Reliability of myotonometry to measure gastrocnemii and Achilles tendon stiffness in unloaded and loaded conditions.

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received : 08 August 2023
accepted: 01 December 2023

ISSN: 2823-989X
DOI: 10.52057/erj.v4i1.42

ABSTRACT

Background: Clinically accessible and reproductible musculotendinous stiffness measurements are important for research and clinical monitoring of muscle-tendon injuries, in particular for plantar flexor muscles, including the gastrocnemii and the Achilles tendon, despite their crucial role in both walking and running. **Objectives**: To establish the reliability of stiffness measurements using the MyotonPro® and to determine clinically relevant points of measurement for the gastrocnemii muscle and Achilles tendon. **Methods**: Design: cross-sectional study. Stiffness was evaluated using the MyotonPro® device on both legs in 22 healthy individuals. Achilles tendon stiffness was measured at 4 points, and gastrocnemius stiffness at 6 points. Two conditions were assessed: at rest (lying) and under load (standing). All participants underwent 2 measurement sessions, one week apart, to evaluate intra- and inter- reliability. **Results**: Intraclass correlation coefficients demonstrated moderate to excellent reliability in lying, with coefficients ranging from 0.735 to 0.933 depending on the location. The standard error of measurement varied from 3.7% to 19.8% depending on the location, and the minimal detectable change ranged from 10.3% in the best condition to 54.5% in the worst. In standing, measurement reliability was poor to good for the Achilles tendon (ranging from 0.000 to 0.636) and excellent for the gastrocnemii (ranging between 0.902 and 0.986). **Conclusion**: Reliability of stiffness measurements of the ankle plantar flexor muscles using the MyotonPro® was good to excellent. Achilles tendon stiffness should be measured at least 6 cm above the distal insertion to the bone, both in lying and standing.

KEYWORDS: clinical monitoring, recommendation, reliability, reproducibility, stiffness

Introduction

Assessment of muscle and tendon stiffness can be useful to monitor pathological conditions (eg, orthopaedic disorders, stroke, etc) or for athletes seeking performance optimization [1]. Muscle and tendon stiffness can be defined as the relationship between the load applied to the tissue and the amount of deformation that occurs within it [2]. Specifically, musculotendinous tissue may be excessively or insufficiently stiff, that both may increase the risk of injury [3]. Shear wave elastography (SWE) can be used to quantify musculotendinous stiffness. This technique has been used in both healthy and pathological conditions and has demonstrated excellent reliability for the assessment of Achilles tendon stiffness [4]. It can also measure stiffness in muscles such as the infraspinatus, erector spinae, and gastrocnemii [5, 6]. However, the cost and the level of expertise required to use the SWE device hinder its adoption in the clinical setting [4]. For clinicians, trainers and athletes, devices for the quantification of musculotendinous stiffness must be accessible,

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user-friendly and quick to operate [7].
The MyotonPro© device is a handheld, non-invasive device that can record stiffness in human tissues, including muscles and tendons [2]. The MyotonPro© has been used in various conditions, both in the upper and lower limbs [8, 9]. It has been shown to be valid for measuring stiffness [2] and provides results similar to SWE [10]. Several studies have demonstrated good to excellent reliability and agreement in healthy subjects for the measurement of stiffness in both the medial and lateral gastrocnemii muscles [4, 11] as well as the Achilles tendon [8, 9, 12]. It has also shown good reliability in people with neurological disorders [12].
However, we found no studies of the reproducibility of measurements in the plantar flexor muscle complex in functional conditions, such as when standing. The assessment of stiffness is relevant in various clinical conditions. For instance, Morgan et al. (2016) found that individuals with tendinopathy exhibited reduced stiffness compared to healthy individuals in a standing position. Furthermore, neurological clinicians and researchers have emphasized the significance of reporting quantitative assessments of muscle stiffness rather than qualitative measurement to enhance the monitoring and treatment of muscle tone (eg spasticity) [13, 14, 15].
Therefore, the first aim of this study was to evaluate the reliability and agreement of measurements for the *triceps surae*, specifically the *medialis gastrocnemius* (MG), *lateralis gastrocnemius* (LG), and Achilles tendon (AT), in two standardized conditions: lying, i.e., unloaded and standing,i.e., loaded, in healthy individuals. The second aim was to determine the reliability and clinically relevant measurement site for the MG, LG and AT. We hypothesised that measurements taken in loaded would be less reliable than those taken unloaded.

