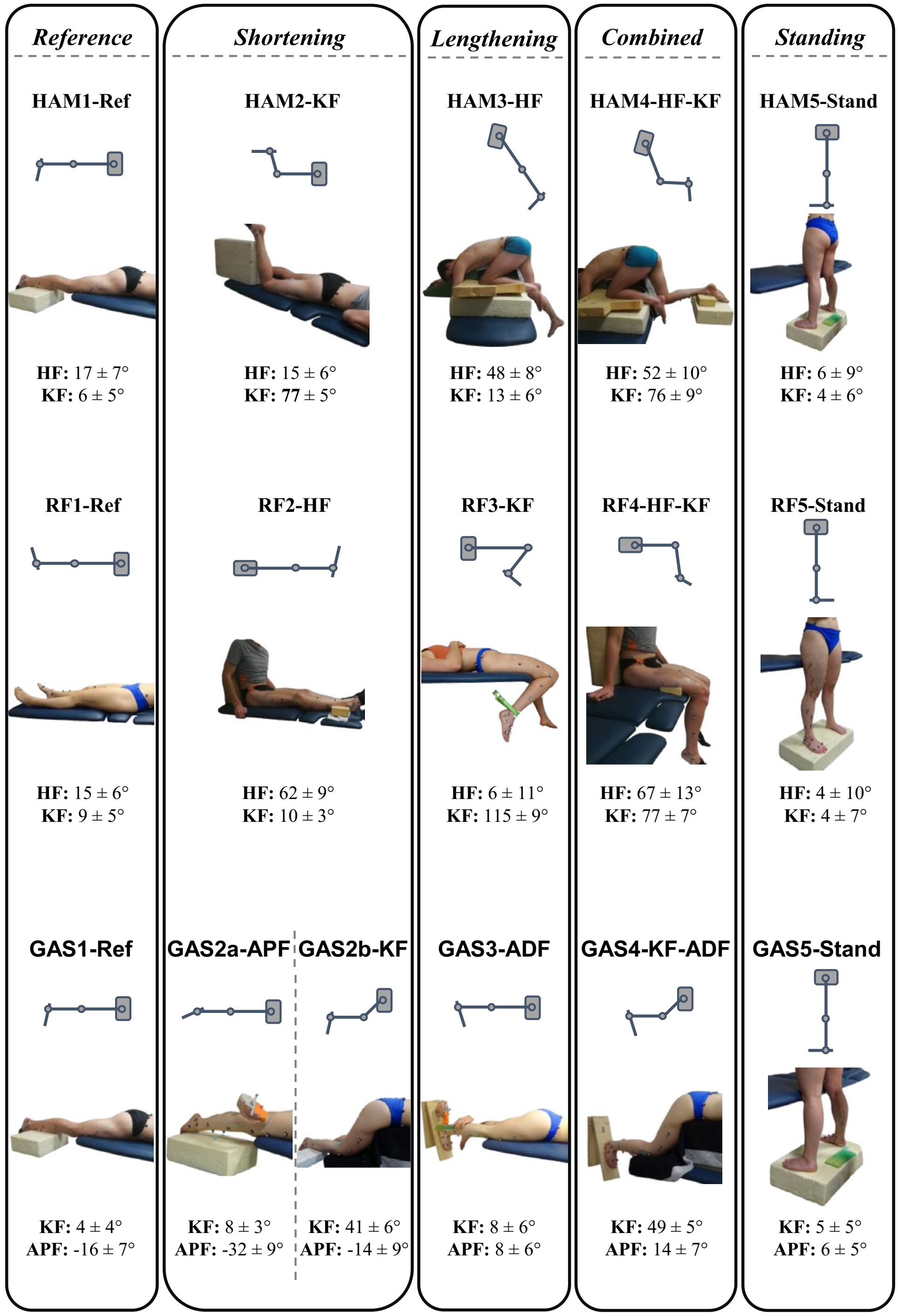

Fig. 1. Hip, knee and ankle joint angles in each position. *HF* hip flexion, *KF* knee flexion, *APF* ankle plantar flexion

the first position was a lying passive condition with joints relaxed (HAM1-Ref, RF1-Ref, and GAS1-Ref). Compared with the first position, the second was a shortening passive condition achieved by moving one joint (HAM2-KF, RF2-HF, GAS2a-ADF and GAS2b-KF). The third position was a lengthening passive condition achieved by moving the other joint (HAM3-HF, RF3-KF and GAS3-ADF). The fourth position was a passive condition that combined simultaneous shortening and lengthening by moving both joints (HAM4-HF-KF, RF4-HF-KF and GAS4-KF-ADF). The fifth position was standing upright (HAM5-Stand, RF5-Stand, GAS5-Stand), which was the only position where participants had to produce a small amount of force to keep their balance. After being placed in each position, participants' MTUs were scanned using 3D US.

