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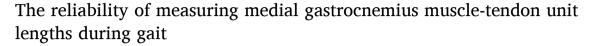
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### Short communication



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#### ABSTRACT

Background: Ultrasound imaging combined with 3D motion analysis allows for in-vivo assessment of muscletendon unit lengths during gait. The clinical relevance of analysing the medial gastrocnemius (MG) and Achilles muscle-tendon junction (MTJ), MG mid-muscle belly fascicles (FAS) and muscle thickness was shown. However, their reliability error estimations over the gait cycle is unknown.

Research question: What are the intra- and inter-session errors associated with extracting MG belly, thickness, FAS and tendon lengths using ultrasound during gait in healthy participants?

Methods: 3D gait analysis was carried out in ten healthy adults as they walked on an instrumented treadmill at a comfortable walking speed. An ultrasound probe was secured on the leg and tracked by 3D motion analysis. Images were collected during two walking trials with the probe on the MG muscle-belly to estimate FAS lengths and muscle thickness, and during two trials with the probe on the MTJ to estimate muscle-belly and tendon lengths. A second session was performed after  $5\pm4$  days where a different operator placed the ultrasound probe. The standard deviation (SD) of absolute and relative lengths changes during the gait cycle over different trials were calculated per participant. SD values averaged over participants represented intra- and inter-session errors. Results: For all assessed variables, the intra-session errors were <2.2 mm, except for the FAS lengths (3.1 mm). The inter-session errors were larger than the intra-session, with the highest values found for the absolute muscle-tendon unit lengths (5.6 mm). Relative length errors were smaller than absolute length errors.

Significance: Intra-session errors, which may reflect natural variability and data processing errors, seem more critical when extracting absolute FAS than muscle-tendon lengths. Standardized probe positioning on the MTJ between sessions may improve the inter-session reliability. Expressing the lengths relative to their lengths as the beginning of the gait cycle reduces the inter-session errors.

#### 1. Introduction

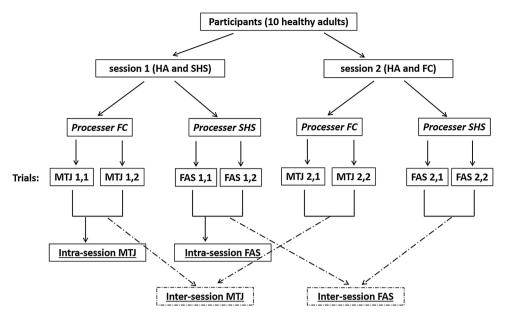
Conventional clinical 3D gait analysis (3DGA) can be enhanced by the addition of muscle-specific outcomes [1]. Musculoskeletal modelling provides insight in the relation between muscle-tendon unit lengths and joint-level parameters, but its accuracy is not yet satisfactory, especially in pathological conditions [2]. A direct assessment of muscle-tendon unit lengths during gait can be performed by combining ultrasound (US) imaging with 3DGA [3].

Studies have already shown the clinical relevance of adding US

imaging to 3DGA, measuring muscle-belly, fascicle (FAS) and tendon length changes during gait in children with cerebral palsy [4,5]. In addition, tracking changes in muscle thickness may be useful for understanding several aspects of a muscle's functional capabilities [6] and (along with the pennation angle) may offer an alternative when FAS are less visible. Estimation of muscle-tendon variables using US is affected by errors that arise from natural variability between gait cycles, probe positioning, integration with 3DGA and data processing [7]. Quantification of these errors is required for appropriate clinical interpretation. This study quantifies the intra- and inter-session errors introduced by

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**Fig. 1.** The repeated-measures experimental design. For each participant, two sessions were acquired on separate days. The same experienced clinician (HA) placed the reflective markers. Operator SHS placed the probe in session 1 and Operator FC placed the probe in session 2. For each session, 4 trials were acquired (2 MTJ and 2 FAS). Processer FC analysed the MTJ data whilst processer SHS analysed the FAS data. Intra- and inter-sessions errors were derived.

probe positioning while using US on the medial gastrocnemius (MG) during 3DGA in healthy adults.

#### 2. Methods

#### 2.1. Participants

A convenience sample of ten healthy adults (5 male/5 female, age 25  $\pm$  3 years, height 1.70  $\pm$  0.10 m, body-weight 65.7  $\pm$  4.8 Kg) were recruited [8,9]. The University Hospital ethics committee approved the study (s57384) and written informed consent was acquired from participants. All data were anonymised.