Methods

This cross-sectional reproducibility study was conducted at the facilities of the University Catholic of Louvain (UCLouvain - Belgium) and received approval from our local ethical committee (CEHF Nº: B403201942384). The present study adhered to the ethical principles for medical research involving humans as outlined in the Declaration of Helsinki. To prevent gender bias, an equal number of women and men were selected. Each participant enrolled voluntarily and provided written, signed consent for participation. Participants did not receive any financial compensation.
To be included in the study, individuals were required to self-describe themselves as healthy, meaning that had not experienced any disease or musculoskeletal disorders in the last 6 months. Additionally, each participant had to be > 18 years old and without any neurological pathologies. We excluded people who had experienced any form of lower limb pain within the last 6 months.

Variables and material

The primary variable measured in this study was the passive stiffness of the triceps surae muscle, assessed using myotonometry. For this purpose, the MyotonPro© device was employed using standard settings. Specifically, the probe applied a pre-force of 0.18 N and released a single impulse of 0.4 N for a duration of 15 ms. Subsequently, the maximal acceleration (a_{max}) of the resulting free oscillations was recorded for 400 ms. This process was repeated 5 times, with a total estimated duration of 2.15 s. The stiffness (S), expressed in $N.m^{-1}$, was determined as the mean value of 5 acceleration measurements calculated as follows: $S\ (N.m^{-1}) = a_{max}\ .m\ .\ l^{-1}$, where a_{max} is the maximal acceleration of the free oscillation of the tested tissue, m is the mass of the probe and l is the displacement [2].

Procedure

After enrolling in this study, participants provided general information including age, height, medical history, limb dominance, and their usual physical activities. An investigator measured their weight, lower limb length, and ankle dorsiflexion range of motion (ROM). Ankle dorsiflexion ROM was assessed using the weight bearing lunge test [16], and both legs were systematically tested.
Ten marks were drawn on each leg. Four of them were positioned at 2 (AT2), 4 (AT4), 6 (AT6), and 8 (AT8) cm above the distal Achilles tendon insertion on the calcaneus. Three additional marks were drawn on each gastrocnemius. The marks on the MG were positioned at 15%, 30%, and 45% of the distance between the medial epicondyle and the lateral malleolus, measured using a flexible ruler. Similarly, the marks on the LG were placed at 15%, 30%, and 45% of the distance between the head of the fibula and the centre of the posterior aspect of the calcaneus. All marks were consistently positioned by the same investigator (B) (Fig 1).

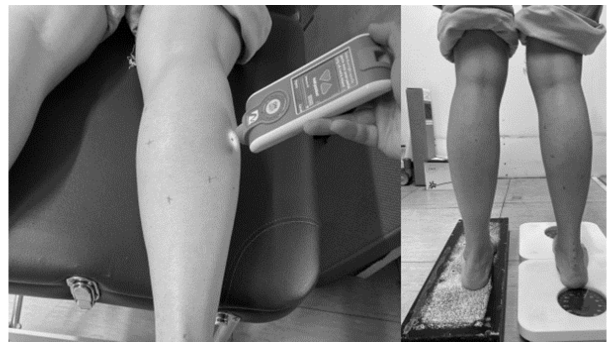

Figure 1 Location of markers and measurements using the Myoton-pro® device in lying and standing conditions.

Two measurement positions were recorded. The first position involved assessing stiffness in lying. The participant lay on their belly with a cushion placed beneath their ankles, and their feet in a spontaneous resting position without specific instructions. The second position involved assessing stiffness under mechanical stress. To apply external stress, the standing position was used. To standardize this position, 4 weighing scales were placed under the participant's feet - 2 under the right foot and 2 under the left foot. Under each foot, the scales were positioned equally along a longitudinal plane. At all times, each scale should indicate one-quarter of the total weight of the participant.
The measurements were taken at rest and under stress by 2 novice investigators, JL and AF, during a single session. Each measurement session followed this order: the first rater (Rater A), the second rater (Rater B) and then, the first rater (Rater A). Participants were asked to leave the marks in place for the second measurement session, which was scheduled one week later. The second session was scheduled at a similar time as the first, either early or late morning, or early or late afternoon. For the second session, Rater A took a single measurement.

