Title: Repeatability and sensitivity of passive mechanical stiffness measurements in the triceps surae muscle-tendon complex

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Abstract

Measurements of muscle-tendon unit passive mechanical properties are often used to illustrate acute and chronic responses to a training stimulus. The purpose of this study was to quantify the inter-session repeatability of triceps surae passive stiffness measurements in athletic and non-athletic populations, with the view to discussing its usefulness both as a muscle-tendon profiling tool and a control measure for studies with multiple data collection sessions. The study also aimed to observe the effects of quiet standing on passive stiffness parameters. Twenty-nine men (10 cyclists, nine triathletes, 10 controls) visited the laboratory on three separate occasions, where passive stiffness tests were carried out using an isokinetic dynamometer and B-mode ultrasound. Participants were fully rested on two of the sessions and subjected to 20 min of quiet standing in the other. The passive stiffness assessment generally showed only moderate inter-session repeatability but was still able to detect inter-group differences, with triathletes showing higher passive stiffness than cyclists ($p < 0.05$). Furthermore, quiet standing impacted passive stiffness by causing a reduction in ankle joint range of motion, although mechanical resistance to stretch in the muscle-tendon unit at a given joint angle was relatively unaffected. These findings show that passive stiffness assessment is appropriate for detecting inter-group differences in the triceps surae and even the effects of a low-intensity task such as quiet standing, despite showing some inter-session variation. However, the inter-session variation suggests that passive stiffness testing might not be suitable as a control measure when testing participants on multiple sessions.

Keywords
muscle-tendon mechanics, passive stiffness, range of motion

1 INTRODUCTION

Joint range of motion (ROM) and the passive resistance to muscle-tendon unit (MTU) elongation are commonly reported attributes in the field of musculoskeletal biomechanics.[1] In particular, passive stiffness (*kp*) is often used as a measure of passive resistance to MTU stretch and is a major contributing factor to joint and MTU ROM.[2] For a given joint, *kp* is calculated from the slope of the passive joint moment-angle curve, either at a specific joint angle or between two points.[2-5] Short bouts of continuous stretching have been shown to result in considerable, short-lasting reductions in *kp*,[4,6,7] suggesting an alteration to the material properties of the MTU. This could be due to mechanical factors within the muscle (eg, actin-myosin decoupling) or changes to the viscosity of the muscle, aponeurosis or tendon due to motion.[5,8] Additionally, neural responses to prolonged stretching may include reduced activity of large-diameter afferents causing a reduced muscle spindle sensitivity[9] or a reduction in tendon reflex amplitude.[10] Even a low-intensity activity such as quiet standing has been shown to require intermittent muscle activity, and subsequent muscle length changes, in MTUs at the ankle to maintain stability. Over time, this might influence material properties of the contractile or series-elastic elements,[11-13] although changes in *kp* have yet to be established after periods of quiet standing. Indeed, muscle fibers have thixotropic properties,[5,10] meaning their stiffness can change as a result of previous activity, which has been shown in previous experiments of low-intensity movements.[5,14] Therefore, it is reasonable to suggest that quiet standing might affect triceps surae stiffness properties due to the elongated position of the MTUs and the requirement for muscles to be intermittently active. In contrast to acute responses, longer-term stretching programs have been shown to elicit an adaptation to MTU ROM in the absence of any change to viscoelastic properties, including *kp*.[6,7,15] This suggests that stretching increases joint ROM by increasing the maximal tolerated joint moment, not via changes to the mechanical properties of a MTU such as overall *kp*, individual muscle or tendon stiffness, or fascicle length. However, data reported for well-trained ballet dancers[3] showed that an increase in maximal passive dorsiflexion is coupled with changes in mechanical properties in the MTU, suggesting that these adaptations only occur after chronic exposure to a stimulus.

It seems that measures of *kp* during controlled passive joint rotation are sensitive enough to detect acute and chronic changes in passive MTU properties after stretching programs. However, the intra-or inter-day repeatability remains underreported. One study reported high repeatability for various passive properties across two tests, separated by one week.[16] Another study presented inter-day reliability for *kp* during slow, passive dorsiflexion,[17] where inter-day intraclass correlation coefficients (ICC) ranged from 0.44 (at 10° plantarflexion) to 0.94 (at 10° dorsiflexion), suggesting that *kp* is more repeatable at greater MTU lengths, where passive joint moment is typically greater. The two aforementioned studies[16,17] that reported *kp* repeatability did so in untrained (ie, non-athletic) populations, not in an athletic population who undergo large training volumes of various modalities (eg, cycling and running). It could be speculated that higher levels of resting *kp* could imply higher repeatability, as the threshold for change caused by low activity (such as during quiet standing) would in theory be higher, thus also affecting *kp* sensitivity. However, comparisons between populations with different *kp* have yet to be carried out. This information would be important for many biomechanical studies that test muscle-tendon mechanical characteristics in trained populations, either in passive tests or in active conditions such as isometric strength testing or during locomotion, as strenuous training leads to neural and muscular fatigue.[18] In addition, comparing different endurance training modalities would further develop our understanding of passive properties in trained populations. Running, for example, requires larger lower limb MTU forces than cycling, and includes distinct phases of energy absorption. As such, it is possible that different adaptations to MTU properties occur in response to different endurance modalities.[19] A further reason to ascertain the degree of *kp* repeatability in trained populations is to explore the possibility of using passive stiffness testing as a way of monitoring the neural and mechanical condition of participants' MTUs in such studies. This is particularly important when repeated strength or other functional measurements on the same participant are needed as part of a study design. Finally, to develop a better understanding of the factors affecting the repeatability of *kp*, it is essential to appreciate the sensitivity of this measurement, such as its capacity to detect responses to low-intensity intermittent activity such as quiet standing. This would offer knowledge about the effects of different mechanical stimuli on *kp* values, particularly "subtle" stimuli that research studies often do not consider to be important. Therefore, the aims of this study were: (1) to quantify the inter-session repeatability of *kp* and relevant mechanical parameters in both athletic (cyclists and triathletes) and untrained populations; and (B) to investigate the effects of a 20-min bout of quiet standing on *kp* and relevant mechanical parameters.