3D Ultrasonography

All the information needed to implement the 3D US scanning performed in the present study are described in detail in the supplementary materials of a previous paper [11]. Two-dimensional B-mode US images were collected using a US scanner (Aixplorer version 12.3, SuperSonic Imagine, Aix-en-Provence, France) with a 10–2 linear probe (40-mm field of view; Vermon, Tours, France). Image depth was set at 6.5 cm and US images were recorded using a video grabber (ElGato Cam Link; Corsair Components; Fremont, CA). The situation of the probe (position and orientation) was assessed by tracking a 3D-printed rigid body with four markers attached to the probe, using the Optitrack motion capture system. Data from the US images and motion capture system were streamed and synchronized using the open source software PlusServer (Public software library for US imaging research; v. 2.8.0: Kingston, Canada) [27], and recorded using the open source software 3D Slicer (slicer.org ; v. 4.10.1; Perth, Australia) [10, 27].

Temporal and spatial calibration were performed using 3D Slicer, as previously described [11]. All the 3D US processes and analyses were performed by an operator experienced in the use of the US technique for the lower limb.

For each muscle, proximal and distal insertions were identified using 2D ultrasound. For the RF, these were the anterior inferior iliac spine and base of the patella, while the patellar tendon was not considered because the musculoskeletal model used did not consider it. For the GM and GL, the insertions were the proximal surfaces of the lateral and medial condyles of the femur, as well as the calcaneus osteotendinous junction, were identified. For the proximal insertions of the three bi-articular hamstring heads, the ischial tuberosity was identified. The distal insertions were also identified for each hamstring: the posteromedial corner of the tibia (just below the joint line) for the SM, the *pes anserinus* for the ST [30], and the fibular head for the BF.

Then, 3D US sweeps were performed at a constant speed in the transverse plane. We used a copious amount of gel between the transducer and the skin to ensure that the US image was not distorted due to tissue compression. When the structure of interest was not easily visible (e.g. the ischial tuberosity in some cases), a mark was digitized directly on a specific tracked US image to indicate the structure location and thus aid identification within the 3D reconstruction. Volume reconstruction was performed for each MTU, while their length was calculated in each position using 3D Slicer software (v. 4.10.1). The full MTU path was assessed by calculating the centroid of muscle and tendon cross-sectional area every 2 cm [20]. The length was then calculated as the sum of the cumulative distances, between successive 3D centroid points.

Five participants (age 26.2 ± 1.6 years, height 181.4 ± 6.2 cm, mass 85.2 ± 20.4 kg) returned on a second day so we could examine the reliability of the reference position for each muscle, which was done using the same process as for the first measurements. These participants were chosen for their wide range of training status. Standard errors of the MTU length measurements were 1.1 mm, 0.7 mm, 1.3 mm, 2.4 mm, 2.6 mm and 1.5 mm, for SM, ST, BF, RF, GM and GL, respectively. Minimal detectable change ($\mathrm{SEM} \times \sqrt{2} \times 1.96$), which indicates the maximum sensitivity of the measurement, was 3.0 mm, 1.9 mm, 3.6 mm, 6.7 mm, 7.2 mm and 4.2 mm, for SM, ST, BF, RF, GM and GL, respectively.

Musculoskeletal modeling

A lower limb musculoskeletal model was used in OpenSim [22]. The hamstrings (SM, ST, and BF), RF and *gastrocnemii* (GM, GL) were modeled using muscle insertions and path points with local coordinates that were defined in five body frames: pelvis, femur, patella, tibia and calcaneus. Each muscle was composed of two insertions (proximal and distal) and one (SM, BF, GM and GL), two (RF) or three (ST) path points. The generic model was scaled for each participant (right side) using the standing static acquisition. For the five bodies, the scaled coefficients through the three axes were stored for next steps.

The inverse kinematic calculation was performed for the right lower limb for all the 16 positions recorded during the experimentation so that the joint angles were computed. The *'Body Kinematics'* toolbox was used to extract the coordinates of lower limb bodies (pelvis and right femur, patella, tibia and calcaneus) for each position. The joint angles and body orientation for each position were then considered as the average of the angles and body orientation, respectively, for the whole frames of acquisition.

MTU length

The MTU length was calculated for each position using 3 models: the OpenSim scaled model (OS), OpenSim implemented with personalized insertions (OS + INSER) and OpenSim implemented with personalized insertions and MTU path (OS + INSER + PATH) (Fig. 2). The two personalized models were developed to better understand whether it is more important to measure the insertion coordinates or the MTU path. The MTU lengths for each position (Fig. 1) were computed using the *'Muscle Analysis'* toolbox of OpenSim.