Statistical analysis

Statistical analysis was performed using Medcalc software. Reliability was assessed using the intraclass coefficient (ICC), which included within-session intra-operator (ICC 2.1), inter-operator reliability (ICC 3.1), and between-session inter-operator reliability (2.1). Following the recommendations of Koo et al. [17] an ICC below 0.5 was considered as poor, between 0.5 and 0.75 was considered as moderate, between 0.75 and 0.9 was considered good, and above 0.9 was considered as excellent. Agreement was estimated by evaluating the standard error of measurement (SEM) and the minimal detectable change (MDC).The SEM calculated as followed: $SEM = SD.\sqrt{1-ICC}$ with the SD representing the pooled standard deviation and the ICC of the corresponding analysis. The MDC was calculated as follows: $MDC = 1.96\ .\sqrt{2}\ .\ SEM$. The significance level was set at $\alpha = 0.05$.

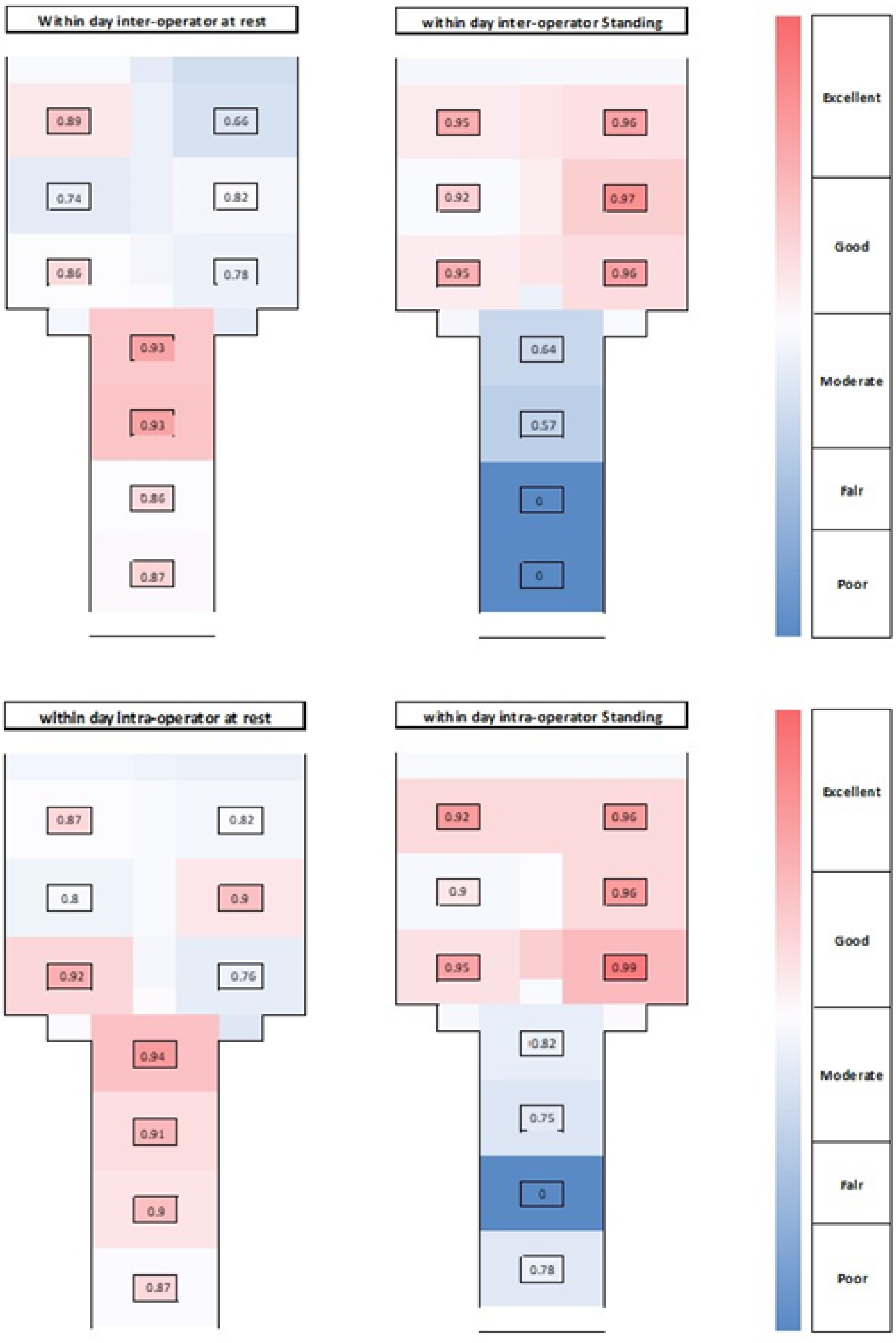

Figure 2 Heat map of within day reliability measurement.

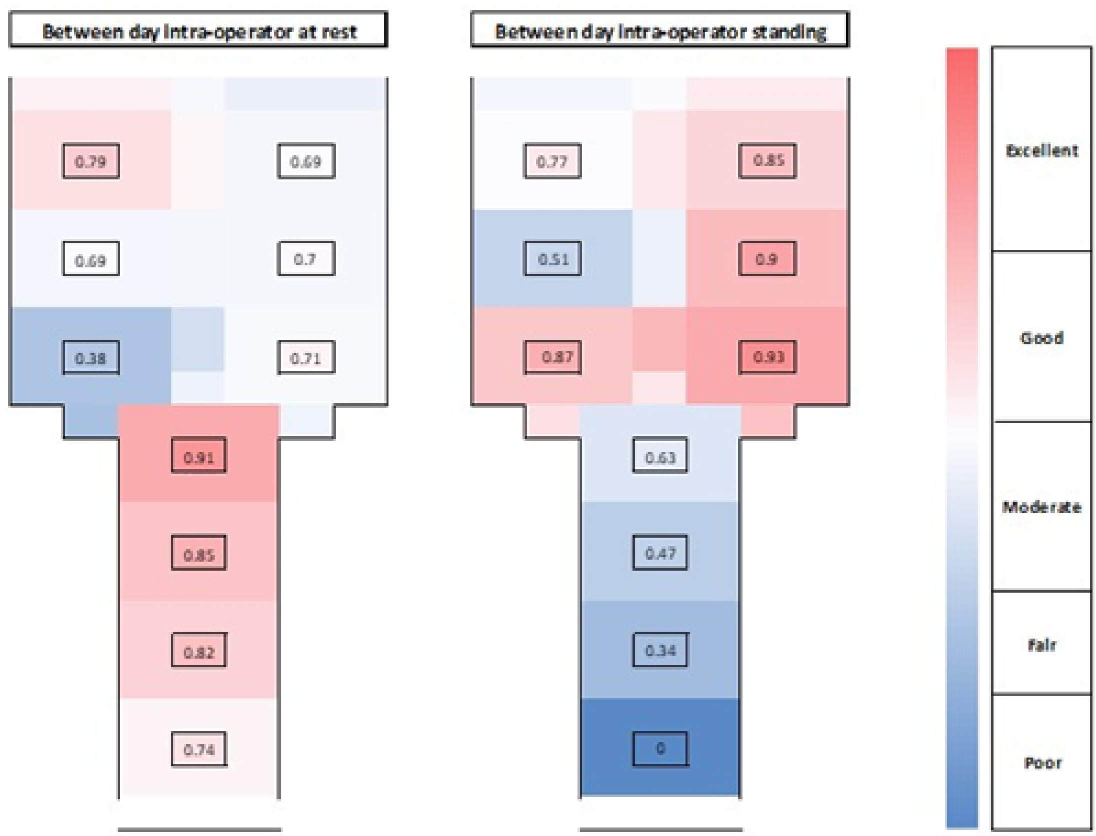

Figure 3 Heat map of between day reliability measurement.