2 MATERIALS AND METHODS

2.1 Participants

Twenty-nine healthy men volunteered as participants for this study, comprising 10 trained road cyclists (CYC; age: 39 ± 16 years; stature: 1.80 ± 0.09 m; body mass: 76.1 ± 9.3 kg), nine trained triathletes (TRI; 38 ± 11 years; 1.85 ± 0.09 m; 81.1 ± 8.6 kg) and 10 untrained but physically active controls (CON; 25 ± 4 years; 1.78 ± 0.03 m; 82.4 ± 9.1 kg). CYC and TRI had a minimum one year's experience participating in their respective events, which naturally vary in their training modality requirements (ie, cycling only vs. cycling plus swimming and running). Before data collection, all participants completed a medical screening questionnaire, provided written informed consent, and had been free from musculoskeletal injury for at least six months before participation. Ethical approval for this study was granted by the university ethics committee and research was carried out in accordance with the Declaration of Helsinki.[20]

2.2 Data collection and processing

Participants were required to attend the laboratory on three occasions at a similar time of day, separated by a minimum interval of 48 h. Participants were also encouraged to abstain from any strenuous or unfamiliar exercise for at least 24 h before each testing session. In the first session, *kp* measurements were taken when the participant was in a rested state (REST1). In the second session, *kp* measurements were obtained after 20 min of quiet standing (STAND1) to investigate its effects on *kp* and other mechanical parameters. Finally, the third session replicated REST1, where *kp* measurements were obtained in a rested state without quiet standing (REST2), providing an indication of inter-session repeatability. Assessments of *kp* were carried out in all three sessions for the right triceps surae muscle-tendon complex using an isokinetic dynamometer (System 4 Pro; Biodex Medical Systems) equipped with an attachment for rotating the ankle joint in the sagittal plane. Participants were seated in the dynamometer with their hip joint fixed at approximately 65° of flexion (0° in anatomical standing position), their right knee joint fully extended with their right leg parallel to the ground. Straps were placed across the pelvis and proximal to the knee joint of the involved limb to restrict movement at the hip and knee joints, and the foot was firmly fixed to the attachment using ratchet straps and additional Velcro wrapping, as similar methods have been shown to notably reduce heel lift during maximal voluntary plantarflexion assessments.[21] Ankle joint center of rotation (approximated using the lateral malleolus) was aligned with the dynamometer axis of rotation. Once in the correct position, the participant's ankle joint ROM was determined by the researcher manually rotating the joint from peak plantarflexion to peak dorsiflexion whilst the participant verbally indicated where the limits of their ROM were. This process was repeated independently on each of the three sessions. No participants, on any occasion, were restricted by the mechanical stops of the dynamometer. In this test, ankle angle was defined as the angle between the foot (first metatarsal to the calcaneus) and leg segments (set as 0° in the anatomical standing position). A gravity correction, taken at 10° plantarflexion, was applied to the dynamometer before *kp* measurements. The test was carried out by passively dorsiflexing the right ankle joint throughout its predefined ROM at a slow, constant angular velocity of 10°/s. Six full repetitions were performed (from maximum plantarflexion to maximum dorsiflexion), with the final one being used for analysis. Between repetitions, the ankle was returned to maximum plantarflexion passively by the dynamometer at 10°/s. Passive joint moment (*Mp*) and *kp* were computed by fitting a fourth-order polynomial through the joint moment-angle curve from maximum plantarflexion to maximum dorsiflexion (Figure 1).[2,3] *Mp* was calculated at a common ankle angle (5° dorsiflexion) and at each participant's individual maximum dorsiflexion angle, whereas *kp* was calculated at the same points from the slope of the leading edge of the polynomial fit.

