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Intra- and inter-session reliability of the MyotonPro in measuring the biomechanical and viscoelastic properties of the knee extensor and flexor muscles

Erhan Secer[1*], Kazım Bayram[2] and Derya Ozer Kaya[3,4]

Abstract

Background The MyotonPro is a widely used, safe, and non-invasive digital device for assessing muscle biomechanical and viscoelastic properties. This study aimed to determine the intra- and inter-session reliability of the MyotonPro in measuring the biomechanical and viscoelastic properties of the knee extensor and flexor muscles.

Methods Eighty-four young male recreational athletes, with a mean age of 21.42 ± 1.37 years, participated in a four-stage repeated-measure study. Both sessions followed the same protocol: rectus femoris (RF) and biceps femoris (BF) measurements were taken twice per side and averaged, with a 30-min interval between sets. Two different intraclass correlation coefficient (ICC) models were used: $ICC_{3,1}$ (two-way mixed effects, absolute agreement) for intra-session reliability and $ICC_{2,1}$ (two-way random effects, absolute agreement) for inter-session reliability. Standard error of measurement (SEM) was calculated using $SEM = SD_{pooled} \times \sqrt{(1\text{-ICC})}$. The minimal detectable change (MDC_{95}) was calculated using the formula $MDC_{95} = 1.96 \times \sqrt{2} \times SEM$. Bland–Altman plots were generated to visually represent the agreement between the two sessions, complementing the ICC analysis.

Results The MyotonPro demonstrated high to excellent intra-session reliability across all measured properties (tone, stiffness, elasticity, relaxation time, and creep) for both the RF (ICCs ≥ .95, ranging from .95 to .99; SEM: 0.01 to 6.14; MDC_{95}: 0.04 to 17.03) and the BF (ICCs ≥ .97, ranging from .97 to .99; SEM: 0.02 to 5.27; MDC_{95}: 0.05 to 14.62). The MyotonPro demonstrated excellent inter-session reliability across all measured properties for both the RF (ICCs ≥ .96, ranging from .96 to .99; SEM: 0.02 to 4.38; MDC_{95}: 0.05 to 12.14) and the BF (ICCs ≥ .94, ranging from .94 to .99; SEM: 0.02 to 5.17; MDC_{95}: 0.07 to 14.54). The Bland–Altman analysis confirmed near agreement for all measured properties across sessions 1 and 2 for both the RF and BF. The limits of agreement encompassed most data points, and the overall systematic mean bias was negligible, remaining close to zero.

Conclusions This study establishes the MyotonPro as a highly reliable instrument for assessing muscle properties, evidenced by excellent intra-session and inter-session consistency across all measured biomechanical and viscoelastic

*Correspondence:
Erhan Secer
erhnscr86@hotmail.com

Full list of author information is available at the end of the article

properties. The negligible systematic bias indicates high temporal measurement stability, supporting the potential utility of myotonometry for monitoring changes in muscle mechanical properties in longitudinal assessments.

Keywords Hamstring muscle, Quadriceps muscle, Muscle tone, Reliability, Stiffness, Elasticity

Background

Accurate measurement of muscle biomechanical and viscoelastic properties is essential for transitioning from theoretical models to practical applications, such as monitoring tissue health, tracking training adaptations, and personalizing intervention protocols [1]. These properties directly determine skeletal muscle functional characteristics, including force production capacity and contractile velocity [2]. Quantifying these traits holds direct and profound clinical and performance implications, providing essential metrics for maintaining long-term athletic health, mitigating injury risk, optimizing athletic performance, and accelerating muscle recovery [3]. Given that these mechanical traits govern both musculoskeletal health and athletic potential, there is an urgent need for robust and reliable measurement techniques to ensure objective clinical and performance assessments.

The biomechanical and viscoelastic profile of muscle tissue is quantified by five key parameters: tone, stiffness, elasticity, mechanical stress relaxation time, and creep [4]. These metrics reflect distinct physiological aspects, ranging from the neurogenic basal tension maintained by the central nervous system [5, 6] to structural resistance and collagen-dependent recovery dynamics [2, 7]. Furthermore, viscoelastic behavior is captured through stress relaxation time and creep, which quantify the tissue's response to persistent mechanical loading [8, 9]. Because these properties are fundamentally interdependent, relying on isolated measurements fails to capture the complex interplay of muscle behavior. Consequently, a simultaneous assessment is clinically essential to provide a holistic view of tissue health and neuromuscular integrity, offering a comprehensive clinical profile that significantly exceeds the limitations of single-parameter analysis [2].

The assessment of muscle biomechanical and viscoelastic properties utilizes techniques classified by invasiveness and data output, including advanced methods like Shear Wave Elastography (SWE), Magnetic Resonance Elastography (MRE), Doppler Laser Vibrometry, and shear wave propagation accelerometry [1, 10]. While informative, the widespread use of these modalities is restricted by significant limitations. SWE suffers from high operator dependency and reduced accuracy in anisotropic tissues, requiring precise probe alignment [11]. MRE, despite offering superior depth, is prohibitively expensive and logistically complex, limiting clinical accessibility [12]. Surface-based methods (vibrometry, accelerometry) face signal attenuation, which obscures data from deeper muscle layers [13]. Furthermore, property calculations often rely on oversimplified mass-spring models that fail to capture the complex, non-linear biological behavior [13]. Researchers must therefore carefully weigh the trade-offs regarding cost, portability, and depth of penetration to characterize the muscle's mechanical state accurately.

The MyotonPro (Myoton AS, Tallinn, Estonia) is a prominent, non-invasive technology for the comprehensive characterization of muscle biomechanical and viscoelastic properties, providing detailed insights into muscular mechanical behavior and functional implications [2]. The device operates by applying a brief (15 ms) and low-intensity (0.58 N) mechanical impulse to the skin surface over the muscle and recording the subsequent oscillatory tissue response [14]. Proprietary internal software analyzes the resulting acceleration curve to quantify key properties: stiffness, tone, elasticity, mechanical stress relaxation time, and creep [15]. It is important to interpret parameters such as 'mechanical relaxation time' and 'creep' not as isolated biological constants, but as quantitative indices derived from the tissue's damped oscillation response. In this context, 'creep' serves as a proxy for the time-dependent mechanical reconfiguration of the tissue's viscoelastic components, specifically the interplay between collagen fibers and the extracellular matrix, following the mechanical impulse. While these metrics are calculated via proprietary algorithms, they provide a standardized, reproducible assessment of the muscle's passive mechanical state in vivo [16], reflecting its inherent viscoelastic integrity and recovery capacity. Therefore, these parameters are utilized herein as holistic markers of viscoelastic behavior, offering meaningful insights into the tissue-level mechanical dynamics rather than representing absolute, independent biological variables.

The MyotonPro has demonstrated robust construct and concurrent validity in assessing both muscular and tendinous tissues, showing strong alignment with established clinical measures [17, 18]. Furthermore, recent investigations have provided rigorous empirical support for the device's fidelity, particularly concerning the assessment of stiffness. Shan et al. substantiated that the MyotonPro provides a highly reproducible and consistent biomechanical profile, confirming its capacity to accurately capture tendon stiffness in both in vivo and in situ conditions based on the fundamental physics of damped oscillation [16]. By effectively distinguishing

these genuine mechanical states from stochastic measurement noise, the technology ensures that stiffness and tone measurements represent reliable physiological tissue behavior. However, while stiffness is a well-validated construct, derived viscoelastic indices such as creep and relaxation time should be interpreted with caution. The extent to which these specific parameters reflect distinct biomechanical constructs in living human skeletal muscle remains less clear, necessitating further validation compared to the more established measures of tissue stiffness.

While MyotonPro, similar to SWE, is influenced by the anisotropic nature of skeletal muscle, the two modalities differ fundamentally in their biomechanical underpinnings. Unlike SWE, which estimates tissue elasticity by measuring the propagation velocity of shear waves, MyotonPro quantifies the acceleration of the tissue's response to a mechanical impulse, reflecting dynamic viscoelastic properties such as muscle tone and stiffness rather than Young's modulus [19]. Comparative research indicates that while both methods are inherently sensitive to factors such as probe orientation, compression force, and precise measurement site, they yield distinct data profiles; specifically, MyotonPro provides a locally-focused assessment of mechanical behavior that complements the elasticity mapping of SWE [19]. Furthermore, it is essential to acknowledge that these modalities may not be directly interchangeable for all muscle groups; recent evidence suggests that MyotonPro and SWE readings for the Rectus Femoris may lack comparability due to the interference of subcutaneous adipose tissue, which significantly influences the impulse-response characteristics of the MyotonPro [20]. The device's widespread adoption stems from significant advantages over more cumbersome methods, specifically its simplicity of operation, affordability, portability across variable conditions, and ease of measurement. These attributes position the MyotonPro as a highly efficient tool for serial, objective assessment in both clinical and research settings [21]. Building on these technical capabilities, the MyotonPro provides a valuable approach for assessing the rectus femoris (RF) and biceps femoris (BF), muscles that play significant roles in knee stability and injury dynamics. Utilizing this objective measurement method for these muscle groups helps to broaden our quantitative understanding of their mechanical health and functional contributions to the lower kinetic chain.