Table 1 Within day inter-rater reproducibility

		OP A (N/m)	OP B (N/m)	ICC	95%CI	SEM (N/m)	SEM (%)	MDC (N/m)	MDC (%)
Lying	AT2	878.9	861.1	0.866	0.793 to 0.913	85.1	9.8	235.9	27.1
	AT4	817.2	799.4	0.859	0.781 to 0.908	79.8	9.9	221.1	27.4
	AT6	753.8	752.4	0.933	0.896 to 0.956	53.4	7.1	148.1	19.7
	AT8	677.5	674.2	0.927	0.887 to 0.952	50.7	7.5	140.5	20.8
	MG-15	254.2	243.8	0.886	0.787 to 0.938	16.5	6.6	45.8	18.4
	MG 30	280.5	265.1	0.735	0.506 to 0.857	17.8	6.5	49.3	18.1
	MG +15	343.9	330.1	0.861	0.741 to 0.925	24.8	7.4	68.9	20.4
	LG-15	288.3	273.9	0.661	0.364 to 0.819	40.2	14.3	111.5	39.7
	LG 30	284.3	275.2	0.820	0.664 to 0.903	17.3	6.2	47.9	17.1
	LG +15	315.3	314.3	0.789	0.606 to 0.886	24.4	7.7	67.5	21.4
Standing	AT2	1121.1	1115.5	0.000	-0.231 to 0.641	217.7	19.5	603.4	54.0
	AT4	1133.1	1089.0	0.000	-0.423 to 0.595	189.7	17.1	525.8	47.3
	AT6	993.8	997.3	0.570	0.200 to 0.768	111.3	11.2	308.4	31.0
	AT8	887.9	883.6	0.636	0.323 to 0.804	91.5	10.3	253.7	28.6
	MG-15	389.0	373.8	0.947	0.900 to 0.971	33.9	8.9	94.1	24.7
	MG 30	440.4	432.1	0.920	0.851 to 0.957	24.8	5.7	68.9	15.8
	MG +15	525.3	523.6	0.946	0.899 to 0.970	34.8	6.6	96.5	18.4
	LG -15	438.5	422.6	0.956	0.918 to 0.976	30.7	7.1	85.0	19.7
	LG 30	447.2	448.8	0.971	0.946 to 0.984	23.8	5.3	65.9	14.7
	LG +15	498.2	507.0	0.959	0.924 to 0.978	30.3	6.0	84.0	16.7

OP A: first operator, OP B: second operator. Standard error of measurement (SEM) is presented in both absolute (N/m) and relative (%) values. Minimal detectable change (MDC) is expressed in both absolute (N/m) and relative (%) values. The rows represent the different measurement locations including AT (Achilles tendon) measurements at 2 (AT2)-4 (AT4)-6 (AT6) -8 (AT8) cm above the distal insertion of the Achilles tendon. *Medialis Gastrocnemius* (MG) and *lateralis gastrocnemius* (LG) measurements at 3 points: 15% above, 30% (centered), and 15% below the distance between the knee and the ankle.

Table 2 Within day test-retest reproducibility

		OP A - 1st (N/m)	OP A - 2nd (N/m)	ICC	95%CI	SEM (N/m)	SEM (%)	MDC (N/m)	MDC (%)
Lying	AT2	878.9	846.7	0.867	0.793 to 0.913	83.5	9.7	231.5	26.8
	AT4	817.2	803.1	0.899	0.844 to 0.934	67.7	8.4	187.6	23.2
	AT6	753.8	742.7	0.907	0.857 to 0.939	58.6	7.8	162.4	21.7
	AT8	677.5	679.5	0.943	0.911 to 0.962	44.0	6.5	122.0	18.0
	MG-15	254.2	245.8	0.869	0.755 to 0.9230	17.1	6.8	47.3	18.9
	MG 30	280.5	274.3	0.804	0.638 to 0.894	14.3	5.1	39.6	14.3
	MG +15	343.9	339.0	0.916	0.844 to 0.955	18.4	5.4	51.0	14.9
	LG-15	288.3	285.1	0.824	0.672 to 0.905	19.7	6.9	54.7	19.1
	LG 30	284.3	279.4	0.897	0.809 to 0.945	14.3	5.1	39.6	14.0
	LG +15	315.3	324.2	0.756	0.550 to 0.869	27.5	8.6	76.2	23.8
Standing	AT2	1121.1	1060.6	0.776	0.584 to 0.880	107.1	9.8	296.9	27.2
	AT4	1133.1	1101.8	0.000	-0.197 to 0.654	190.8	17.1	528.8	47.3
	AT6	993.8	989.2	0.751	0.538 to 0.866	83.7	8.4	232.1	23.4
	AT8	887.9	880.4	0.818	0.661 to 0.902	61.3	6.9	169.9	19.2
	MG-15	389.0	382.1	0.961	0.928 to 0.978	29.6	7.7	82.0	21.3
	MG 30	440.4	424.5	0.902	0.816 to 0.948	29.1	6.7	80.7	18.7
	MG +15	525.3	516.5	0.954	0.914 to 0.975	32.0	6.1	88.7	17.0
	LG -15	438.5	423.5	0.961	0.927 to 0.979	29.0	6.7	80.4	18.6
	LG 30	447.2	437.4	0.961	0.928 to 0.979	28.0	6.3	77.7	17.6
	LG +15	498.2	492.2	0.986	0.973 to 0.992	18.3	3.7	50.8	10.3