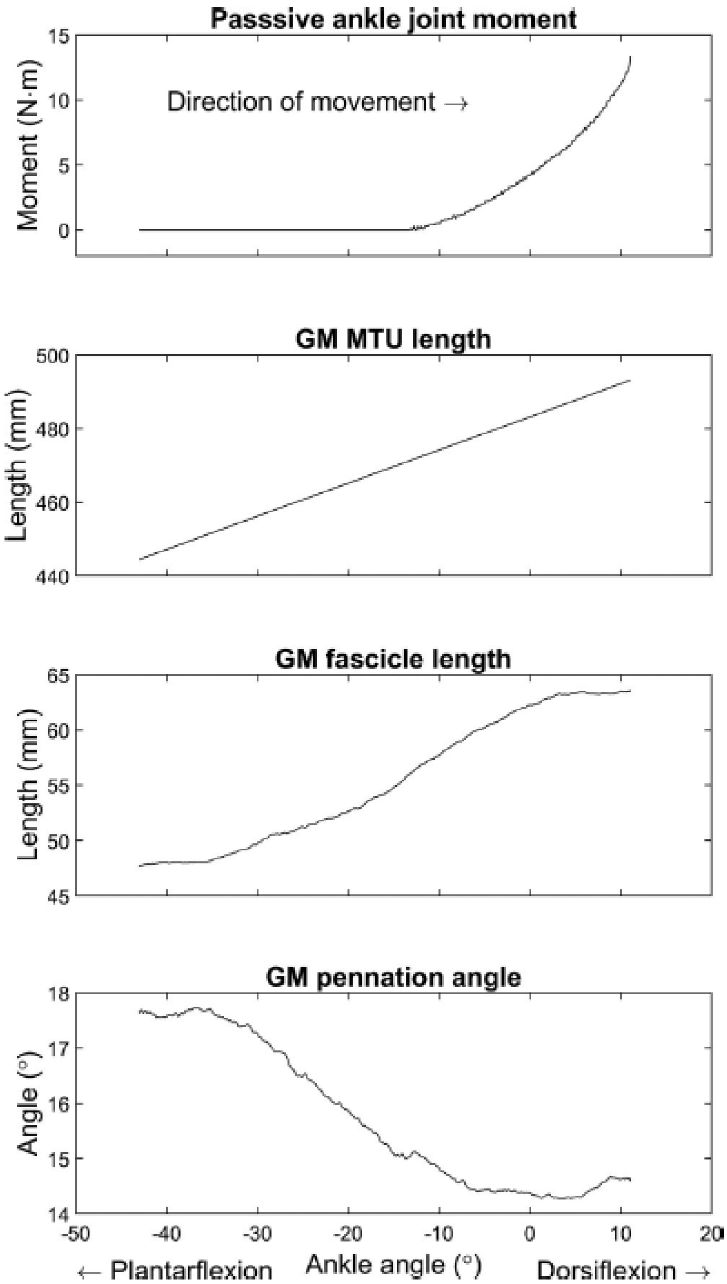

Figure 1 Typical example of *kp* assessment from one participant. As joint moment increases, MTU and fascicle lengths increase, whilst pennation angle decreases. Data plotted against ankle angle from maximum plantarflexion (beginning of the trial) to maximum dorsiflexion (end of the trial)

B-mode ultrasound videos of the gastrocnemius medialis (GM) were recorded at 60 Hz using a 60-mm, 128-element linear array probe (LV7.5/60/128Z-2, 5.0–8.0 MHz; EchoBlaster 128 CEXT-1Z; Telemed UAB) throughout the full repetition. The probe was placed directly over the muscle belly, in line with the direction of muscle fibers, and was fixed in position using a bespoke polystyrene casing and elasticated bandages. Ultrasound provided *in vivo* measurements of GM fascicle length and pennation angle changes throughout joint rotation (Figure 1), which were obtained using UltraTrack (version 4.2).[22-24] Fascicle length was defined as the straight line distance between one fascicle's insertions on the superficial and deep aponeuroses.[25-27] Absolute MTU length of the GM was computed from ankle angle data (provided by the dynamometer) using regression equations[28] and the participants' measured shank lengths (measured with a tape measure from the lateral femoral epicondyle to the lateral malleolus: 446 ± 22 mm). Ultrasound data combined with MTU length data also permitted the estimation of contractile (CE) and series-elastic (SEE) length changes, which were computed using previously established techniques.[29] All MTU and fascicle data were presented as length changes from the beginning of the movement, instead of absolute values. This is because comparing absolute length data between sessions is subject to the assumption that the same fascicle is labelled in the same way. This manual labelling of fascicles in semi-automated tracking algorithms can lead to a systematic offset in tracked length over time.[22] However, these algorithms show very high waveform similarity, despite any systematic offsets, when comparing tracked data on more than one occasion,[24] meaning length-change data are more valid for multiple comparisons. Finally, a high-speed video camera (100 Hz; HS3; Fastec Imaging), synchronized with ultrasound, was placed in the sagittal plane of motion and used to track ankle joint angle to synchronize ultrasound data with dynamometer signals.

2.3 Statistical analysis

All statistical analyses were carried out in SPSS (version 26, IBM). First, a two-way mixed analysis of variance (ANOVA) with repeated measures was used to detect main effects of session and group (as well as session × group interactions) for REST1 and REST2. Post-hoc Bonferroni assessments were used to determine individual differences between sessions or groups, if any main effects were found. A Mauchly's test was used to confirm sphericity of discrete variables. Additionally, a paired-samples *t*-test observed the systematic effects of quiet standing by comparing data from STAND1 with the mean of REST1 and REST2 ($REST_{mean}$). The significance level for all statistical tests was set at $p < 0.05$. $ICC_{3,1}$ were used to show general agreement between REST1 and REST2 for all participants and for each individual group.[30] $ICC_{3,1}$ values were interpreted as: 0.00–0.49 = "poor", 0.50–0.74 = "moderate", 0.75–0.89 = "good", and 0.90–1.00 = "excellent".30,31 In addition to the ANOVA, systematic bias and 95% random error (limits of agreement) were computed to compare REST1 with REST2 and $REST_{mean}$ with STAND1.32,33