## 2.2. Acquisition protocol and research design

A 12-camera motion analysis system (Vicon Motion Systems Ltd., UK) operating at 100 Hz was used. Participants walked on an instrumented treadmill (Motek Medical, Netherlands). A computer-based Bmode US device (Telemed EchoBlaster 128 Ext-1Z system, Lithuania) with a 59 mm linear probe was used to record images at 30 Hz. When needed, participant-specific fine-tuning of the predefined US acquisition parameters (focus 17 mm, depth 50 mm, dynamic range 38 dB, power 100 %, gain 52 %, frequency 8 MHz) was performed to maximise visibility of the MG FAS and most distal MG fascicle-Achilles tendon transition, or muscle-tendon junction (MTJ). The US and motion-analysis systems (Nexus 2.6 and Echowave II) were synchronized and used for data acquisition and pre-processing. The same experienced clinician (HA) placed reflective markers to the body following the lower-limb Plug-in-Gait model (Vicon Motion Systems Ltd., UK) plus additional markers on the head of fibula, tibial tuberosity and medial malleolus to define a local shank reference frame [10].

A cluster of four reflective markers was rigidly attached to the US probe [11], which was then secured to the leg using an elastic bandage to minimise probe movement relative to the skin [5] (Figure S1). While standing, the US probe was used as pointer to image the location of the medial femoral condyle as the MG origin [8]. Two consecutive trials were first collected with the US probe on the muscles' mid-belly to image FAS, followed by two trials with the US probe on the MTJ, along the pulling direction of the Achilles tendon (Figure S2) [9,12]. After a period of habituation (minimum 3 min), data were recorded for 10 s per

#### Table 1

Average intra-session and inter-session errors (from the respective standard deviations over participants). The ratio (r) between inter- and intra-session errors reveals the influence of experimental (extrinsic) errors (for example, r=1 indicates no experimental errors, while r>1 indicates the proportion of the experimental error with respect to natural variability) [14]. For each variable, the average value (avg) and its corresponding minimum (min) and maximum (max) values are reported. Relative values are the length changes from the corresponding values at initial foot contact. Muscle-tendon unit (MTU).

	intra-session errors	inter-session errors	r	Avg (min,max)
knee angles [deg]	1.8	3.0	1.7	21.2
ankle angles [deg]	1.3	2.4	1.8	(4.9,57.4) 4.5 (-9.3,15.2)
muscle length change [mm]	1.3	4.8	3.7	246.9 (241.1,250.6)
relative muscle length change [mm]	1.8	2.1	1.2	2.6 (-3.1,6.3)
tendon length change [mm]	1.3	3.0	2.3	180.7 (176.5,184.8)
relative tendon length change [mm]	2.2	2.4	1.1	-0.6 (-4.9,3.5)
MTU length change	1.0	5.6	5.6	426.4 (417.1,431.6)
relative MTU length change [mm]	1.9	2.0	1.1	1.8 (-7.4.7.1)
fascicle length change	3.1	3.3	1.1	51.8 (43.7,55.0)
relative fascicle length change [mm]	1.7	1.9	1.1	-2.7 (-10.7,0.6)
thickness change [mm]	0.6	1.0	1.7	16.7 (15.2,17.8)
relative thickness length change [mm]	0.4	0.5	1.3	-0.4 (-1.9,0.7)

trial at a self-selected comfortable gait velocity (1.0  $\pm$  0.1 m/s). Data acquisition was repeated during a second session (average 5.1  $\pm$  4.0 days after the first) during which a different operator placed the US probe (FC or SHS). The experimental design is described in Fig. 1.

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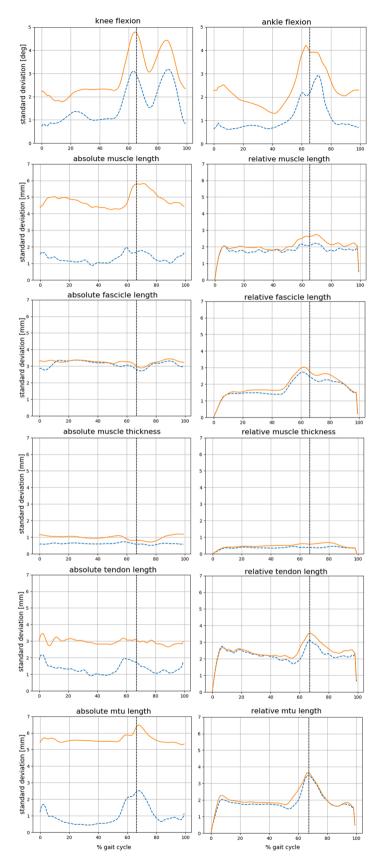


Fig. 2. Absolute (left) and relative (right) average SD for the intra-session (blue, dashed lines) and inter-session (orange, solid lines) assessment for muscle belly, fascicle, thickness, Achilles tendon and muscle-tendon unit (MTU) length changes for 10 healthy participants over the gait cycle. Average SD of knee and ankle sagittal kinematics are also reported. The corresponding averages of these variables are reported in figure S4.