The RF and BF are central to lower limb function, making them critical targets for establishing performance-sensitive metrics that govern joint stability, optimize force production, and predict injury susceptibility [22, 23]. The RF, a bi-articular quadriceps component, is essential for hip flexion and knee extension during dynamic activities [24]. Its biomechanical properties serve as sensitive indicators of neuromuscular efficiency, and its superficial location facilitates accurate, non-invasive assessment [25]. The BF, a major bi-articular hamstring muscle, is vital for hip extension, knee flexion, deceleration, and postural stability. Given its high eccentric demands and frequent injury incidence, particularly in sprinting, the BF's viscoelastic and stiffness properties are key indicators for assessing injury risk [26]. Methodologically, the RF and BF serve as the dominant agonist–antagonist pair acting on the knee joint, enabling a comparative and holistic assessment of muscle health, performance capacity, and differential pathology, thereby providing a robust basis for longitudinal reliability studies.

A systematic synthesis of the existing literature confirms that while the characterization potential of both the RF and BF properties is substantial, the reliability landscape remains incomplete [19, 23, 27–38]. Specifically, previous research consistently highlights stiffness as the most robust and reproducible metric across diverse populations [intraclass correlation coefficient (ICC) ≥ 0.90] [23, 27, 28, 30]. However, the reliability of other critical properties shows variability; tone and elasticity reliability significantly suffer in patient cohorts (e.g., elasticity ICC drops to 0.68 in spinal cord injury patients), suggesting these parameters may be susceptible to variations in tissue properties associated with pathological conditions, which requires confirmation using standardized device protocols [31, 33, 34]. Crucially, the literature presents a major methodological gap regarding the viscoelastic properties (mechanical relaxation time and creep). Although high intra-rater reliability is reported for these properties in clinical settings (e.g., ICC ≥ 0.96 in spinal cord injury), their inter-rater reliability remains largely unestablished in most populations, and comprehensive inter-session data is scarce [34, 37, 38].

Current literature regarding MyotonPro reliability in athletic cohorts appears limited regarding the availability of simultaneous, high-resolution data for all five biomechanical and viscoelastic properties, particularly concerning inter-session stability. This methodological gap may complicate the clinical ability to distinguish between genuine physiological adaptations and measurement error. By establishing this methodological bedrock, this study aims to provide clinicians and researchers with evidence that may help clarify this distinction, thereby supporting the use of the MyotonPro for objective, longitudinal clinical monitoring in high-performance populations. Accordingly, this study was designed to examine the intra- and inter-session reliability of the MyotonPro in assessing the biomechanical and viscoelastic properties of the knee extensor and flexor muscles. It was hypothesized that stiffness would demonstrate consistently high reliability across sessions. Furthermore, it was anticipated that the reliability of viscoelastic properties

(mechanical relaxation time and creep) would indicate acceptable levels of stability, addressing current uncertainties regarding their consistency across different sessions. This comprehensive approach may facilitate the establishment of normative data, potentially providing a more robust foundation for the utilization of the MyotonPro in research and clinical practice.

Methods

Experimental approach to the problem

A four-stage repeated-measures study was systematically executed to assess the intra-session and inter-session reliability of the MyotonPro device. The primary objective was to quantify the consistency of measurements obtained for the biomechanical and viscoelastic properties of the knee extensor and knee flexor muscles across both the dominant (DE) and non-dominant extremities (NDE). The testing protocol spanned two consecutive days (between 2 and 5 pm), designated as Session 1 and Session 2. Within each of these two sessions, the complete measurement protocol was performed twice (Measurement Set 1 and Measurement Set 2). A fixed 30-min rest interval was strictly enforced between Measurement Set 1 and Measurement Set 2 to introduce a controlled time lag, which was crucial for the intra-session reliability assessment [39]. The selection of measurement intervals was guided by established protocols in recent myotonometric literature. A 30-min intra-session interval was implemented to ensure the stabilization of tissue viscoelastic properties and to mitigate any lingering effects of prior mechanical impulses. This interval is supported by recent evidence demonstrating its efficacy in achieving measurement independence [14, 39, 40]. Regarding inter-session reliability, a 24-h interval was adopted to assess test–retest stability while minimizing the confounding effects of day-to-day biological variability, such as circadian rhythms and activity levels. This interval is consistent with widely accepted reliability procedures in the field, as evidenced by studies utilizing similar protocols for longitudinal clinical monitoring [14, 34, 41]. During the 30-min rest interval between intra-session measurements, participants remained seated in a controlled environment. They were closely monitored by the researchers to ensure that no physical activities potentially affecting muscle tone, such as stretching or prolonged standing, were performed. For every individual measurement set (e.g., Set 1 in Session 1), the assessment of each muscle (extensor and flexor) on each side (DE and NDE) involved two immediate repetitions conducted without interruption. The mean value derived from these two repetitions was subsequently calculated and utilized as the representative data point for that specific measurement set. This structured approach, involving both immediate repetitions and a controlled 30-min interval within the sessions, alongside the testing on consecutive days, allowed for a robust determination of measurement consistency both within a single day and across different days. A schematic representation of the experimental protocol is presented in Fig. 1.

Ethical considerations

The study protocol received approval from the Manisa Celal Bayar University Faculty of Medicine Health Sciences Ethics Committee (Approval Number: 20.478.486/3465, Decision Date: 15.10.2025). All participants were thoroughly informed about the study procedures and experimental protocols. All subjects signed an informed consent form before enrollment. The treatment of human research subjects complied with the ethical principles outlined in the Declaration of Helsinki.

Subjects

To ensure methodological rigor and sufficient statistical power, an a priori power analysis was conducted using G*Power software (version 3.1.9.7) based on the framework for ICC recommended by Koo and Li [42]. Setting the null hypothesis (H_0) at a minimum acceptable ICC of 0.50 ('moderate' reliability) and the alternative hypothesis (H_1) at an expected ICC of 0.80 ('good' reliability), with an alpha level of 0.05 and a stringent power of 0.90, the analysis indicated a minimum requirement of 38 participants. The recruitment process initially involved 89 young male recreational athletes. Following the application of exclusion criteria, specifically a history of time-loss injuries ($n = 3$), major sports injuries ($n = 1$), and lower extremity pathology ($n = 1$), the final analysis was conducted with 84 athletes (mean age = 21.42 ± 1.37 years). Eligibility was defined by the American College of Sports Medicine's guidelines for regular physical activity and the absence of any previous medical conditions that could compromise physical performance [43]. While a convenience sampling method was employed due to the voluntary nature of recruitment, the final study progression and exclusion process are detailed in Fig. 2. Our final sample size of 84 athletes is more than double the a priori requirement, thereby maximizing the statistical power and ensuring high precision and stability in the observed ICC estimates. Notably, this sample size is substantially larger than those reported in established MyotonPro reliability studies, which typically range from 20 to 52 participants [19, 23, 28, 29], thereby strengthening the generalizability and robustness of our findings.

Procedures

All myotonometric measurements were performed under controlled ambient conditions, specifically at a consistent room temperature of 22°C and a relative humidity of 50% [1]. Before the procedure, subjects rested for 24 h