3 RESULTS

When comparing REST1 and REST2, there were no significant main effects of session for any parameter analyzed (Table 1). There was also no main effect of group for ankle joint ROM, maximum dorsiflexion, as well as MTU, fascicle, CE and SEE length change (Table 1). However, there was a significant main effect of group for *Mp* at 5° dorsiflexion (F2,26 = 5.60, p = 0.009) and individual maximum dorsiflexion (F2,26 = 4.50, p = 0.021), with post-hoc assessments showing TRI to possess greater *Mp* than CYC at both locations (5°: p = 0.008; maximum dorsiflexion: p = 0.024). Similarly, there was also a significant main effect of group for *kp* at 5° dorsiflexion (F2,26 = 4.84, p = 0.016) and individual maximum dorsiflexion (F2,26 = 6.06, p = 0.007), with post-hoc assessments also showing TRI to possess greater *kp* than CYC at both locations (5°: p = 0.24; maximum dorsiflexion: p = 0.007). For *kp* at 5° dorsiflexion, TRI was also larger than CON (49% across REST1 and REST2), although there was no post-hoc difference (p = 0.055). There was no significant session × group interaction detected for any MTU mechanical parameter ($p \geq 0.518$).

Table 1 Mean ± SD for ankle joint kinematic data, MTU, fascicle, CE and SEE length changes, and Mp and kp at both locations (5° dorsiflexion and individual maximum dorsiflexion) for each group on both rested sessions (REST1, REST2)

				95% LoA ('ALL')
	REST1	REST2	$ICC_{3,1}$	REST1 – REST2
Ankle ROM (°)				
ALL (p = 0.519)	61 ± 5	60 ± 7	0.810^{G}	0.62 ± 9.69
CYC	62 ± 4	62 ± 8	0.712 M	
TRI	58 ± 2	58 ± 5	0.632 M	
CON	62 ± 7	61 ± 7	0.880^{G}	
Individual maximum dorsiflexion (°)				
ALL (p = 0.800)	13 ± 4	13 ± 5	0.856^{G}	0.14 ± 6.29
CYC	14 ± 2	14 ± 5	0.992^{E}	
TRI	12 ± 5	11 ± 6	0.701 M	
CON	13 ± 4	14 ± 4	0.868^{G}	
MTU length change (mm)				
ALL (p = 0.474)	58.09 ± 4.37	57.42 ± 5.51	0.705 M	0.67 ± 9.34
CYC	59.41 ± 2.91	59.26 ± 5.39	0.153^{P}	
TRI	57.41 ± 3.85	56.88 ± 4.95	0.787^{G}	
CON	57.36 ± 5.91	56.01 ± 6.12	0.823^{G}	
Fascicle length change (mm)				
ALL (p = 0.122)	20.56 ± 7.34	18.78 ± 4.97	0.681 M	1.78 ± 11.93
CYC	19.17 ± 7.21	17.07 ± 5.52	0.832^{G}	
TRI	21.76 ± 483	18.47 ± 4.15	0.120^{P}	
CON	20.88 ± 9.54	20.77 ± 4.82	0.712 M	
CE length change (mm)				
ALL (p = 0.146)	21.51 ± 8.00	19.77 ± 5.46	0.718 M	1.73 ± 12.45
CYC	19.69 ± 7.51	17.68 ± 5.78	0.838^{G}	
TRI	23.08 ± 5.21	19.70 ± 4.34	0.232^{P}	

				95% LoA ('ALL')
	REST1	REST2	$ICC_{3,1}$	REST1 – REST2
CON	21.90 ± 10.59	21.92 ± 5.71	0.775^{G}	
SEE length change (mm)				
ALL (p = 0.266)	37.14 ± 7.30	38.62 ± 5.46	0.647 M	-1.48 ± 13.80
CYC	40.11 ± 7.11	42.06 ± 5.91	0.751^{G}	
TRI	35.20 ± 4.89	38.87 ± 5.68	0.120^{P}	
CON	35.92 ± 8.88	35.84 ± 6.75	0.637 M	
M_p at 5° (N·m)				
ALL (p = 0.127)	8.23 ± 2.47	7.50 ± 3.32	0.794^{G}	0.72 ± 4.65
CYC††	6.87 ± 1.69	5.70 ± 2.06	0.677 M	
TRI	10.03 ± 2.62	9.64 ± 3.30	0.857^{G}	
CON	7.96 ± 2.17	7.38 ± 3.48	0.794^{G}	
M_p at maximum dorsiflexion (N·m)				
ALL (p = 0.077)	14.06 ± 3.53	12.45 ± 3.89	0.395^{P}	1.61 ± 8.85
CYC†	12.24 ± 2.96	10.36 ± 4.33	0.596 M	
TRI	15.50 ± 2.30	14.08 ± 3.81	0.672 M	
CON	14.59 ± 4.39	13.07 ± 4.68	0.206^{P}	
k_p at 5° (N·m/°)				
ALL (p = 0.800)	0.59 ± 0.23	0.58 ± 0.31	0.733 M	0.01 ± 0.50
CYC†	0.48 ± 0.17	0.49 ± 0.22	0.259^{P}	
TRI	0.75 ± 0.23	0.79 ± 0.39	0.706 M	
CON	0.56 ± 0.22	0.48 ± 0.23	0.787^{G}	
k_p at maximum dorsiflexion (N·m/°)				
ALL (p = 0.386)	0.89 ± 0.33	0.83 ± 0.34	0.531 M	0.07 ± 0.75
CYC††	0.67 ± 0.28	0.64 ± 0.29	0.575 M	
TRI	1.05 ± 0.21	1.00 ± 0.39	0.280^{P}	
CON	0.98 ± 0.38	0.86 ± 0.28	0.223^{P}	