For the OS + INSER model, we used both the proximal and distal insertion locations measured with 3D US in the reference position (HAM1, RF1 and GAS1). The 3D locations in the overall frame were expressed in local

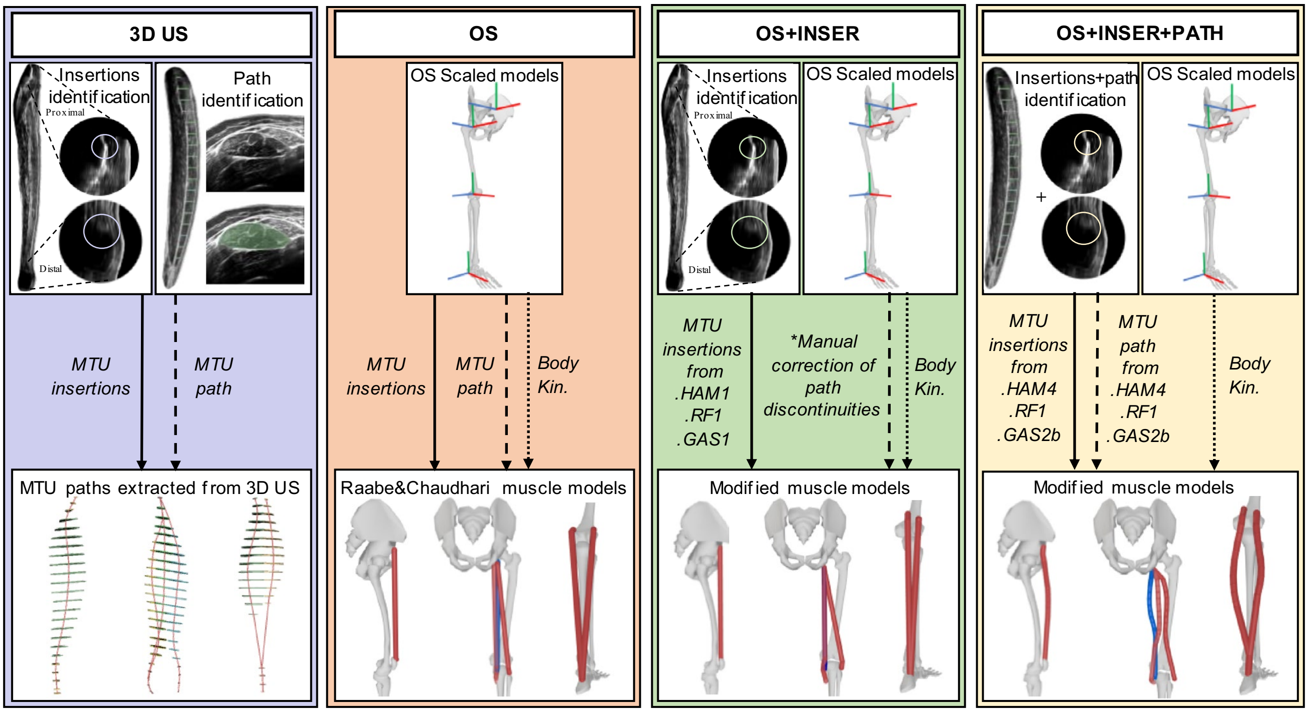

Fig. 2. Methods used to assess muscle-tendon unit length. 3D US: 3D Ultrasound is used as the reference imaging method used. *OS* OpenSim generic model scaled to the participant's antropometry, *OS + INSER* OpenSim scaled model with personalized MTU insertions of the participant assessed by 3D US, *OS + INSER + PATH* OpenSim scaled model with personalized MTU insertions and path of the participant assessed by 3D US. *The proximal and distal path discontinuities were corrected using the nearest 3D US point (to HAM1, RF1 and GAS1) to replace coordinates of the generic path points inducing the path break.

lower limb frames that relied on the body orientations. The coordinates of the proximal and distal insertions were then modified in the participant-specific scaled model. Then, a manual correction was performed to remove the proximal and distal discontinuities that were generated between the personalized insertions and the OpenSim generic path. This was done using the nearest 3D US point (to HAM1, RF1, and GAS1) to replace coordinates of the generic path points inducing the path break. For the OS + INSER + PATH model, the MTU insertions and whole path that were acquired during a single 3D US acquisition, were implemented in the musculoskeletal model in two steps. First, the most suitable position to perform the 3D US scan was determined for each muscle group through a method presented in Supplementary Data 1. Second, some discontinuities that were observed when the bodies moved relative to each other were removed because the insertions were defined in different body frames from the path points. As an example, the RF insertions were located in the pelvic (proximal) and tibial (distal) frames, while the path points were located in the frame fixed on the femur, which leads to a collision of the proximal path points during hip flexion. To erase these discontinuities, some proximal and distal path points were not considered in the musculoskeletal model.