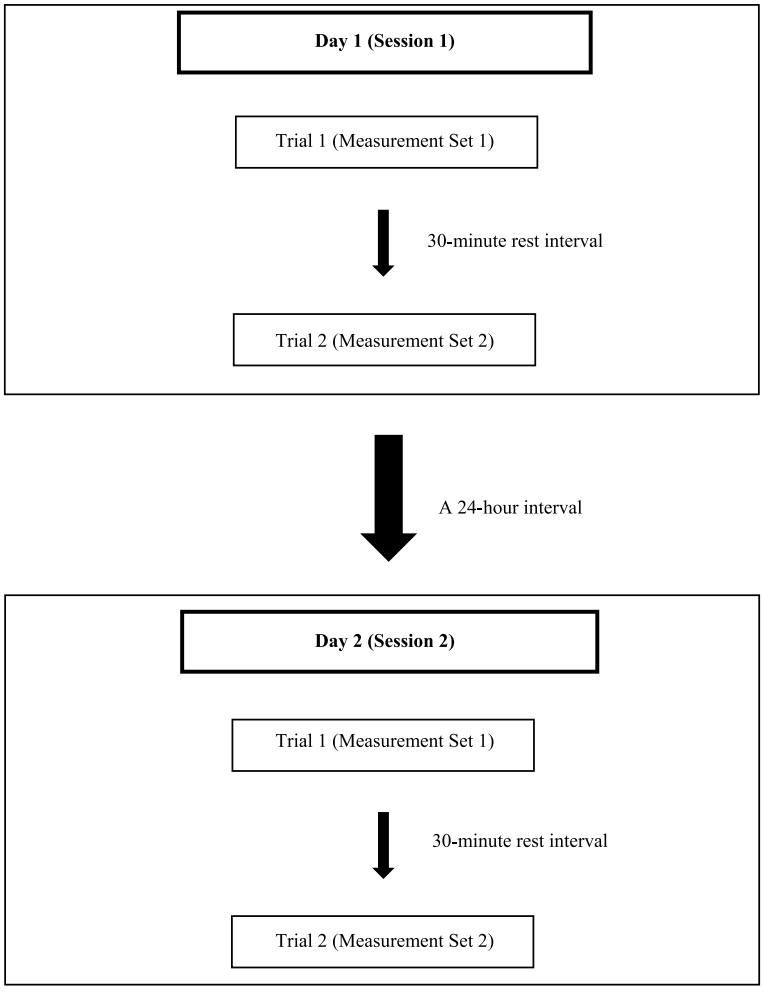

Fig. 1 A schematic representation of the experimental protocol

or refrained from vigorous physical activity. Each measurement at a site involved three successive immersions of the myotonPro probe head, ensuring it was perpendicular to the muscle surface; any deviation triggered an automatic error alert from the device, requiring a repeat measurement. To standardize the measurement location for the RF, a line was first drawn between the anterior superior iliac spine and the medial patellar border, with the measurement point set at the midpoint. For the RF measurement, the subject was placed in a supine position with a 20 cm diameter roller under the knees [44]. (Fig. 3). For the BF measurement, the subject was positioned prone, with an oval pillow under the ankles to facilitate hamstring relaxation, and was instructed to remain still throughout the procedure [45]. Measurements for the BF were systematically taken at the midpoint of the long head's longitudinal axis to ensure consistency across subjects [46] (Fig. 3). Once identified, the 3-mm measurement sites were marked using an indelible marker. These marks were maintained throughout the study period to ensure that the MyotonPro probe was applied to the identical anatomical location on both assessment days. After a 5-min acclimatization period with subjects remaining still, the measurements were performed. All measurements were conducted by the same physiotherapist, who had over ten years of experience in manual therapy. Once the anatomical sites were marked, all procedures were strictly followed according to the equipment manufacturer's methodology and guidelines [4]. To reduce technical artifacts, real-time guidance features integrated into the MyotonPro device were used to detect unwanted motion, muscle contractions, and excessive compression force. Additionally, the evaluator kept their arm steady by resting their elbow on a level surface. According to the manufacturer's standard procedures, any measurement set with a coefficient of variation over 3% was considered unreliable [4, 47]. Such data sets were immediately discarded and re-measured to maintain the accuracy and reliability of the final myotonometric results.

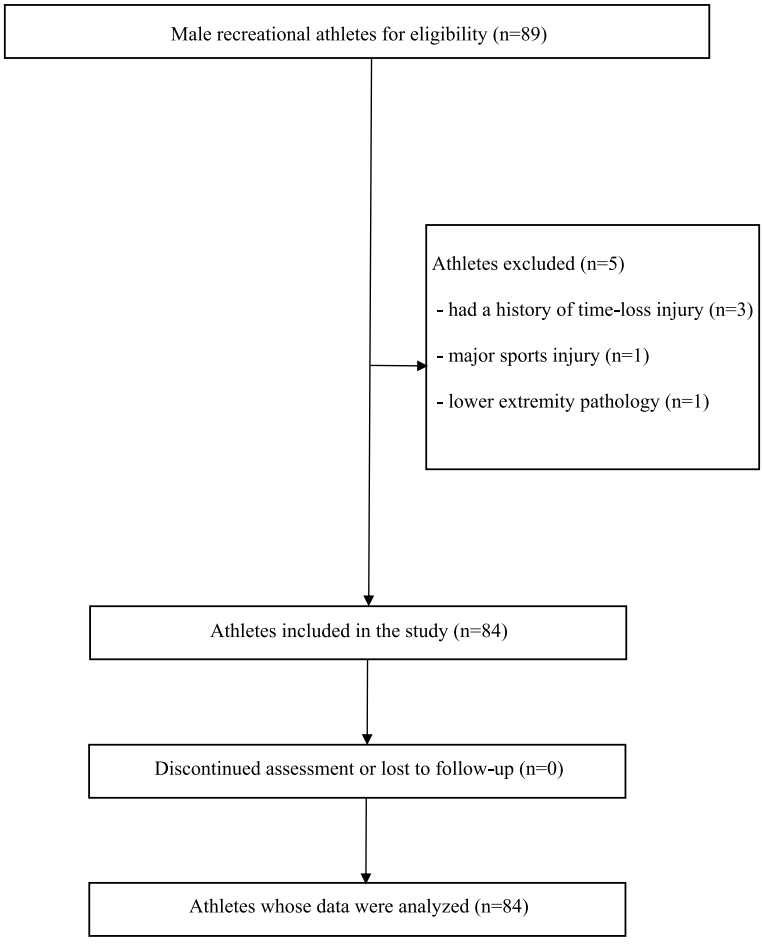

Fig. 2 Flowchart of the athletes

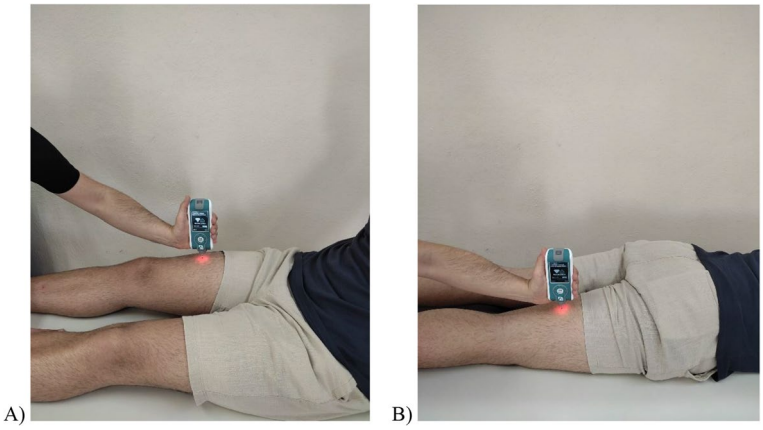

Fig. 3 Measurements of the biomechanical and viscoelastic properties of the RF (**A**) and BF (**B**)

Measurement equipment

Measurements were performed using the MyotonPro (Myoton AS, Tallinn, Estonia). The MyotonPro is a widely used, safe, and non-invasive digital device for quantitatively assessing muscle biomechanics and viscoelastic properties [2]. Structurally, the device comprises a main body and a 3-mm diameter depth probe. The measurement sequence initiates with the probe applying a consistent initial pressure of 0.18 N to the tissue surface. This static force is immediately followed by the release of a focused mechanical pulse (0.4 N delivered over 15 ms), which imparts a dynamic shear force to the underlying soft biological tissue, causing rapid deformation [1]. The device then accurately records the resulting

damped natural vibrations of the tissue in the form of an acceleration signal. Crucially, this signal is simultaneously analyzed to calculate parameters indicative of the stress state alongside the specific biomechanical and viscoelastic properties of the muscle. The resulting units of measurement are derived using established logarithmic formulas [37]. In the standardized data collection protocol, the probe is applied only once to the designated tissue measurement site; however, it executes the tissue deformation pulse three times in succession to ensure robust and reliable data acquisition.

Measurement parameters

The mechanical state of the target muscles was characterized using the MyotonPro, a device that assesses soft tissue properties based on the principles of damped oscillation. The reliability and measurement consistency of this technology in quantifying muscle properties have been extensively documented in recent systematic literature reviews [2]. It is critical to note that the parameters derived by the device are quantitative indices calculated from the tissue's acceleration response to a brief (15 ms), perpendicular mechanical impulse following the manufacturer's standard protocol; thus, they are interpreted here as biomechanical markers of the tissue's passive state rather than absolute physiological constants. The experimental protocol involved the assessment of five key variables: Tone (Hz), which serves as an indicator of the muscle's inherent tension, representing the natural frequency of the tissue oscillation following the mechanical impulse; an elevation in this value is interpreted as a marker of increased muscle tension or passive stiffness [45]. Stiffness (N/m) quantifies the muscle's transverse resistance to deformation; unlike subjective interpretations of "hardness," this parameter represents the tissue's mechanical response to a defined perpendicular impulse, reflecting the structural integrity and passive resistance of the muscle–tendon unit. Elasticity, quantified via the logarithmic decrement of the natural oscillation, reflects the rate of energy dissipation during the damped oscillation of the tissue; rather than a direct, absolute measure of elasticity, it functions as a viscoelastic index where higher values signify greater energy loss during oscillation, potentially associated with diminished viscoelastic efficiency. Relaxation time (ms) is calculated as the time required for the tissue to return to its equilibrium state following the deformation; this parameter is a computational index reflecting the tissue's damping capacity, interpreted as a marker of the time-dependent relaxation behavior of the tissue, where prolonged values are discussed in the context of altered viscosity. Finally, creep is derived as a proxy for the time-dependent mechanical reconfiguration of the tissue's viscoelastic components, calculated as the ratio of the deformation time to the relaxation time; it is utilized as a holistic indicator of the tissue's viscoelastic consistency, reflecting the interplay between the collagenous matrix and ground substance. All interpretations of these parameters are framed within the theoretical framework of the damped oscillation model, and while these indices provide standardized, reproducible data for serial assessment, their translation into absolute biological properties is interpreted with caution, acknowledging that they serve as plausible markers of viscoelastic behavior rather than direct, isolated measurements of specific biological components [2, 45].