Note: $ICC_{3,1}$ across sessions as well as 95% limits of agreement (bias ±random error) for each variable are also reported.
ALL = whole-group data. p-values next to 'ALL' are main effects of session. † and †† indicate group values were significantly different from TRI at the $p < 0.05$ and $p < 0.01$ level, respectively. For $ICC_{3,1}$ values, P, M, G and E indicate poor, moderate, good and excellent agreement, respectively. 95% LoA values are presented as bias ± random error.

Agreement between REST1 and REST2 for all participants was generally interpreted as moderate or good for all parameters except *Mp* at maximum dorsiflexion, which was poor (Table 2). Individual maximum dorsiflexion was generally the most repeatable variable ($ICC_{3,1}$ = 0.856, good). CYC showed the best repeatability here ($ICC_{3,1}$ = 0.992, excellent) whilst TRI showed the worst ($ICC_{3,1}$ = 0.701, moderate). TRI showed the lowest agreement between session for fascicle, CE and SEE length changes, as well as ankle joint ROM (poor to moderate; Table 2). Repeatability varied between groups for *Mp* and *kp* data, although they were generally higher at 5° dorsiflexion than at maximum dorsiflexion (Table 2).

Paired-sampled *t*-tests showed a difference between $REST_{mean}$ and STAND1 for ankle ROM (Table 2; $p < 0.001$), which was lower in STAND1 (Figure 3). MTU length change was also lower in STAND1 than $REST_{mean}$ ($p < 0.001$). There were no other differences between $REST_{mean}$ and STAND1 for MTU mechanical parameters (Table 2), although there was a tendency for length changes to be lower in STAND1, with *Mp* and *kp* being higher.

Table 2 Mean ± SD for ankle joint kinematic data, MTU, fascicle, CE and SEE length changes, and Mp and kp at both locations (5° dorsiflexion and individual maximum dorsiflexion) during the mean rested session (RESTmean) and after 20 min of quiet standing (STAND1)

	$REST_{mean}$	STAND1	Sig.	95% LoA $REST_{mean}$ – STAND1
Ankle ROM (°)	61 ± 6	57 ± 7	$p < 0.001$	3.93 ± 8.20
Individual maximum dorsiflexion (°)	13 ± 4	12 ± 5	$p = 0.076$	1.20 ± 6.85
MTU length change (mm)	57.75 ± 4.37	54.04 ± 6.71	$p < 0.001$	3.71 ± 8.10
Fascicle length change (mm)	19.67 ± 5.48	18.08 ± 6.74	$p = 0.147$	1.59 ± 11.25
CE length change (mm)	20.64 ± 6.07	19.19 ± 7.45	$p = 0.234$	1.45 ± 12.56
SEE length change (mm)	37.88 ± 5.95	35.43 ± 8.89	$p = 0.092$	2.45 ± 14.82
Mp at 5° (N·m)	7.86 ± 2.68	8.53 ± 2.58	$p = 0.130$	-0.67 ± 4.53
Mp at maximum dorsiflexion (N·m)	13.26 ± 2.95	13.62 ± 4.68	$p = 0.628$	-0.37 ± 7.90
kp at 5° (N·m/°)	0.58 ± 0.24	0.62 ± 0.26	$p = 0.210$	-0.04 ± 0.30
kp at maximum dorsiflexion (N·m/°)	0.86 ± 0.28	0.88 ± 0.35	$p = 0.647$	-0.02 ± 0.56

Note: 95% limits of agreement (bias ± random error) for each variable are also reported. *p*-values are from paired-samples *t*-test between RESTmean and STAND1. 95% LoA values are presented as bias ± random error.

4 DISCUSSION

The aims of this study were to quantify the repeatability of a dynamometry-based assessment for *kp* in athletic and non-athletic populations and to observe the impact of quiet standing on parameters obtained from *kp* assessments. As these tests have previously been shown to display adequate sensitivity to detect acute and chronic changes to the passive properties of a MTU,[2-4,6,7,15] we examined whether the same test could be used to illustrate the passive "neuromuscular state" (ie, neural and mechanical characteristics at the time of measurement) in the GM MTU, which would ideally be similar between sessions in studies with >1 testing session. Overall, inter-session repeatability varied between MTU mechanical parameters, with *Mp* calculated at maximum dorsiflexion showing the poorest repeatability, although repeatability was better when calculated at 5° dorsiflexion. Despite inter-session variation, *kp* assessments detected between-group differences, with TRI showing higher stiffness-related parameters than CYC, but not CON. Finally, quiet standing caused a reduction in ankle joint ROM (and thus GM MTU length change). However, this reduction in ROM was not attributable to any individual component of the MTU, such as the CE or SEE.