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#### 2.3. Data processing

Experienced operators performed all MTJ (FC) and FAS (SHS) data processing (Fig. 1). Data were manually extracted from each US image with the MTJ as a single point [9], FAS as the most visible straight-line distance between the superficial and deep aponeuroses (one fascicle per image), and thickness as the perpendicular distance between superficial and deep aponeuroses (Figure S2) [13]. Open-source software was used to process all data [11]. The locations of the MTJ in the images were mapped into the shank reference frame. Absolute muscle and tendon lengths were defined as straight-line distances between the medial femoral condyle (visualised by the probe-as-pointer [8]) and the MTJ, and between the MTJ and the marker on the calcaneus, respectively, with the sum providing the muscle-tendon unit (MTU) length. Muscle-tendon variables were normalized by subtracting the corresponding length at initial contact from the actual lengths over the gait cycle [5]. All variables are in mm.

Kinematic and muscle-tendon variables were expressed over five time-normalised gait cycles (100 points) per trial. For each participant, the intra- (between the two trials) and inter-session (between the two sessions, four trials in total) mean and standard deviations (SD) of the variables over the gait cycles were calculated. Intra- and inter-session SDs were then averaged over all the participants. The ratio (r) SD inter-session/SD intra-session was calculated [14].

#### 3. Results

Joint kinematic inter-session errors were larger than the intra-session (Table 1), with average values  $<\!3.0^\circ$ . The intra-session errors in muscletendon lengths were  $<\!2.2$  mm in all cases, except for FAS lengths (3.1 mm). The inter-session errors were larger than intra-session, with the highest values for the absolute muscle and MTU lengths (Table 1). For all relative lengths as well as FAS absolute length, r was close to 1 (1.1–1.3). The inter- and intra-session errors over the gait cycle are shown in Figs. 2 and S3. Joint kinematics and relative muscle-tendon lengths are reported in Figures S4 and S5.

#### 4. Discussion

The present study estimated the intra- and inter-session errors (from SD) of extracting muscle-tendon unit lengths using US during gait in healthy participants. The use of SD is in line with previous gait literature [14], allowing comparison with normative datasets (figure S4 and S5). The intra- and inter-session errors include natural gait cycle variability, 3DGA and data processing. integration with operator-dependent probe positioning errors are only found inter-session. The intra- and inter-session kinematic errors (Table 1) were similar to those already reported [14], albeit in the present study a probe was attached to the leg and the participants were walking on a treadmill [15]. For muscle-tendon variables, the intra-session errors associated with the absolute belly lengths were lower than those for FAS. Assuming that the natural gait cycle variability and integration with 3DGA are comparable between these muscle features, this highlights higher processing variability for MG-FAS than MG-MTJ US images, despite experienced operators performing a manual process to limit errors. Similar findings were shown when these US variables were extracted in static conditions [9]. As for inter-session, the errors were larger for the data derived from MTJ than from FAS. This may indicate that probe positioning over the MTJ, along with landmark detection for the MG origin and insertion, is more prone to errors than probe positioning on the FAS. In any case, for both MTJ and FAS related data, inter-session errors were further reduced (by 50 %) when length changes were normalized relative to their lengths at initial contact. Smaller absolute and relative errors than FAS were found for muscle thickness.

It should be noted that the level of inter-session errors reported here are obtained with two different operators performing probe positioning.

For single operator studies, lower errors are expected. Such errors can be used to ascertain whether the accuracy of extracting MTU lengths during gait using US in a healthy adult population is acceptable for research and/or clinical purposes. Study limitations are included as supplementary material. More work is required to define intra- and inter-sessions errors in pathological populations, where it is expected to be more challenging [15].

#### **Declaration of Competing Interest**

The authors declare that we have no financial or personal relationships with other people or organizations that could inappropriately influence (bias) our work.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.gaitpost.2021.09.198.

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