Statistical analysis

Descriptive statistics are presented as mean and standard deviation (SD) for continuous variables and as number (percentage) for categorical variables. The Shapiro–Wilk test was applied to confirm the normality of the data. The ICC with a 95% confidence interval (95% CI) was calculated to assess reliability. To ensure methodological transparency and rigor, this study was reported in strict adherence to the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) [48]. We utilized the following ICC models: The $ICC_{3,1}$ (two-way mixed effects, absolute agreement, single measures) model was used to determine intra-session reliability. This model was selected because the rater in this study is considered a fixed effect, representing the specific experimental condition. For inter-session reliability, the $ICC_{2,1}$ (two-way random effects, absolute agreement, single measures) model was employed. This choice is justified as it treats both subjects and time points/sessions as random effects, allowing for the generalization of our reliability findings to other similar clinical settings and raters. Importantly, we consistently applied the "absolute agreement" definition rather than "consistency" for both intra- and inter-session analyses. This decision was driven by the clinical emphasis on the interchangeability of MyotonPro measurements; absolute agreement is a more stringent criterion that accounts for systematic errors, ensuring that the device provides consistent absolute values essential for longitudinal clinical monitoring. ICC values were interpreted on the following scale: below 0.50 (poor reliability), 0.50 to 0.75 (moderate), 0.75 to 0.90 (good), and above 0.90 (excellent) [42]. Standard error of measurement (SEM) was calculated using the formula $SEM = SD_{pooled} \times \sqrt{(1\text{-ICC})}$, where SD_{pooled} represents the pooled SD of trial 1 and trial 2 for intra-session reliability, and session 1 and session 2 for inter-session reliability. While it is recognized that this formula represents a simplification compared to calculations based directly on ANOVA mean-square error terms, it was applied consistently across both the consistency $ICC_{3,1}$ and absolute agreement $ICC_{2,1}$ models to ensure a uniform approach

for all comparisons. This method allows for a standardized interpretation of measurement precision across the different reliability models used in this study. The minimal detectable change (MDC_{95}) was calculated using the formula $MDC_{95} = 1.96 \times \sqrt{2} \times SEM$. The significance level (α) for all statistical tests was set at $p < 0.05$. Additionally, Bland–Altman plots were generated to visually represent the agreement between the two sessions, complementing the ICC analysis [49]. All calculations were performed using IBM SPSS Statistics Standard Concurrent User Version 26 (IBM, Armonk, New York, USA).

Results

The data in Table 1 outline the demographic and sporting characteristics of the subjects, who include 84 athletes. All characteristics are reported as the mean ± SD.

Regarding intra-session reliability, the MyotonPro device demonstrated high stability across all biomechanical and viscoelastic properties for both the knee extensor and flexor muscle groups. ICC values for all properties ranged from 0.95 to 0.99, which are classified as 'excellent' according to established reliability scales. This measurement stability was accompanied by low SEM and MDC_{95} values and consistent mean scores across all trials. Detailed numerical distributions and specific reliability indices for each muscle and property are systematically presented in Table 2.

The inter-session reliability of the MyotonPro device demonstrated high consistency across all properties evaluated between sessions 1 and 2 for both the knee extensor and flexor muscle groups. For the RF, ICC values for all properties ranged from 0.96 to 0.99, while the BF showed ICC values between 0.94 and 0.99, both of which are classified as 'excellent' reliability. Precision indices, including SEM and MDC_{95}, remained low for both muscle groups, indicating measurement stability over time. Detailed inter-session reliability indices, including specific values for all properties, are systematically presented in Table 3.

Table 1 Demographic and sporting characteristics of the athletes

Characteristics	Total ($n = 84$)
Age, (year)	21.42 ± 1.37
Height, (cm)	174.76 ± 8.52
Weight, (kg)	68.49 ± 11.84
BMI, (kg/m^2)	22.36 ± 2.97
Primary sport	n (%)
Football	28 (33.3)
Basketball	24 (28.6)
Volleyball	19 (22.6)
Handball	13 (15.5)

Abbreviation: *BMI* Body mass index. Data are expressed as mean ± standard deviation (SD) for continuous variables and as numbers (percentages) for categorical variables

Lastly, the Bland–Altman plots demonstrated favorable agreement for all measured properties across sessions 1 and 2 for both DE and NDE RF and BF. Systematic biases remained near zero for all variables, particularly for viscoelastic properties, and no significant proportional bias was observed. The Limits of Agreement (LoA) encompassed the vast majority of data points, suggesting high inter-session stability. Detailed systematic bias values and LoA ranges for tone, stiffness, elasticity, relaxation time, and creep are illustrated in Figs. 4 and 5. While these results confirm high reliability within this specific cohort, they reflect consistency within this experimental context and do not necessarily imply clinical validity.

Discussion

This study aimed to evaluate the intra- and inter-session reliability of the MyotonPro in measuring the biomechanical and viscoelastic properties of the knee extensor and flexor muscles under controlled, standardized conditions. The findings suggest that the MyotonPro demonstrated favorable test–retest reliability for assessing these specific muscle properties within the scope of this experimental setup. While our results demonstrate consistent measurements in repeated assessments, it is important to clarify that these findings are focused on point-in-time reliability. Further investigations are necessary to determine the device's long-term evaluative capacity and its practical feasibility across broader clinical settings.

The reliability of five key MyotonPro-derived properties (tone, stiffness, elasticity, relaxation time, and creep) has been examined in previous literature [19, 23, 27–30, 33, 34, 36–38]. Existing data suggest that measurement reproducibility may vary depending on the specific parameter and the patient cohort investigated. Tone and stiffness measurements appear to demonstrate consistent reproducibility; tone ICCs generally range from 0.89–0.96 (intra-rater) and 0.68– ≥ 0.81 (inter-rater), while stiffness measurements often indicate favorable intra-rater (0.91–0.96) and inter-rater (0.90–0.93) reliability. Conversely, the reliability of viscoelastic properties (elasticity, relaxation time, and creep) appears to be more contingent upon the study population. Intra-rater elasticity variations have been observed (ICC range: 0.68–0.99), whereas relaxation and creep parameters generally indicate high intra-rater consistency (ICC: 0.98–0.99). However, the scarcity of inter-rater data, particularly regarding relaxation time, warrants a cautious approach to drawing definitive conclusions. Collectively, while these properties may offer favorable reproducibility under controlled conditions, their evaluative potential appears to remain dependent on study design and patient profile, underscoring the need for cautious interpretation of these biomechanical markers. The reliability of MyotonPro-derived biomechanical and viscoelastic properties of the