Repeatability values between rested sessions (REST1 and REST2) were generally good across the whole participant group, although some inter-group variation was seen for both ankle ROM and maximum dorsiflexion (Table 1), with TRI showing the lowest repeatability. This may be due to the differences in training modalities for TRI (cycling plus swimming and running) compared with CYC, especially as running exposes the triceps surae to higher-magnitude forces than cycling.[34,35] However, this is only a valid concern if participants exercised the day before testing,[36] which they were required not to do for 24 h before each testing session. It is possible that chronic exposure to running in TRI has led to changes in mechanical parameters such as aponeurosis compliance, which could have impacted ROM repeatability data here, although this is beyond the scope of this study. Nonetheless, it is not entirely clear how much of the observed inter-session variation is physiological and how much is due to variability in the measurement technique (ie, determination of joint ROM). Previous literature has claimed that ankle isokinetic dynamometry is highly reproducible,[37,38] although reproducibility data were limited to peak joint moments and angle of peak moment during maximal-effort contractions, with no data reported on ROM. An additional consideration here is the method used to determine ankle ROM. In the current study, ROM was determined by the operator slowly rotating the ankle joint, and the participant verbally indicating their ROM limits. Other studies have previously dorsiflexed the angle to "full volitional dorsiflexion" (ie, the position at which the participant could no longer tolerate the discomfort caused by dorsiflexion).[1] This is a subtle yet important distinction in methodology. Because of our study design, we are unable to make recommendations about which approach is more appropriate, but this factor could explain why maximum dorsiflexion was lower than in some previous studies and might have affected the consistency of ROM measurements. Another methodological consideration regarding the use of commercial dynamometers for range of motion assessment is the approach taken to minimize heel lift in the extreme dorsiflexed positions. Significant heel lift has been documented in the literature, especially for maximal voluntary plantarflexion contractions,[39] although this can be reduced with appropriate fixation of the foot to the dynamometer attachment.[21] Whilst our mode of testing (passive dorsiflexion) differed significantly from maximal dynamic contractions, we still controlled for heel lift by using the additional Velcro wraps round the foot and the dynamometer.

The length change of individual MTU components showed more variation than whole MTU length change between REST1 and REST2, although there was still no main effect of session on any length change parameter (Table 1). Greater variation in values such as fascicle length change would perhaps be expected given potential errors in its measurement. For instance, labelling of the initial fascicle considerably determines the absolute fascicle length analyzed over the course of a movement by the semi-automated tracking algorithm used in the current study.[22-24] However, as fascicle length change (from the start of the trial) was used instead of absolute fascicle length, the impact of this error has been somewhat mitigated. Nonetheless, other sources of error and assumptions in these length change estimations still exist, particularly the calculation of CE and SEE length changes.[29] For example, the tracking of fascicles *in vivo* using ultrasound is carried out in a two-dimensional plane, whereas fascicles lengthen and shorten in a three-dimensional manner,[40-43] inducing an error that might differ in magnitude between participants and between groups. This could, at least in part, explain some of the discrepancies in $ICC_{3,1}$ between groups for different length change parameters (Table 1).

Regarding the parameters quantifying the MTU passive resistance to stretch (Mp and kp), repeatability was higher at the common ankle angle (5° dorsiflexion) than at individual maximum dorsiflexion (Table 1; Figure 2). Given that maximum dorsiflexion was determined independently on both REST1 and REST2, repeatability of passive mechanical measurements will have been compounded by the joint angle it was measured at. Therefore, using a common ankle angle (such as 5° dorsiflexion) appears to be a more robust way of monitoring the "neuromuscular state" of a given participant. Furthermore, inter-session variation in *Mp* and *kp* appears to be somewhat random (Figure 2). That is, an increase in *Mp* does not necessarily mean an increase in *kp*. Repeatability for some of these parameters (eg, *Mp* at maximum dorsiflexion) appears to be slightly lower than previously reported.16 In addition, *Mp* values presented previously were somewhat higher than presented here, which could be explained by the greater maximum dorsiflexion angle achieved. As the groups with higher *Mp* and *kp* in the current study generally showed better repeatability between REST1 and REST2, the difference in agreement between studies could be explained by the assumption that inter-session variation is lower in stiffer MTUs. However, this would imply a heteroscedastic relationship between absolute differences and grand means, which does not appear to be the case (Figure 2). Nonetheless, further investigation is clearly warranted to explore the association between the mechanical stiffness of a tissue and the day-to-day variation of the measurement. Despite this, these measurements were still able to detect a main effect of group at both locations (Table 1). Post-hoc testing showed that TRI had higher *Mp* and *kp* than CYC ($p < 0.05$). This finding, apart from the primary observation that this test can detect between-group differences despite day-to-day variation, also indicates that the addition of running, as well as swimming, to an athlete's training program could lead to chronic mechanical changes in the triceps surae MTU.