OP A: first operator, OP B: second operator. Standard error of measurement (SEM) is presented in both absolute (N/m) and relative (%) values. Minimal detectable change (MDC) is expressed in both absolute (N/m) and relative (%) values. The rows represent the different measurement locations including AT (Achile tendon) measurements at 2-4-6-8 cm above the distal insertion of the Achilles tendon. *Medialis gastrocnemius* (GM) and *lateralis gastrocnemius* (LG) measurements at 3 points: 15% above, 30% (centered), and 15% below the distance between the knee and the ankle.

Table 3 Between day intra-rater reproducibility

		OP A - 1st (N/m)	OP A - 3rd (N/m)	ICC	95%CI	SEM (N/m)	SEM (%)	MDC (N/m)	MDC (%)
Lying	AT2	842.7	908.3	0.744	0.628 to 0.822	105.5	12.0	292.4	33.4
	AT4	811.3	849.3	0.818	0.738 to 0.873	85.6	10.3	237.2	28.6
	AT6	755.6	767.0	0.850	0.784 to 0.895	71.9	9.4	199.4	26.2
	AT8	683.5	689.8	0.907	0.866 to 0.935	53.6	7.8	148.5	21.6
	MG-15	250.5	249.5	0.790	0.483 to 0.915	22.9	9.2	63.5	25.4
	MG 30	280.1	274.4	0.689	0.244 to 0.874	19.1	6.9	53.0	19.1
	MG +15	344.6	337.9	0.376	-0.565 to 0.752	51.3	15.0	142.1	41.6
	LG -15	285.6	287.9	0.690	0.212 to 0.877	22.2	7.7	61.5	21.5
	LG 30	280.0	283.8	0.696	0.213 to 0.880	23.7	8.4	65.7	23.3
	LG +15	311.5	328.9	0.707	0.288 to 0.882	29.1	9.1	80.6	25.2
Standing	AT2	1173.9	1011.6	0.000	-1.091 to 0.268	215.0	19.7	596.0	54.5
	AT4	1126.4	1120.8	0.343	-0.255 to 0.655	156.5	13.9	433.7	38.6
	AT6	1027.2	964.4	0.465	0.0271 to 0.711	123.8	12.4	343.0	34.4
	AT8	896.1	869.8	0.630	0.311 to 0.803	86.3	9.8	239.1	27.1
	MG-15	373.4	399.0	0.774	0.441 to 0.909	76.5	19.8	212.2	54.9
	MG 30	425.4	440.3	0.509	-0.220 to 0.804	67.3	15.6	186.6	43.1
	MG +15	520.2	521.7	0.874	0.678 to 0.950	54.7	10.5	151.6	29.1
	LG -15	415.6	448.0	0.847	0.620 to 0.939	59.6	13.8	165.2	38.3
	LG 30	430.9	454.8	0.896	0.737 to 0.958	46.7	10.6	129.5	29.3
	LG +15	476.9	515.4	0.932	0.789 to 0.975	39.5	8.0	109.4	22.0

OP A: first operator, OP B: second operator. Standard error of measurement (SEM) is presented in both absolute (N/m) and relative (%) values. Minimal detectable change (MDC) is expressed in both absolute (N/m) and relative (%) values. The rows represent the different measurement locations including AT (Achile tendon) measurements at 2-4-6-8 cm above the distal insertion of the Achilles tendon. *Medialis Gastrocnemius* (MG) and *lateralis gastrocnemius* (LG) measurements at 3 points: 15% above, 30% (centered), and 15% below the distance between the knee and the ankle.