Table 2 Intra-session reliability of the MyotonPro

Session-1						
Muscles	**Properties**	**Trial-1 (mean ± SD)**	**Trial-2 (mean ± SD)**	**ICC (95% CI)**	**SEM**	**MDC_{95}**
Knee extensor muscle						
DE Rectus Femoris	Tone, (Hz)	12.03 ± 1.00	12.02 ± 0.94	.96 (.94-.97)	0.19	0.54
	Stiffness, (N/m)	195.25 ± 43.61	196.38 ± 43.21	.98 (.97-.99)	6.14	17.03
	Elasticity	1.16 ± 0.21	1.17 ± 0.21	.97 (.96-.98)	0.04	0.12
	Relaxation time, (ms)	23.81 ± 2.66	23.86 ± 2.54	.98 (.97-.99)	0.37	1.02
	Creep	1.40 ± 0.15	1.41 ± 0.15	.95 (.93-.97)	0.03	0.09
NDE Rectus Femoris	Tone, (Hz)	11.70 ± 1.50	11.79 ± 0.99	.97 (.95-.98)	0.22	0.61
	Stiffness, (N/m)	184.95 ± 40.13	186.05 ± 40.62	.99 (.98-.99)	4.04	11.19
	Elasticity	1.13 ± 0.17	1.13 ± 0.18	.97 (.95-.98)	0.03	0.08
	Relaxation time, (ms)	24.12 ± 2.43	24.16 ± 2.51	.98 (.97-.99)	0.35	0.97
	Creep	1.44 ± 0.14	1.44 ± 0.15	.98 (.97-.99)	0.02	0.05
Knee flexor muscle						
DE Biceps Femoris	Tone, (Hz)	13.03 ± 1.16	12.99 ± 1.14	.98 (.97-.99)	0.16	0.45
	Stiffness, (N/m)	226.91 ± 43.48	226.92 ± 51.71	.99 (.98-.99)	4.78	13.24
	Elasticity	1.11 ± 0.20	1.11 ± 0.21	.98 (.97-.99)	0.02	0.08
	Relaxation time, (ms)	21.38 ± 2.90	21.37 ± 2.86	.98 (.97-.99)	0.41	1.13
	Creep	1.28 ± 0.16	1.29 ± 0.17	.97 (.96-.98)	0.02	0.07
NDE Biceps Femoris	Tone, (Hz)	12.93 ± 1.04	12.93 ± 1.02	.97 (.96-.98)	0.18	0.49
	Stiffness, (N/m)	221.77 ± 52.44	221.80 ± 53.00	.99 (.98-.99)	5.27	14.62
	Elasticity	1.11 ± 0.21	1.12 ± 0.21	.98 (.97-.99)	0.03	0.08
	Relaxation time, (ms)	21.48 ± 2.99	21.55 ± 2.95	.99 (.98-.99)	0.30	0.82
	Creep	1.29 ± 0.16	1.29 ± 0.19	.98 (.97-.99)	0.02	0.06
Session-2						
Muscles	**Properties**	**Trial-1 (mean ± SD)**	**Trial-2 (mean ± SD)**	**ICC (95% CI)**	**SEM**	**MDC_{95}**
Knee extensor muscle						
DE Rectus Femoris	Tone, (Hz)	12.04 ± 0.91	12.08 ± 0.96	.96 (.94-.98)	0.19	0.52
	Stiffness, (N/m)	195.76 ± 44.15	195.95 ± 44.73	.99 (.98-.99)	4.44	12.32
	Elasticity	1.16 ± 0.22	1.16 ± 0.21	.97 (.96-.98)	0.03	0.10
	Relaxation time, (ms)	23.74 ± 2.49	23.71 ± 2.48	.97 (.96-.98)	0.43	1.19
	Creep	1.39 ± 0.14	1.39 ± 0.15	.99 (.98-.99)	0.01	0.04
NDE Rectus Femoris	Tone, (Hz)	11.97 ± 0.94	11.91 ± 0.98	.97 (.96-.98)	0.17	0.46
	Stiffness, (N/m)	185.86 ± 41.25	185.32 ± 41.61	.99 (.98-.99)	4.14	11.49
	Elasticity	1.12 ± 0.18	1.14 ± 0.18	.95 (.94-.96)	0.04	0.11
	Relaxation time, (ms)	23.96 ± 2.41	24.08 ± 2.42	.98 (.97-.99)	0.34	0.95
	Creep	1.43 ± 0.14	1.43 ± 0.15	.97 (.96-.98)	0.02	0.07
Knee flexor muscle						
DE Biceps Femoris	Tone, (Hz)	12.89 ± 1.75	13.02 ± 1.20	.98 (.97-.99)	0.21	0.59
	Stiffness, (N/m)	227.28 ± 51.29	227.00 ± 51.01	.99 (.98-.99)	5.12	14.18
	Elasticity	1.10 ± 0.24	1.11 ± 0.20	.98 (.97-.99)	0.03	0.08
	Relaxation time, (ms)	21.21 ± 3.64	21.37 ± 2.77	.98 (.97-.99)	0.46	1.27
	Creep	1.28 ± 0.16	1.28 ± 0.15	.97 (.96-.98)	0.02	0.07
NDE Biceps Femoris	Tone, (Hz)	12.96 ± 1.02	12.99 ± 1.04	.97 (.96-.98)	0.18	0.49
	Stiffness, (N/m)	222.42 ± 52.42	222.61 ± 52.36	.99 (.98-.99)	5.24	14.52
	Elasticity	1.11 ± 0.21	1.12 ± 0.21	.98 (.97-.99)	0.03	0.08
	Relaxation time, (ms)	21.46 ± 3.01	21.50 ± 2.91	.99 (.98-.99)	0.51	1.42
	Creep	1.28 ± 0.15	1.28 ± 0.14	.98 (.97-.99)	0.02	0.05

The intra-session SEM was calculated using the pooled SD of trial 1 and trial 2, reflecting the measurement precision within a single testing session

DE Dominant extremity, *NDE* Non-dominant extremity, *ICC* intraclass correlation coefficient, *95% CI* 95% confidence interval, MDC_{95} Minimal detectable change at the 95% confidence level, *SD* Standard deviation, *SEM* Standard error of measurement

Table 3 Inter-session reliability of the MyotonPro

Muscles	Properties	Session-1 (mean ± SD)	Session-2 (mean ± SD)	ICC (95% CI)	SEM	MDC_{95}
Knee extensor muscle						
DE Rectus Femoris	Tone, (Hz)	12.02 ± 0.95	12.06 ± 0.92	.96 (.94-.98)	0.19	0.52
	Stiffness, (N/m)	195.81 ± 43.24	195.85 ± 44.31	.99 (.98-.99)	4.38	12.14
	Elasticity	1.16 ± 0.21	1.16 ± 0.22	.98 (.97-.99)	0.03	0.08
	Relaxation time, (ms)	23.83 ± 2.57	23.72 ± 2.45	.97 (.96-.98)	0.44	1.21
	Creep	1.41 ± 0.15	1.39 ± 0.14	.96 (.94-.98)	0.03	0.08
NDE Rectus Femoris	Tone, (Hz)	11.74 ± 1.11	11.94 ± 0.95	.96 (.94-.97)	0.21	0.57
	Stiffness, (N/m)	185.50 ± 40.22	185.59 ± 41.27	.99 (.98-.99)	4.07	11.30
	Elasticity	1.13 ± 0.17	1.13 ± 0.18	.96 (.95-.97)	0.03	0.09
	Relaxation time, (ms)	24.14 ± 2.44	24.02 ± 2.39	.97 (.95-.98)	0.42	1.16
	Creep	1.44 ± 0.14	1.43 ± 0.14	.98 (.97-.99)	0.02	0.05
Knee flexor muscle						
DE Biceps Femoris	Tone, (Hz)	13.01 ± 1.14	12.96 ± 1.35	.95 (.94-.96)	0.28	0.77
	Stiffness, (N/m)	226.92 ± 52.45	227.14 ± 51.04	.99 (.98-.99)	5.17	14.34
	Elasticity	1.11 ± 0.20	1.10 ± 0.21	.96 (.94-.98)	0.04	0.11
	Relaxation time, (ms)	21.38 ± 2.87	21.29 ± 3.07	.96 (.94-.97)	0.59	1.65
	Creep	1.29 ± 0.16	1.28 ± 0.15	.94 (.93-.95)	0.03	0.11
NDE Biceps Femoris	Tone, (Hz)	12.93 ± 1.02	12.97 ± 1.02	.97 (.95-.98)	0.18	0.49
	Stiffness, (N/m)	221.79 ± 52.60	222.52 ± 52.29	.99 (.98-.99)	5.24	14.54
	Elasticity	1.12 ± 0.21	1.12 ± 0.22	.98 (.97-.99)	0.03	0.08
	Relaxation time, (ms)	21.51 ± 2.96	21.48 ± 2.95	.98 (.97-.99)	0.42	1.16
	Creep	1.29 ± 0.16	1.28 ± 0.15	.97 (.96-.98)	0.02	0.07

The inter-session SEM was calculated using the pooled SD of session 1 and session 2, quantifying the absolute measurement stability across different days

DE Dominant extremity, *NDE* Non-dominant extremity, *ICC* intraclass correlation coefficient, *95% CI* 95% confidence interval, MDC_{95} Minimal minimal detectable change at the 95% confidence level, *SD* Standard deviation, *SEM* Standard error of measurement

BF has been evaluated across various populations [19, 31, 32, 35]. Tone and stiffness measurements generally demonstrate favorable intra-rater reliability (ICC range: 0.91–0.99). Inter-rater reliability for these parameters appears to fall within the moderate-to-good range, with ICC values between 0.76 and 0.81 across healthy, spinal cord injury, and stroke cohorts. Regarding viscoelastic properties, intra-rater elasticity appears reliable (ICC range: 0.82–0.99), and high intra-rater consistency (ICC: 0.96) has been observed for relaxation and creep in spinal cord injury patients; however, inter-rater data for these measures remain limited. Collectively, these findings suggest that while BF properties, particularly tone and stiffness, may offer consistent intra-rater reproducibility, inter-rater agreement for stiffness and elasticity appears moderate, warranting cautious application in clinical practice. In synthesis, stiffness appears to serve as the most consistent biomechanical metric for both the RF and BF, demonstrating reliable reproducibility across raters and patient cohorts. While tone and elasticity exhibit favorable intra-rater consistency, their reliability often varies by population, likely reflecting pathology-induced biomechanical changes. Crucially, the scarcity of inter-rater data for relaxation time and creep currently limits their routine inclusion in clinical research and evidence-based practice, suggesting a clear need for further research to address these methodological gaps.