Thus, for the OS + INSER + PATH model of the RF, the best 3D US scan position was determined to be 'RF1'. For the discontinuities in this model to be removed, the three proximal and five distal path points were erased. For the hamstrings, the optimal 3D US scan position was determined to be 'HAM4-HF-KF'. For the ST, the three last path points were found in the tibial frames, delineating the *pes anserinus*. For the SM, ST and BF, the four proximal and two distal path points were removed to erase the discontinuities. For the *gastrocnemii*, the best 3D US scan position was found to be 'GAS2b-KF'. The path points between the distal muscle-tendon junction and the distal insertion were removed so that discontinuities within this model could be erased while the bodies moved. These models obtained using a single 3D US scan position, were then used to compute the length of each MTU in all the other positions.

The MTU lengths were expressed in percent of segment length. The body length for the RF and hamstrings was considered as the distance between hip and knee joint centers. For the *gastrocnemii*, the body length was considered as the distance between knee and ankle joint centers. The absolute body length of each participant is given in Supplementary Data 2.

The change in the MTU length was then computed by subtracting the length assessed during the positions HAM2 to HAM5, RF2 to RF5 and GAS2 to GAS5 from the length assessed during the reference position of each MTU (see Fig. 1). A positive value indicates an MTU lengthening, while a negative value indicates an MTU shortening relative to the reference position.

Statistical analysis

Biases between 3D US and the three OpenSim models were examined using Bland–Altman analysis and the 95% limits of agreement method for each position and MTU [4]. RStudio v2022.07.2 + 576 software (R Development Core Team 2011) was used to perform the statistical analyses, with the *'nlme'* package to implement mixed effect models. Linear mixed effect models were used to determine significant differences between the three OpenSim models and 3D US (6 MTU × 6-7 positions × 3 models - random variable 'participant'). When the interaction terms were significant, contrast analyses were computed using the Tukey method with the '*emmeans'* R package. Statistical significance was set at 0.05.

Results

The main results on length are reported in Table 1 and Fig. 3, and those on change in length measurements in Fig. 4. Additional detailed results are presented in Supplementary Data 3.

Length measurements

Non-significant effects were found for the hamstrings regarding the OS model, notably for ST (− 1.5%) and BF (− 1.9%), while the SM just crossed the alpha level (− 3.4%, $p = 0.049$) (Fig. 3A–C). Significant differences in MTU length were found between the OS model and 3D US for RF, GM and GL, with biases of − 10.9%, − 7.4% and − 6.3%, respectively (Fig. 3D–F). The OS + INSER model reduced the magnitude of biases by an average of 4% for RF, GM and GL, while the OS + INSER + PATH model showed the smallest biases in length estimates, which made them negligible and non-significant for all MTU (≤ 2.2%). The OS + INSER + PATH model reduced the LoA for each MTU, particularly for GM and GL, with a decrease from 10.5% and 8.9% to 3.4% and 3.3%, respectively (Table 1).

Change in length measurements

Regarding the OS model, non-significant effects were reported for the RF and hamstrings, notably for ST. This latter showed the smallest bias, at 0.4%, while the highest was found for RF, at − 2.5% ($p = 0.683$) (Fig. 4A–D). Significant differences in MTU change in length were found between the OS model and 3D US for GM and GL, with biases of 1.3%, and 1.2%, respectively (Fig. 4. E, F). The OS + INSER + PATH model showed no significant differences compared with 3D US for any of the MTUs and all biases were ≤ 1.7% (Table 1).

Table 1: Bland-Altman analysis of each model compared to 3D Ultrasound (US) for the estimation of both MTU length and MTU change in length

MTU	OpenSim models *VS* 3D US	Length			Change in length		
SM	OS	− 3.4	9.7	0.049	− 1.7	7.1	0.606
	OS + INSER	6.7	11.1	< 0.001	− 4.6	11.5	0.005
	OS + INSER + PATH	− 2.2	8.6	0.297	− 0.5	8.4	0.989
ST	OS	− 1.5	10.8	0.705	1.4	6.6	0.829
	OS + INSER	− 2.3	8.0	0.293	− 1.4	8.8	0.819
	OS + INSER + PATH	− 1.3	8.6	0.751	1.7	8.5	0.709
BF	OS	− 1.9	10.4	0.539	0.4	5.0	0.992
	OS + INSER	− 2.0	7.1	0.385	0.1	7.9	0.999
	OS + INSER + PATH	0.7	8.1	0.945	0.7	7.6	0.943
RF	OS	− 10.9	11.3	< 0.001	− 2.5	9.4	0.683
	OS + INSER	− 6.4	14.5	0.001	− 6.2	15.0	0.016
	OS + INSER + PATH	− 1.7	8.3	0.744	− 1.7	9.2	0.854
GM	OS	− 7.4	10.5	< 0.001	1.3	3.6	0.018
	OS + INSER	− 3.4	4.1	< 0.001	− 0.8	4.4	0.244
	OS + INSER + PATH	0.9	3.4	0.284	0.5	3.0	0.834
GL	OS	− 6.3	8.9	< 0.001	1.2	3.3	0.018
	OS + INSER	− 2.5	4.0	< 0.001	− 0,8	4.1	0.244
	OS + INSER + PATH	0.8	3.3	0.284	0.3	2.7	0.834

Datas are expressed in % of the bone segment length.