The period of quiet standing in STAND1 caused a reduction in ankle joint ROM when compared with $REST_{mean}$ ($p < 0.001$; Table 2). The 20 min of quiet standing might have altered the passive mechanical properties of the triceps surae, either through a change in the viscoelastic response to stretch in the SEE or by an alteration to the muscle spindle sensitivity via reduced tendon reflex amplitude[10] or lower activity of large-diameter afferents.[9] Previous research has shown that quiet standing requires the ankle plantarflexors and dorsiflexors to be at least intermittently active,[11-13,44-46] which could lead to a change in spindle sensitivity and some neural or muscular alterations that did not occur in REST1 or REST2. This might be corroborated by the argument that quiet standing with a neutral ankle angle and fully extended knee joint causes a relative GM MTU length of ~1.09 (9% strain) based on the regression equations used in the current study.[28] Although this does not directly imply intermittent muscle activity, it does show that either the CE or SEE were stretched beyond slack length, which over time could lead to a change in neural and mechanical properties of the MTU.[5,10,14] Frictional forces within the joint capsule are also likely to contribute to *Mp* measured in a dynamometer, but were not experienced by the MTU. It is possible that these frictional forces were higher in STAND1, but this is somewhat speculative. Additionally, physiological factors such as blood pooling in the lower extremities have been observed after prolonged standing.[47] Although

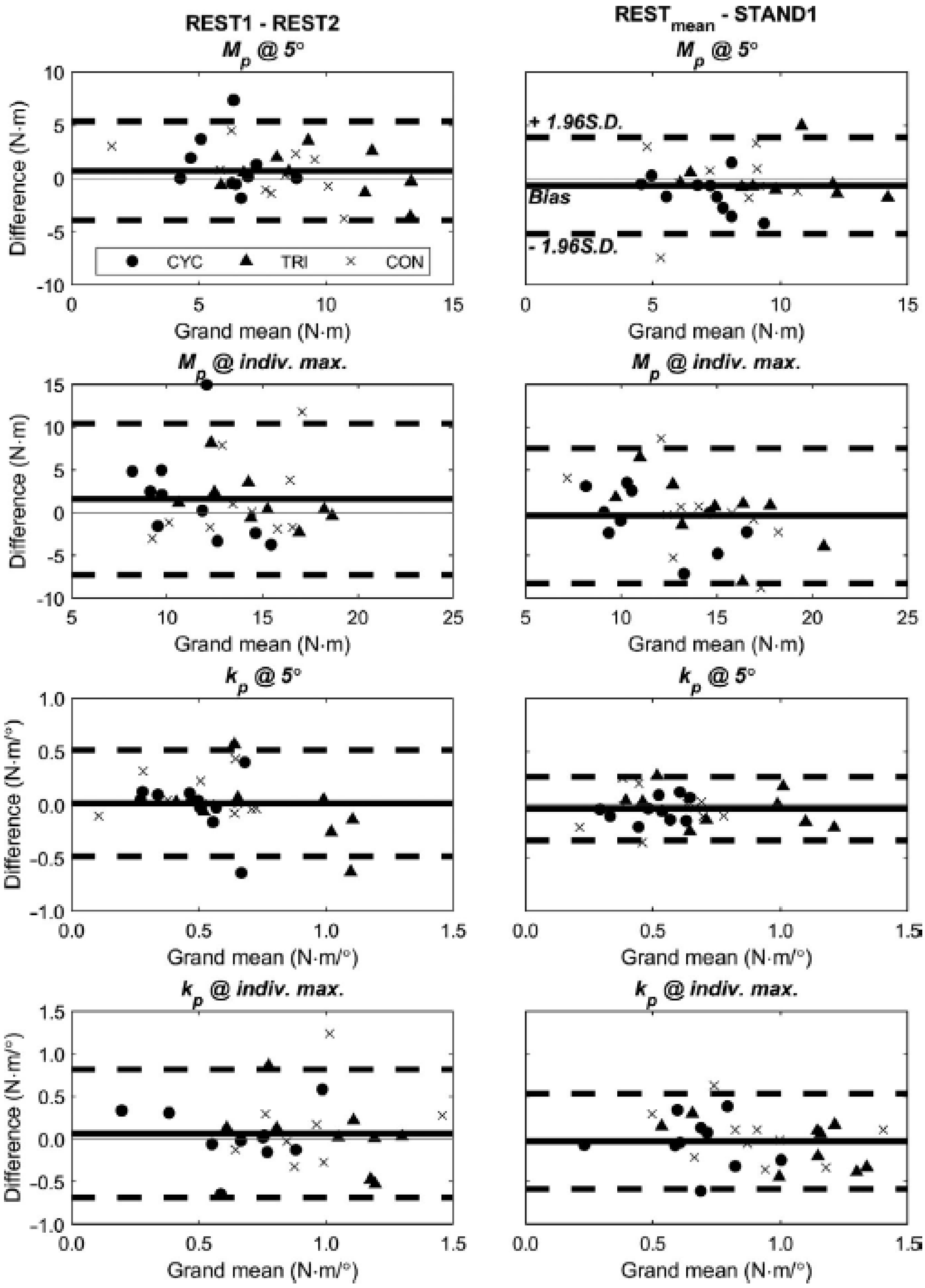

Figure 2 Bland-Altman plots for individual session comparisons (REST1 versus REST2: left-hand- side subplots; RESTmean versus STAND1: right-hand- Side subplots) for *Mp* (upper four subplots) and *kp* (lower four subplots). Black dots = individual CYC participants; black triangles = TRI; crosses = CON

unlikely in healthy and active populations, any blood accumulation could cause a reduction in the joint ROM through increased compression about the joint capsule. This would also likely impact maximum dorsiflexion that, although lower in STAND1, was not different from $REST_{mean}$ (p = 0.076; Table 2). Therefore, any effects of quiet standing might have altered MTU parameters in predominantly plantarflexed positions (Figure 3). This in turn could explain why *kp* measures were unaffected by the change in joint ROM, as they were quantified in dorsiflexed positions.