In this study, the reliability of the MyotonPro for measuring the biomechanical and viscoelastic properties of the DE and NDE RF and BF muscles was evaluated under standardized conditions. The findings suggest a high degree of measurement reproducibility, with intra-session ICC values consistently demonstrating strong stability (predominantly ≥ 0.95). For the RF, ICC values ranged from 0.95 to 0.99, with stiffness and creep frequently reaching 0.99 in both limbs. Similarly, the BF showed robust agreement, with ICCs ranging from 0.97 to 0.99. Inter-session reliability also appears strong, supporting the potential for longitudinal monitoring; ICC values for the RF remained high (0.96–0.99), and the BF demonstrated consistent values ranging from 0.94 to 0.99. Stiffness consistently emerged as the most reliable property across all muscle groups, often yielding ICC = 0.99. While these results indicate that the MyotonPro provides robust measurement reliability under standardized conditions, they do not extend to determining the device's long-term monitoring capabilities or its definitive role in clinical decision-making. Thus, further investigation in diverse clinical populations appears necessary to determine the device's practical applicability. These reliability assessments appear to align with existing literature regarding the stability of RF and BF biomechanical and viscoelastic properties [19, 23, 27–38]. The observed intra- and inter-session consistency across all five parameters suggests

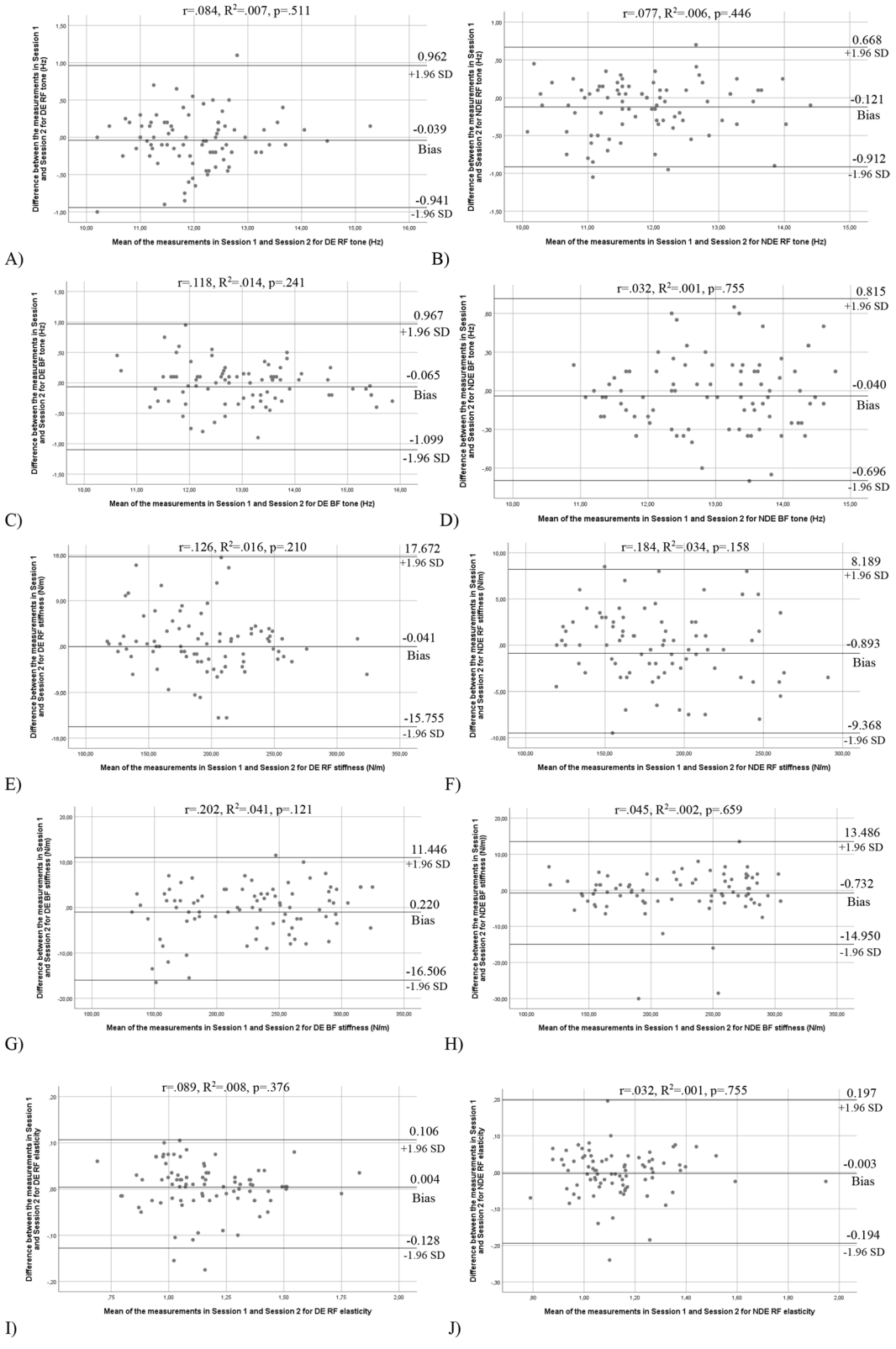

Fig. 4 (See legend on next page.)

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Fig. 4 Bland–Altman plots for the MyotonPro measurements across sessions 1 and 2. Panel **A**: DE RF tone; Panel **B**: NDE RF tone; Panel **C**: DE BF tone; Panel **D**: NDE BF tone; Panel **E**: DE RF stiffness; Panel **F**: NDE RF stiffness; Panel **G**: DE BF stiffness; Panel **H**: NDE BF stiffness; Panel **I**: DE RF elasticity; Panel **J**: NDE RF elasticity. The central line represents the absolute average difference between measurements, while the upper and lower lines represent ± 1.96 standard deviation (SD). The low R^2 values, along with the non-significant correlation coefficients (r) and p values ($p > 0.05$), indicate that the MyotonPro measurements from the device show no systematic tendency to overestimate or underestimate the reference values for muscle tone, stiffness, and elasticity. The Bland–Altman plots indicated favorable agreement for DE and NDE RF and BF tone, stiffness, and DE and NDE RF elasticity [ranges from 0.962 to −0.941 (Panel **A**), 0.668 to −0.912 (Panel **B**), 0.967 to −1.099 (Panel **C**), 0.815 to −0.696 (Panel **D**), 17.672 to −15.755 (Panel **E**), 8.189 to −9.368 (Panel **F**), 11.446 to −16.506 (Panel **G**), 13.486 to −14.950 (Panel **H**), 0.106 to −0.128 (Panel **I**), 0.197 to −0.194 (Panel **J**)] across sessions 1 and 2. Additionally, the identified systematic bias between the measurements in sessions 1 and 2 is −0.039, −0.121, −0.065, −0.040, −0.041, −0.893, 0.220, −0.732, 0.004, and −0.003 for DE and NDE RF and BF tone, stiffness, and DE and NDE RF elasticity, respectively

that the MyotonPro may serve as a reliable instrument for capturing muscle characteristics under controlled conditions. In conclusion, the study demonstrates significant measurement reliability for the RF and BF, particularly regarding the stability of stiffness and the inter-session reproducibility of viscoelastic markers. However, as these findings are limited to measurement consistency, the methodological properties of these parameters warrant further examination before their potential utility can be considered in clinical settings.

The assessment of intra-session reliability, which reflects measurement consistency within a single testing environment, demonstrated favorable results across all measured properties in this study. For the RF, stiffness exhibited notable precision metrics relative to its measurement scale. The SEM for stiffness ranged from 4.04 to 6.14, and the MDC_{95} spanned 11.19 to 17.03. From a clinical perspective, the calculated MDC_{95}, representing approximately 5–8% of baseline muscle stiffness, serves as a pragmatic benchmark for interpreting measurement results. This level of measurement resolution indicates that the MyotonPro possesses sufficient precision to track subtle variations in muscle stiffness, such as those following stretching, fatigue, or recovery protocols. These findings underscore the potential utility of the device for clinicians aiming to monitor longitudinal changes in musculoskeletal status. Tone also demonstrated high internal consistency, with SEM values between 0.17 and 0.22 and MDC_{95} values between 0.46 and 0.61. Among the viscoelastic properties, the MDC_{95} for creep was notably low (ranging from 0.04 to 0.09). This low variability suggests that the MyotonPro possesses sufficient resolution to identify subtle shifts in this specific tissue characteristic within a single session. The overall low intra-session variability across different muscle groups suggests the MyotonPro is a reliable instrument for the instantaneous assessment of muscle properties within the scope of this study. Regarding inter-session reliability, which quantifies the stability of measurements taken across different testing sessions, the device maintained consistent precision, supporting its potential for longitudinal monitoring within this study. Stiffness continued to demonstrate the greatest overall stability, with its inter-session MDC_{95} values remaining consistently tight (ranging from 11.30 to 14.54). This low measurement error across days supports confidence in detecting potential physiological changes in muscle stiffness over time. Tone and creep also maintained stability between sessions, with MDC_{95} values ranging from 0.49 to 0.77 and 0.05 to 0.11, respectively. These results indicate that the instrument captures the inherent biological variability of these properties. While relaxation time presented the largest MDC_{95} values (ranging from 1.16 to 1.65), potentially reflecting natural physiological fluctuations over time, its measurement error remains within acceptable limits for research use. The consistently low SEM and MDC_{95} values observed across all five properties and muscle groups support the MyotonPro's potential for objective evaluation in settings where monitoring tissue characteristics over subsequent sessions is investigated.