LoA 95% Limits of agreements. The *p*-value referred to the bias significance.

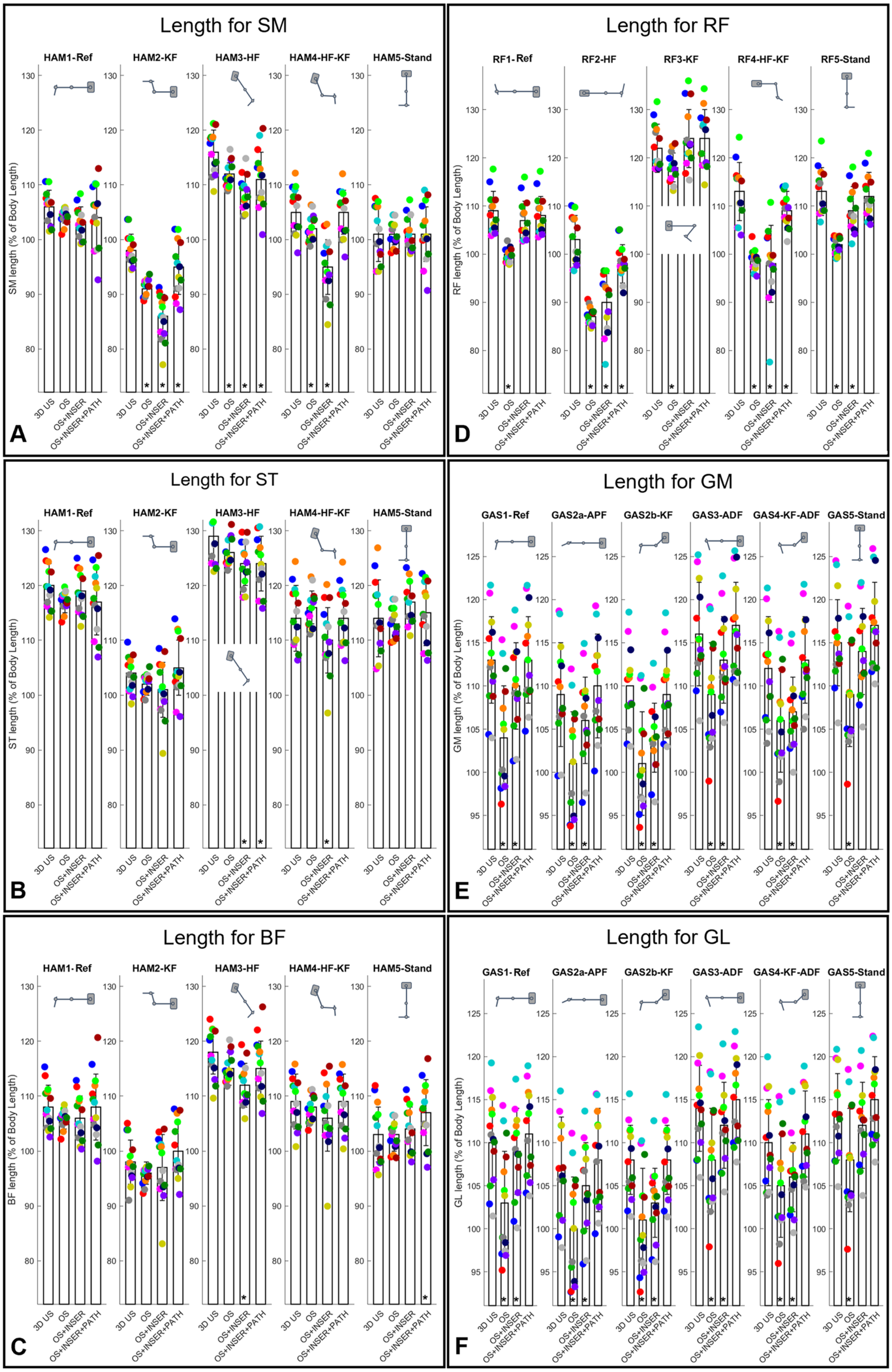

Fig. 3. MTU length for each model and position. *Significant difference.