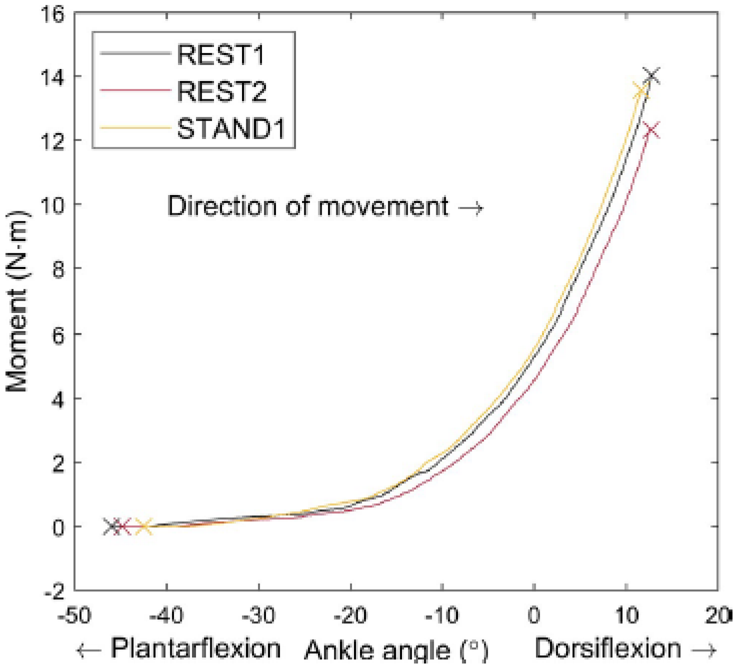

Figure 3 Group moment-angle curves for the three sessions (REST1 in black, REST2 in red, and STAND1 in gold). Crosses indicate maximum plantarflexion and dorsiflexion

The reduction in ankle joint ROM, and thus MTU length change, was not attributable to any individual MTU element (CE, SEE). Both CE and SEE length changes reduced equally (~6.5–7%), implying that quiet standing caused a reduction in stretch tolerance with an increase in stretch resistance at a given MTU length (termed "sensory theory"[15]). This is supported by the absence of any differences between $REST_{mean}$ and STAND1 for *Mp* or *kp* at either joint angle (Table 2), which agrees with research analyzing the effects of static stretching.[6,7,15] However, there was a substantial amount of variation within the groups for *Mp* and *kp* (Figure 2; Table 2), so it is possible that changes might have been found in a more homogenous sample (repeated measurements in a single participant, for example). It should also be acknowledged that the lack of differences observed for CE and SEE length change could be caused by methodological assumptions required to carry out these estimations. For example, the simple binary decoupling of SEE from whole MTU assumes uniform stiffness properties in both tendon and aponeurosis, which is almost certainly not the case. Additionally, the *kp* assessment carried out in the current study is technically not solely a test of MTU stiffness properties. Various hard-and soft-tissue restrictions about the ankle joint (eg, bones, ligaments, subcutaneous fat, and skin), as well as frictional forces present within the joint capsule,[5] might also influence the passive joint moment and joint ROM recorded by dynamometry, particularly at angles close to maximal dorsiflexion. Although these non-musculotendinous factors may differ between individuals, they would be unlikely to alter between sessions or be impacted by quiet standing. Nonetheless, future research might seek to isolate a MTU (probably *ex vivo*) and monitor the effects of intermittent activity and continuous stretch on passive mechanical properties. A limitation of the current methodology is the lack of control over involuntary and even voluntary muscle activity during the *kp* test itself. Indeed, previous evidence[3,48] suggests that some low-level activity in the ankle plantarflexors would be expected during passive dorsiflexion, although this is difficult to control for. Only by isolating the mechanical response to passive stretch from the neural response (for example, via the use of nerve blocks) could this be controlled, which is a potential avenue for future research. Therefore, the impact of this limitation on the current results is unknown and should be taken into consideration when interpretating findings.

5 PERSPECTIVE

The findings of this study show that there is some inter-session variation in the passive stiffness parameters of both athletic and non-athletic populations, even when participants were requested to refrain from strenuous exercise for 24 h before to testing. Therefore, associations or comparisons of MTU data across multiple sessions should be interpreted with some caution. However, despite this variation, variables derived from these assessments were still able to detect differences between populations who undergo different forms of repetitive loading, showing that the test for *kp* is sensitive enough to be used for studies of athlete or population profiling. Furthermore, just 20 min of quiet standing considerably impacted ankle joint ROM during passive dorsiflexion, although stiffness-related metrics (*Mp*, *kp*) appeared to be relatively unchanged. These findings should be considered in future studies that use *kp* as part of a muscle-tendon profiling battery or for studying responses to an intervention.

Conflict of Interest

The authors state they have no conflicts of interest to declare.

Data Availability Statement

Research data are not shared.

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