The discussion of the reported SEM and MDC_{95} values focuses on the internal validation of the MyotonPro's measurement precision and stability, as quantified by the metrics derived from this study's protocol. We have opted not to perform an exhaustive comparison of these error values against those reported in existing literature. Direct cross-study comparisons of SEM and MDC_{95} are often complicated by differences in subject populations, muscle testing conditions, and variations in probe application techniques. Therefore, rather than introducing potential variability through cross-study comparisons, this analysis focuses on the intrinsic repeatability demonstrated across the five biomechanical properties within the confines of this controlled investigation. These findings contribute to the evidence supporting the reliability of the device within the parameters of this study.

The Bland–Altman plot is a standard statistical tool for assessing agreement between repeated measurements by plotting the difference between them against their mean. This method provides a visual and quantitative evaluation of systematic bias and random error, supplementing the information provided by the ICC. In this study, Bland–Altman plots indicated general agreement for all measured properties, tone, stiffness, elasticity, relaxation time, and creep, across both the DE and NDE RF and BF limbs between sessions 1 and 2. The focus of the Bland–Altman analysis on inter-session reliability (comparing session 1 and session 2) was chosen to

evaluate the device's suitability for longitudinal monitoring, where measurements are obtained across different clinical visits. While intra-session consistency was verified by the ICC values (≥ 0.94), inter-session analysis is essential for determining measurement consistency for longitudinal monitoring. By quantifying systematic bias and LoA between sessions separated by time, the Bland–Altman analysis helps clarify whether changes observed over time likely reflect physiological shifts rather than measurement error. Therefore, prioritizing the inter-session Bland–Altman analysis provides relevant evidence regarding the device's potential utility in follow-up assessments. The LoA, encompassing the range of data points (e.g., 0.962 to −0.941 for DE RF tone), suggests that differences between sessions largely fell within an acceptable range of variation. Furthermore, the systematic mean bias values were generally close to zero (e.g., −0.039 for DE RF tone, 0.009 for NDE BF creep), particularly for the viscoelastic properties, suggesting no substantial systematic difference between measurements obtained on separate days. While some biomechanical biases were slightly larger, particularly the −0.893 value for NDE RF stiffness, the overall minimal bias confirms that there is no substantial systematic difference between the measurements taken on separate days. Crucially, this stiffness bias is substantially lower than the reported SEM (4.07) and MDC_{95} (11.30), indicating that the error is clinically negligible. This finding supports the inter-session validity and temporal stability of the measurements obtained by the MyotonPro device. However, it is important to emphasize that these high precision metrics were obtained under strictly controlled conditions with a single experienced assessor. While the low SEM and MDC_{95} values confirm the device's internal stability, they may represent an optimized reliability profile that might not be fully mirrored in diverse clinical settings or across different demographic groups. The specific characteristics of our sample, such as the relatively uniform muscle mass and low subcutaneous fat thickness typically found in young male recreational athletes, could potentially provide a more stable environment for myotonometric assessments. In contrast, factors such as age-related changes in muscle architecture or different adipose tissue distributions in other populations might introduce additional variability. Therefore, while our results demonstrate strong consistency within this specific group, the findings should be interpreted with consideration when applied to more diverse or clinical populations where tissue characteristics may differ. It would be beneficial for future research to include more heterogeneous cohorts to examine whether these findings hold across different tissue characteristics, such as varying levels of subcutaneous fat and muscle mass.

While existing literature has extensively validated the reliability of MyotonPro for measuring muscle tone and stiffness, further evidence is needed regarding the inter-session reliability of viscoelastic properties, specifically mechanical relaxation time and creep. Previous research has limited data to support the consistent use of these parameters in extended clinical observations. This study addresses this gap by evaluating the intra- and inter-session reliability of these viscoelastic metrics. Our findings suggest that mechanical relaxation time and creep exhibit favorable reliability levels, supporting their potential as objective indices for tissue assessment. Consequently, this study contributes to the understanding of Myotonometry as a reproducible tool, providing a foundation for its potential application in follow-up assessments within musculoskeletal rehabilitation and therapeutic evaluation.

This study provides findings regarding the reliability of the MyotonPro device; however, it is essential to consider the methodological context to interpret these results and inform future research properly. The study's design, utilizing a four-stage repeated-measures approach across two consecutive days, enabled the assessment of both intra-session (internal precision) and inter-session (longitudinal stability) reliability. The observed ICC values (≥ 0.94) across all five biomechanical and viscoelastic properties (tone, stiffness, elasticity, relaxation time, and creep) suggest favorable consistency for this specific measurement protocol. Furthermore, the inclusion of SEM and MDC_{95} metrics offers practical insights, helping to quantify measurement error and providing a potential framework for interpreting whether observed changes represent genuine physiological shifts. The use of Bland–Altman plots further complements the ICC analysis by offering an evaluation of systematic bias and agreement between sessions. The strict standardization of measurement conditions, such as controlled environmental factors, a fixed time of day, and a 24-h rest period, combined with the use of a single experienced examiner, likely contributed to minimizing potential confounding variables during this investigation.

This study had several limitations that warrant careful consideration. First, the homogeneity of our sample, which consisted solely of young, healthy male recreational athletes, strictly limits the external generalizability of the findings. Furthermore, while we included participants from various primary sports (see Table 1), we did not perform a sport-specific analysis to account for potential differences in muscle mechanical properties arising from distinct training adaptations (e.g., team sports vs. individual disciplines). Since muscle stiffness and viscoelastic properties can be influenced by sport-specific loading profiles, the normative data presented here should be interpreted with caution when applied to

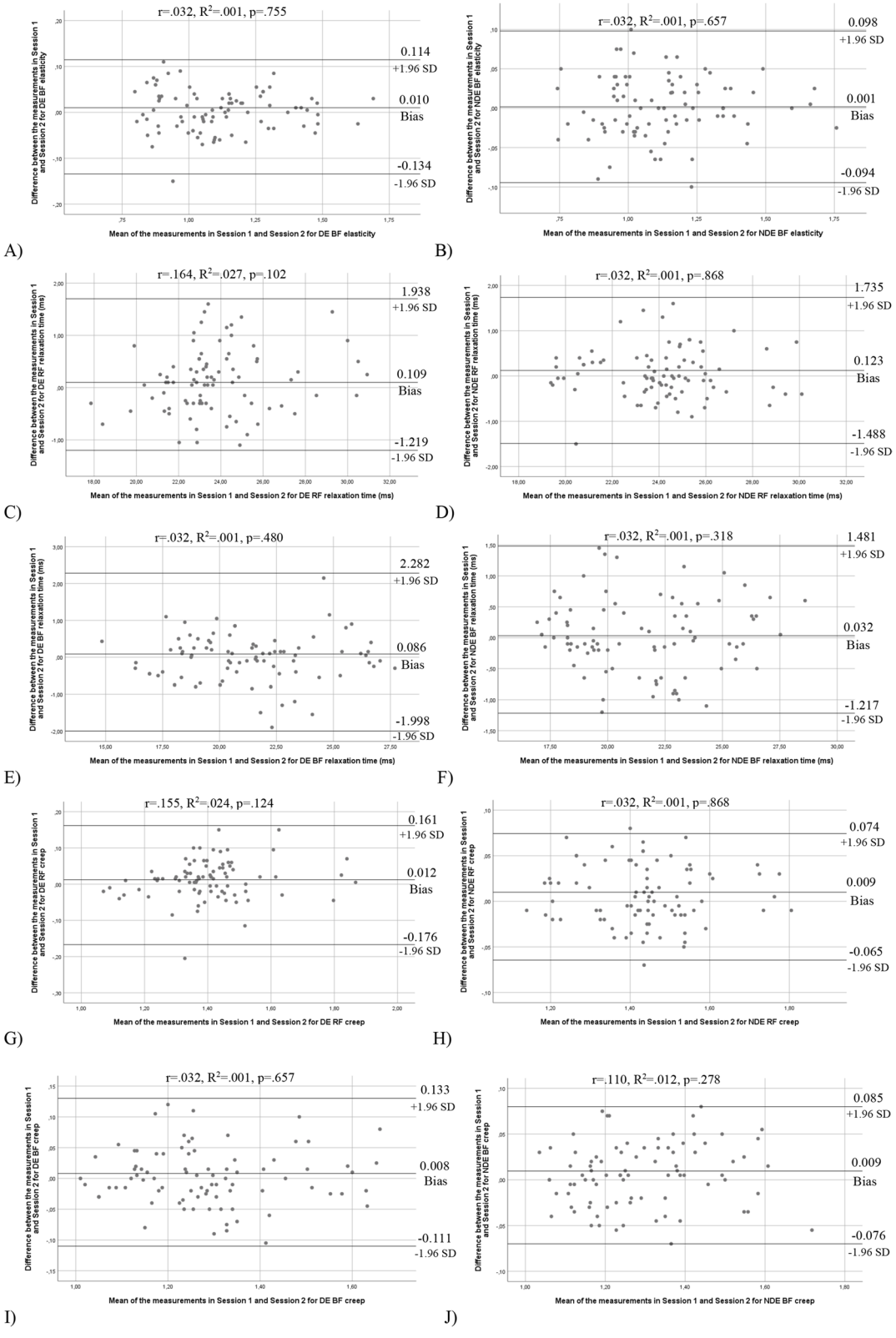

Fig. 5 (See legend on next page.)