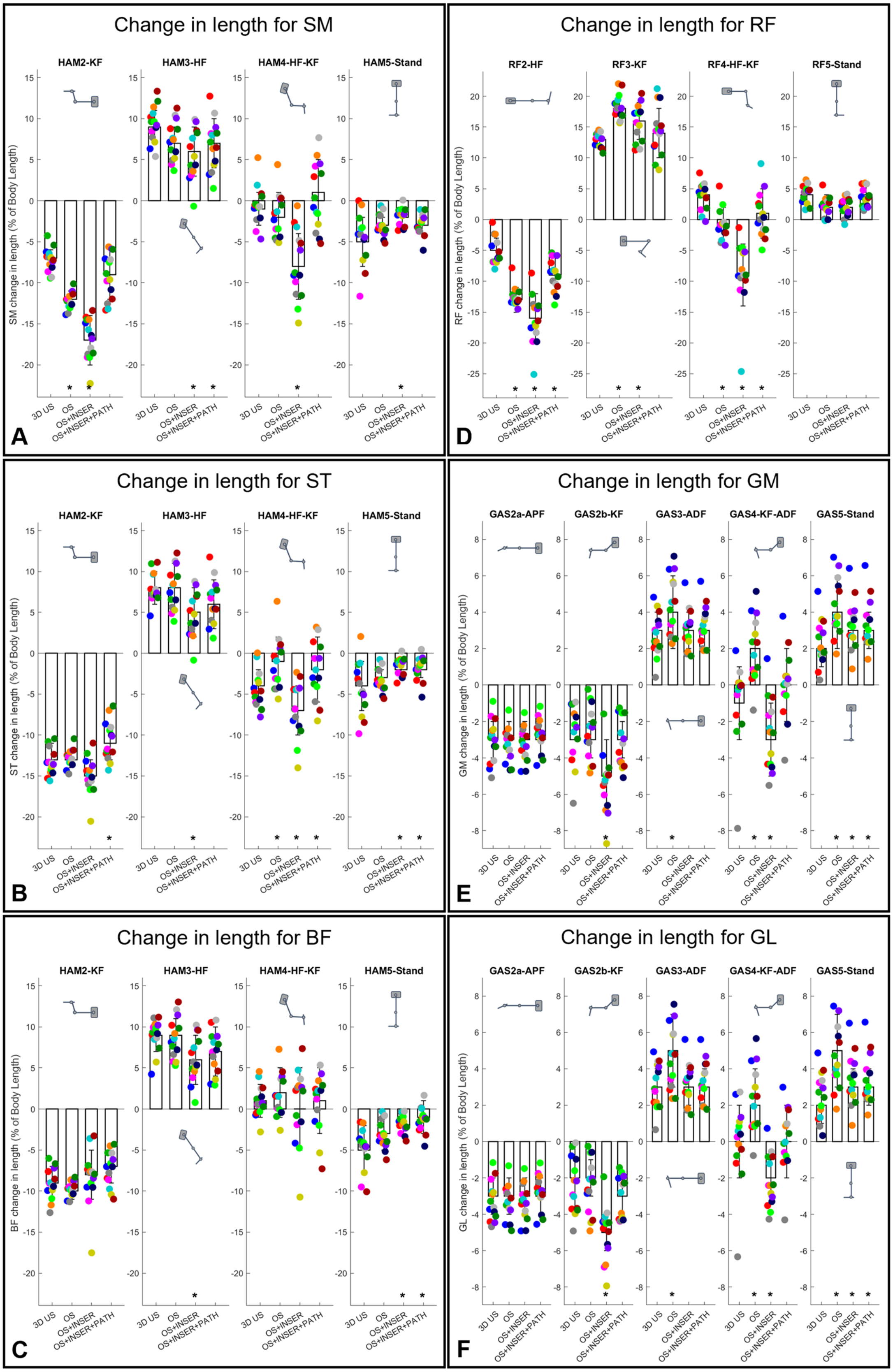

Fig. 4. MTU change in length for each model and position. The change in length is computed with respect to the reference condition. *Significant difference.

Discussion

In our study, a 3D US pipeline was developed and used to assess the MTU length and change in length of the main bi-articular muscles of the lower limb for 16 passive static positions. The personalized MTU insertions and paths were used to develop an OpenSim participant-specific musculoskeletal model. Compared with the OpenSim generic model, our personalized model allowed more accurate MTU length estimation both at the group level and in terms of inter-individual variability.

Our results invalidated our first hypothesis that the OS model would provide unbiased mean length estimation for the group of participants, since for four of the six muscles (SM, RF, GM and GL), a significant bias was found for the MTU length estimated using the OS model. This result strengthens the need for the personalized models that were developed in the present study if accurate MTU length estimates can be expected. The OS + INSER model reduced the magnitude of bias compared with the OpenSim model, while remaining significant. The OS + INSER + PATH model showed non-significant differences with experimental 3D US data, and had lower individual errors.

The overall MTU length measurements that were performed using 3D US (Supplementary Data 2) were consistent with data extracted from dissections or imaging studies. For example, an average length ranging from 42.5 to 47.9 cm was found here for the SM, ST and BF, respectively. In a previous study, Woodley and Mercer [28] reported similar lengths of 44 cm for each bi-articular hamstring MTU. Regarding the *gastrocnemii*, we found an average GM MTU length of 48 cm, while Habersack et al. [13] reported a length of around 44 cm for a similar sample of participants. These differences can be explained by the variances created using different methods. While both these previous studies used bony landmarks and a straight line that reduced the assessed length, we measured the entire GM line of action.