Results

Participants

A total of 22 individuals (11 females, 11 males) were included in the study. The mean (SD) age of the sample was 21.6 (1.78) years, average height was 1.74 (0.11) m and an average weight was 70.1 (11.9) kg. Participants engaged in various physical activities, including running, cycling, swimming, general strengthening, dancing, track and field, basketball, and football, with a mean 2.52 (1.17) sessions per week. Each session lasted approximately 1 hour.

Measurement points for the Achilles tendon demonstrated good to excellent inter-rater reliability in lying but poor to moderate inter-rater reliability in standing. In contrast, measurements for gastrocnemii demonstrated moderate to good inter-rater reliability in lying but excellent inter-rater reliability in standing. (Table 1) The agreement measurements for the SEM and the MDC in lying ranged from 6.5% to 14.3% and from 17.1% up to 39.7% respectively. In the standing position, the SEM and MDC ranged from 5.3% to 19.5% and from 14.7% to 54%.

Intra-rater reliability was good to excellent for the Achilles tendon and the gastrocnemii in lying and in standing, except for AT4, which demonstrated poor test-retest reliability in standing (Table 2). The SEM for the Achilles tendon ranged from 6.5% to 9.7% in lying and from 6.9% to 17.1% in standing. Regarding the measurement of the gastrocnemii in both lying and standing, the SEM ranged from 5.1% to 8.6% and from 3.7% to 7.7%, respectively. The MDC for both the Achilles tendon and gastrocnemii ranged from 14.9% to 47.3%.

Between-day reliability ranged from poor to excellent for both lying and standing (Table 3). In lying, reliability for the Achilles tendon was moderate at AT2 (ICC= 0.744) but excellent at AT8 (ICC=0.907), suggesting better reliability when measuring stiffness further away from the calcaneus. Conversely, reliability was poor for the AT in standing, with moderate reliability for the AT8 location (ICC=0.630).

Reliability was poor to moderate for the gastrocnemii in lying. However, in standing, reliability was moderate to excellent, with ICC values ranging from 0.509 to 0.932. (Table 3). Agreement measurements ranged from 6.9% up to 54.9% depending on the location and conditions assessed.

Discussion

This study is the first to assess the reproducibility of myotonometry measurements of stiffness of the gastrocnemii and the Achilles tendon across multiple measurement points and in unloaded and loaded conditions. Our results suggest that measurements of Achilles tendon stiffness using the MyotonPro© device should be performed at 6 cm or 8 cm above the distal insertion point, as this point had the highest reliability in both lying and standing. Reliability for the Achilles tendon was higher in lying than standing and for the gastrocnemii was higher in standing than lying. It is interesting to note that the stiffness of the Achilles tendon should be assessed away from the distal insertion point. Indeed, the proximity of the bone to the AT2 and AT4 sites could lead to increased stiffness values, which could negatively impact reliability. The proximity to the insertion point could introduce noise in the measurement, for example because of rebound, resulting in higher acceleration values and consequently artificially increased stiffness values and imprecision. The lack of between-day reproducibility leads us to suggest the MyotonPro© should not be used near the bone insertion point to monitor tendon stiffness in standing. Chang et al. [10] reported similar results with an MDC of approximately 177 N/m (18%) at the Achilles tendon bone insertion and 59 N/m (5%) at 6 cm above the insertion point. In contrast, Tas et al. [9] reported a good ICC of 0.83 and 0.9 for Achilles tendon stiffness (AT2) at 0º and 10º of dorsiflexion, respectively, in a population of young healthy males. Although these findings are consistent with our results, the load applied on the Achilles tendon in their study may not be considered clinically relevant. This is because passive stretching of the plantar flexor muscles primarily induces strain on the contractile tissue rather than on the tendon itself [18]. Moreover, ankle dorsiflexion range of motion is not correlated with tendon stiffness [13]. To induce sufficient load on a tendon, the involvement of the contractile tissue is essential.