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Fig. 5 Bland–Altman plots for the MyotonPro measurements across sessions 1 and 2. Panel **A**: DE BF elasticity; Panel **B**: NDE BF elasticity; Panel **C**: DE RF relaxation time; Panel **D**: NDE RF relaxation time; Panel **E**: DE BF relaxation time; Panel **F**: NDE BF relaxation time; Panel **G**: DE RF creep; Panel **H**: NDE RF creep; Panel **I**: DE BF creep; Panel **J**: NDE BF creep. The central line represents the absolute average difference between measurements, while the upper and lower lines represent ± 1.96 standard deviation (SD). The low R^2 values, along with the non-significant correlation coefficients (r) and *p* values ($p > 0.05$), indicate that the MyotonPro measurements from the device show no systematic tendency to overestimate or underestimate the reference values for elasticity, relaxation time, and creep. The Bland–Altman plots indicated favorable agreement for DE and NDE BF elasticity, and DE and NDE RF and BF relaxation time, and creep [ranges from 0.114 to −0.134 (Panel **A**), 0.098 to −0.094 (Panel **B**), 1.938 to −1.219 (Panel **C**), 1.735 to −1.488 (Panel **D**), 2.282 to −1.998 (Panel **E**), 1.481 to −1.217 (Panel **F**), 0.161 to −0.176 (Panel **G**), 0.074 to −0.065 (Panel **H**), 0.133 to −0.111 (Panel **I**), 0.085 to −0.076 (Panel **J**)] across sessions 1 and 2. Additionally, the identified systematic bias between the measurements in sessions 1 and 2 is 0.010, 0.001, 0.109, 0.123, 0.086, 0.032, 0.012, 0.009, 0.008, and 0.009 agreement for DE and NDE BF elasticity, and DE and NDE RF and BF relaxation time, and creep, respectively

specific athletic populations. These results should not be generalized beyond similar populations without further validation, as muscle properties and measurement reliability may differ significantly in older adults, females, or diverse clinical populations (e.g., those with severe spasticity or edema). Second, the short inter-session interval (24 h) may not fully account for long-term physiological fluctuations or potential measurement drift that could occur over extended periods; thus, these findings should be viewed as an assessment of short-term stability rather than long-term reliability. Third, a major limitation is the absence of an inter-rater reliability assessment. Since all measurements were performed by a single, highly experienced assessor, the reported ICC values likely represent an upper bound of reliability. Consequently, these findings may not fully reflect real-world clinical conditions where multiple evaluators with varying levels of experience are involved, and inter-rater variability could introduce a higher degree of measurement error. Fourth, the highly standardized measurement conditions, while essential for minimizing confounding variables in this reliability study, create an idealized environment. This does not fully reflect the environmental challenges, patient variations, and clinical workflow constraints encountered in routine practice, where such stringent standardization may not be feasible. Fifth, while the standardization of the RF and BF measurement points is clearly defined, the precise placement of the probe might still introduce minute variability, even with real-time guidance. Sixth, a potential limitation of this study is the fixed measurement sequence used for all participants. While this approach was chosen to ensure a highly standardized clinical workflow and to minimize procedural variability, we acknowledge that it could theoretically introduce systematic bias.

Based on these findings and the aforementioned limitations, future studies should focus on the following key areas to advance the clinical and scientific application of myotonometry: First, to establish a robust evidence base for the device's measurement accuracy, future investigations should prioritize the concurrent and construct validity of MyotonPro metrics. A critical research priority involves comparing these biomechanical properties against established gold standards, such as SWE or dynamometry, which would provide a more comprehensive validation of the device's methodological rigor and concurrent validity. Furthermore, to expand the generalizability of our findings, research must prioritize investigating the reliability and validity of these measures in diverse cohorts, including elderly individuals, females, and clinical populations (e.g., those with spasticity or edema). Additionally, future investigations should explicitly categorize participants based on their primary athletic discipline to clarify how sport-specific training adaptations influence muscle mechanical properties. Second, given that our findings reflect short-term consistency, future research should assess long-term reliability over extended periods (e.g., weeks or months) to better account for physiological fluctuations and potential measurement drift. Third, further investigation is essential to involve multiple assessors to establish inter-rater reliability. This step is critical for providing a more realistic estimate of the device's assessment reliability and for understanding how the experience levels of different evaluators impact measurement error. Fourth, future studies should evaluate the device's performance in less controlled, real-world clinical environments. This will help determine the feasibility of our standardized protocols in settings where such stringent controls may not be practical and where environmental challenges and patient variability are prevalent. Fifth, to minimize variability associated with probe placement, future studies should investigate the impact of advanced training protocols or enhanced visualization techniques. Additionally, exploring the trade-off between single-measurement protocols and the means of multiple trials could help optimize assessment efficiency in time-sensitive clinical environments without sacrificing necessary precision. Sixth, future studies investigating muscle biomechanics should employ randomized, counterbalanced designs to mitigate potential order effects inherent in fixed measurement sequences.

Conclusions

This study demonstrates that the MyotonPro device is a highly reliable and clinically stable instrument for assessing the biomechanical and viscoelastic properties of the RF and BF within the specific context of young, healthy

male recreational athletes. Across all measured properties, the device demonstrated favorable intra-session consistency, suggesting high precision within a single testing environment for this participant group. Furthermore, inter-session reliability appeared consistent, supporting the device's stability for repeated measurements obtained on separate days. The Bland–Altman analysis corroborated these statistical findings, revealing minimal systematic bias and acceptable LoA between sessions for all properties. However, as the current study relied on a single experienced evaluator and a homogeneous sample, these findings likely represent an upper bound of reliability. Therefore, these results should not be generalized beyond similar populations or different clinical conditions without further validation. Future research involving female participants, older adults, and multiple raters is essential to establish the broader applicability and generalizability of these findings.

Abbreviations

BF	Biceps femoris
CI	Confidence interval
DE	Dominant extremity
ICC	İNtraclass correlation coefficient
GRRAS	Guidelines for Reporting Reliability and Agreement Studies
LoA	Limits of Agreement
MDC_{95}	Minimal detectable change at the 95% confidence level
MRE	Magnetic Resonance Elastography
NDE	Non-dominant extremity
RF	Rectus femoris
SD	Standard deviation
SEM	Standard error of measurement
SWE	Shear Wave Elastography

Protocol and statistical analysis plan availability
The study protocol and statistical analysis plan are available from the corresponding author upon reasonable request.

AI Declaration
During the preparation of this work, the authors used Gemini to improve the language and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Authors' contributions
Erhan Secer **1** **:** Research concept and study design, literature review, data collection, data analysis and interpretation, statistical analyses, writing of the manuscript. **Kazım Bayram** **2** **:** Research concept and study design, data analysis and interpretation, writing of the manuscript. **Derya Ozer Kaya** **3,4** **:** Research concept and study design, data analysis and interpretation, reviewing/editing a draft of the manuscript.

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

Declarations

Ethics approval and consent to participate
The study protocol received approval from the Manisa Celal Bayar University Health Sciences Ethics Committee (Approval Number: 20.478.486/3465, Decision Date: 15.10.2025). All participants were thoroughly informed about the study procedures and experimental protocols. All subjects signed an informed consent form before enrollment. The treatment of human research subjects complied with the ethical principles outlined in the Declaration of Helsinki.

Consent for publication
Written informed consent for the publication of anonymized study data and identifiable images was obtained from all participants.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Health Care Services, Vocational School of Health Services, Manisa Celal Bayar University, Manisa, Türkiye
[2]Department of Physiotherapy and Rehabilitation, School of Health Sciences, Kapadokya University, Nevşehir, Türkiye
[3]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Izmir Katip Celebi University, Izmir, Türkiye
[4]Physiotherapy and Rehabilitation Research and Application Center, Izmir Katip Celebi University, Izmir, Türkiye

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