In addition, the magnitude of differences between the OS model and 3DUS is in line with the previous work of Persad et al. [21], who found average biases up to 4%, as well as high inter-individual variations up to 22% of the OS model compared with MTU length measured during intra-operative dissection of the *gracilis* MTU. For example, in the case of the ST MTU (which is probably the most comparable muscle to *gracilis*), we found an average bias of − 1.5%, with high inter-individual variations up to − 17% depending on the position. However, it should be noted that, on average, the OS model generally underestimates MTU length compared with 3D US (see Table 1). This which was less apparent in the study of Persad et al. [21], possibly due to the differences between the methods used. Using 3D US, it is possible to assess MTU length from the whole MTU path by considering muscle and tendon curvatures, which lead to longer MTU measurements. In contrast, the dissection method does not take this curvature into account and instead considers a straight line. Regarding inter-individual variations in length measurements, the OS model seems to be fairly consistent across all the MTUs (LoA: 8.9% to 11.3%).

By looking at the OS model, small and non-significant biases were found for the length, as well as change in length, for the ST and BF. The SM crossed the alpha level for the length assessment ($p = 0.049$) but not for change in length ($p = 0.606$). This could indicate that the OS model is able to accurately measure the mean length and change in length of a group of participants. However, large errors were found for some participants, with LoA ranging from 5% to 10.8% for hamstring MTU length and change in length, indicating that personalization is required to better describe the inter-individual variability. Overall, the estimate error seems to be quite homogeneous among bi-articular MTUs, which were basically of the same order for the RF, GM and GL (LoA of 8.9% to 11.3%), except for the case of the *gastrocnemii* in the change in length measurements, which was lower (3.5% on average). Although MTUs of the GM and GL showed some of the lowest limits of agreement, small but significant biases were found for their change in length using the OS model.

Contrary to the hamstrings, the length measurements of the RF, GM and GL were significantly biased by a large difference between OS and 3D US (− 6.3 to − 10.9%). This could be partly explained by the curvatures of these MTUs, which were not taken into account in the OpenSim model. This source of error is particularly important for the RF, which presented the highest biases for the two positions that involved a hip flexion (RF2-HF: − 15.8%, RF4-HF-KF: − 14% on average, and up to − 24%; see Supplementary Data 3 for detailed results). Thus, the fact that the RF path is modeled as a straight line in the OS model should have contributed to the higher reported biases that were induced by the substantial hip flexion. In addition, the participant-specific morphology, which is not finely considered during the deformation, induced by the scaling of the model could also explain these results. This happens especially in regard to the inter-participant variability of MTU insertion locations and moment arms, which influence both MTU length and change in length [8]. This type of error is intrinsic to the linear scaling process since there is no exact relationship between the segment dimensions and MTU length. The specificities of individuals' bone geometry could not be taken into account either, which also contributed to the model bias. It should be kept in mind that this kind of modeling does not correspond to precise descriptive anatomy, but to a simple visualization based on rough estimations of both the insertion points and muscle path [26]. For instance, the change in length of the RF was highly overestimated using OS for the RF2-HF position. In addition, for the GAS4-KF-ADF position, the OS model predicted a lengthening for both the GM and GL, while the mean length was unchanged using the 3D US technique. Overall, the differences between the OS approach and the 3D US measurements were not surprising, especially considering that the generic OS model was established on individuals who were male and older [22]. Taking all these points into consideration, our results underline the need for personalized models.

For this purpose, two levels of model personalization were investigated in our study. The OS + INSER model induced a decrease in the magnitude of bias in the length measurement, by an average of 4% for the RF and *gastrocnemii,* i.e. the most biased MTU length estimations using OS. This was not the case for the hamstrings, where the mean improvement potential was limited since both small and non-significant bias were found between OS and 3D US. However, in accordance with our hypothesis, inter-individual variability for the measurement of MTU length was decreased for the ST (from 10.8% to 8.0%) and BF (from 10.4% to 7.1%) with this first level of personalization, although this was not the case for the SM.

The OS + INSER + PATH model, for which both insertions and path were personalized, showed the smallest biases in length measurements, as these became negligible and non-significant for all MTUs ($\leq$ 2.2%; $p > 0.28$). The magnitude of the bias reduction was higher for the muscles that were most biased with the OS model. Therefore, this effect was larger for the RF and *gastrocnemii* (decreases of 9.2% and 6% on average, respectively), compared with the hamstrings (0.9% on average). This model also showed an encouraging reduction of the inter-individual variability error for all MTU. In the case of the *gastrocnemii*, the largest decrease was shown, with LoA from 10.5 to 3.4% for the GM and from 8.9 to 3.3% for the GL. The change in length biases for all MTUs when using this model were either smaller or equal to the biases observed in the OS model. The *gastrocnemii* showed the largest decrease, from 1.3 to 0.4% on average, having shown non-significant values, as well as slightly lower LoA compared with the OS model.