In contrast, we found that the measurement reliability for contractile tissue such as the gastrocnemii was higher in standing than lying. This is consistent with the findings of many studies that have reported good to excellent reliability for the gastrocnemii [4, 6, 11]. Furthermore, we report that the measurement during muscle contraction is more reliable than assessing the loaded tendon in standing. Kelly et al [6] demonstrated that this was also true for different loading conditions, with an ICC of 0.97 at 40% of maximal voluntary contraction and 0.98 at 80%. Similarly, Albin et al. [11] demonstrated excellent reliability of the *medialis gastrocnemius* measurement in both resting and contracted conditions. Although their contraction did not mimic weight-bearing conditions, it is reasonable to assume that contractile tissue reliability is maintained under loaded conditions.

Our results suggest that the assessment of plantar flexor muscle stiffness is reliable in healthy subjects. Finnamore et al. [14] demonstrated that MyotonPro© measurements are reliable in people with Achilles tendinopathy. They selected the most painful area and the thickest part of the tendon, which corresponds to our AT8 site. They reported an ICC of 0.8, a SEM of 43 N/m, and an MDC of 122 N/m, which is equivalent to a 17% MDC according to the findings of our study. Although the results of Finnamore et al. did not exceed the MDC, they were above the SEM. This is crucial in clinical practice as the T8 site has been shown to be the most reproducible site of measurement for the Achilles tendon. In the case of Achilles tendinopathy, the difference in stiffness between lying and standing may be small [15]. For example, Morgan et al. (2019) reported lower stiffness measured with myotonometry in a group with Achilles tendinopathy compared to healthy controls [19]. However, those studies did not rely on reliability standards to estimate differences between people with tendinopathy and healthy controls. The present study fills the gap in the literature, making comparisons and rehabilitation recommendations potentially accessible.

The MyotonPro© could provide reliable quantitative measurements for monitoring the effects of Achilles tendon rehabilitation and offer clinicians improved monitoring possibilities for therapeutic trials. In the context of prevention, the MyotonPro© could offer valuable information about the Achilles tendon for sport stakeholders and provide recommendations for athletes who may be risk of injury. It is worth nothing that the Achilles tendon stiffness measurement is more reliable in lying, whereas the gastrocnemii measurements are more reliable in standing.

The sample studied here consisted of young individuals. It is well known that myofascial tissue quality can change with age [20]. Therefore, caution should be exercised when interpreting agreement measurement values, as they may not be applicable to older individuals or those with pathological conditions. Additionally, we used bony landmarks as reference points for the measurements. Although this approach could enhance the reproducibility of the protocol, it may also yield non-relevant points of measurement in the gastrocnemii depending on the subject's anatomy. Although our results align with the available literature, these factors could explain some discrepancies.

Finally, the standardization of the standing position is somewhat approximate. To our knowledge, only one other study assessed Achilles tendon stiffness in a standing position. Schneebeli et al. [21] described the reproducibility of the MyotonPro© in standing using a Wii balance board, which allowed control over the centre of mass position. They reported an intra-rater reliability of 0.87, an inter-rater reliability of 0.56 and an inter-session reliability of 0.75. These results are remarkably similar to ours. Although the Wii balance board can provide real-time information about the centre of mass, the techniques used in the present study are easier to employ and record.

In summary, we found good to excellent reliability of stiffness measurements of the ankle plantar flexor muscles using the MyotonPro©. Achilles tendon stiffness should be measured at least 6 cm above the distal insertion to the bone, both in lying and standing. The gastrocnemii stiffness measurements were excellent in both lying and standing.

Statement and declaration

Authors' contribution

The authors confirm contribution to the paper as follows:

- APN : Initial concept; Experimentation; Writing
- JV : Planning of recruitment and testing steps; Experimentation; Writing
- AF : Planning of recruitment and testing steps; Experimentation; Writing
- PM : Initial concept; Writing; Project supervision
- CD : Initial concept; Writing; Project supervision

Acknowledgments

The authors would like to express the gratitude to all the voluntary participants, as well as Prof. Paul Fisette and Dr. Simon Hinnekens for their valuable contributions to the development of this project.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

None.

Disclosure statement

All authors declare they have neither financial nor non-financial interests.

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