Considering that 3D US provides great improvements in MTU length estimation, the experimental cost of such measurements should be considered. This technique requires 3D motion capture, as well as an ultrasound system, which are classically available in biomechanics laboratories. However, like all ultrasound methods, it requires operators to have had rigorous training to acquire the appropriate expertise to perform both the scanning and processing steps. The scanning can be performed in the motion capture environment with scanning positions that are relatively easy to set up (see Fig. 1), permitting a fast scanning time of between one to three minutes per MTU. Moreover, based on our results, we consider that one scanning position per MTU is sufficient. Although the data processing could be time consuming when it involves imaging, this duration can be limited since no significant differences were found when comparing paths assessed every 2 cm or every 4 cm. Therefore, apart from the experience required for the scanning, which should not be ignored, we are convinced that the experimental cost of 3D US is relatively low relative to the gains it could provide if accurate MTU length estimates can be obtained. It is important to note that these data were obtained under passive conditions. While EMG was not measured to confirm the absence of muscle activation, we are convinced that the muscle activity was minimal in our experiments for three reasons. (i) All conditions were resting positions maintained without the need for participants to activate their muscles. This was achieved by ensuring that each limb was stabilized while participants were asked to relax completely. If a joint moved during relaxation, the position was adjusted using foam until the limb was stabilized and participant was completely relaxed. (ii) Postures were analyzed and, therefore, the stretch reflex was not elicited. (iii) In a very similar experiment [23], EMG was checked and found to be negligible for all the participants.

Due to possible changes in muscle path, there remains the question of how muscle contraction changes muscle-tendon length. In a pilot experiment, we found that even high isometric contraction levels (50% of MVC) did not influence the muscle-tendon length measured using 3D ultrasound. These pilot tests remain to be confirmed by further work to extend our methods and findings to active conditions.

Two main methodological limitations should be considered to better understand the remaining sources of error in the OS + INSER + PATH model. First, it seems necessary to consider the measurement errors related to the inverse kinematics. We chose to use the 3D US insertion points and path from a single acquisition (Supplementary Data 1), the inverse kinematics then being reapplied to match the other joint positions with the personalized models. Measurement errors related to the inverse kinematics procedure have already been identified [26] and are known to be joint-dependent. The hip joint is often reported as a source of significant errors in both the inverse kinematics and scaling process [16], which could also explain some of the greater noise for the MTUs inserted at the hip (i.e. hamstrings and RF). To better understand the influence of inverse kinematics on MTU length estimates, we computed the change in length for each MTU that could be induced by a given angular error (Supplementary Data 4). Thus, we found that an error of 4.4° at the knee would result in a 3.7 mm change in length for the RF, but only 1.7 mm for the *gastrocnemii*. In comparison, an error of 4.8° at the hip resulted in a 5.5 mm change in length for the ST. The fact that the hip would be more likely to be affected by measurement errors could partly explain the higher LoA we found for hip-inserted MTUs compared with the *gastrocnemii*.

Second, imaging-related measurement errors with the 3D US method were a further source of noise. Although the overall inter-day reliability, obtained on five of our participants, was quite good, we found larger reliability errors for the hamstrings (SEM and MDC of 2 and 6 mm) than for the other muscles (SEM and MDC of 1 and 3 mm, respectively). This was probably due to the anatomic complexity of the hamstrings [11], which would have contributed to the increase in LoA found for hamstring MTUs. Overall, our reliability results for the RF and *gastrocnemii* were similar to those from a previous study that used a similar method for the tendon and found a minimal detectable change of 2 to 3 mm [20].

To conclude, muscle-tendon length of the main six bi-articular MTUs of the lower limb was measured using 3D US and the results compared with the predictive results of a generic musculoskeletal model in OpenSim. This generic model showed an overall underestimation of MTU length in our sample. Small biases were found for the hamstrings, but larger ones for the RF, GM and GL. Inter-individual variation errors were found to be quite large (5 to 11%) across almost all MTUs. The present study provides new insights about the behavior of bi-articular MTUs at various joint angles and also developed a 3D US pipeline that opens up new perspectives for personalizing musculoskeletal models using low-cost user-friendly US scanning. Our results show that the personalization of MTU insertion points and path (OS + INSER + PATH model) resulted in more accurate estimates of MTU length, with a decrease at the group level and in terms of inter-individual variability. Future studies should assess the influence of muscle-tendon length estimates obtained with the methods used in the present study on OpenSim movement simulations. The OS + INSER + PATH model could be highly valuable for future studies that involve populations that probably have specific MTU geometries (e.g., elite athletes, obese patients, tendon transfer patients, those with bone deformations, etc.).

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Declarations

Conflict of interest The authors state that they have no financial or personal affiliations with individuals or organizations that may have exerted inappropriate influence on this study.

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