Agyapong-Badu S, Warner M, Samuel D, Stokes M

Practical considerations for standardised recording of muscle mechanical properties using a myometric device: recording site, muscle length, state of contraction and prior activity.

J Musculoskel Res 2018 In Press

Accepted: accepted 20th June 2018 for publication in *Journal of Musculoskeletal Research*

Title page

(i) Title:

Practical considerations for standardised recording of muscle mechanical properties using a myometric device: recording site, muscle length, state of contraction and prior activity

(ii) Authors:
Sandra Agyapong-Badu PhD[1,2], Martin Warner PhD[1,3], Dinesh Samuel PhD[1], Maria Stokes PhD[1,3]

(iii) Author's affiliations:
[1]Faculty of Health Sciences, University of Southampton, UK

[2]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, UK

[3]Arthrtis Research UK Centre for Sport, Exercise and Osteoarthritis

(iv) Corresponding author:
Dr Sandra Agyapong-Badu
School of Sport, Exercise and Rehabilitation Sciences
University of Birmingham
B15 2TT
United Kingdom
Email: s.agyapong-badu@bham.ac.uk

(v) Running Title
Recording conditions for Myoton technique

Abstract

Purpose: This study aimed to systematically examine the influence of various muscle and experimental conditions on Myoton recordings. **Methods:** A cross-sectional, observational design was used to examine muscle conditions and experimental factors (different recording sites, muscle length, level of contraction and prior physical activity) that may influence reproducibility of Myoton recordings for biceps brachii (BB) and rectus femoris (RF). Fifty-three healthy adults (26 young, 27 older) aged 18-90 years were studied. Muscle stiffness, tone and elasticity were measured using the MyotonPRO device. **Results:** Statistically significant differences in Myoton parameters were found for aspects of all four muscle and experimental conditions compared with the control condition ($p<0.05$). However, clinically relevant differences in tone, stiffness and elasticity were only found for contracted compared to resting muscle, with changes being greater than the minimal detectable change. Elasticity was not affected by prior activity. **Conclusions:** The conditions studied significantly altered Myoton parameters of BB and RF in healthy adults, but only changes in parameters during muscle contraction were clinically relevant. These findings provide evidence to support the need to consider muscle condition and experimental factors for improving the robustness of test protocols for assessing muscle mechanical properties using the MyotonPRO device.

Key Words: Experimental conditions, muscle elasticity, muscle stiffness, muscle tone, validity

1. Introduction

A portable device (MyotonPRO) offers a novel means of assessing muscle tone and mechanical properties such as stiffness and elasticity, in clinical and field environments. The device is hand-held, non-invasive, relatively inexpensive and easy to use. The technique induces oscillations of muscle and records these oscillations, from which various parameters are calculated simultaneously, including non-neural tone, stiffness and elasticity [13]. The reliability of Myoton devices is generally good, as reported in several studies in healthy and clinical populations [2, 6, 9, 10, 12, 22, 28]. The validity of the Myoton technique has received less attention but has been validated against electromyography (EMG) and force in healthy muscles [12], and in Parkinson's disease against EMG and mechanomyography [20]. Parameters computed from the Myoton technique showed good validity when compared to clinical rigidity scores [25].

The sensitivity to change in muscle mechanical properties using Myoton technology has been reported in neurological conditions. Muscle stiffness was more responsive than tone and elasticity to change after upper-extremity rehabilitation in stroke patients [9]. Responsiveness of Myoton parameters to the effects of intervention in people with Parkinson's disease also indicated validity of the Myoton device [19]. Marusiak, Żeligowska (21) reported the discriminant ability of Myoton to identify differences between patients with Parkinson's disease and controls, as well as construct validity (against other measures of muscle) and responsiveness to change with an exercise intervention. The authors also reported a correlation between changes in Myoton parameters and changes in brain chemistry post-intervention [21]. Another study on the effects of exercise programmes on back pain showed that Myoton parameters at 3 months post-intervention were predictive of pain outcome at 6 months [18]. Significant correlations between Myoton recorded stiffness and isometric force development in triceps surae in female netballers has been reported [24].

Despite these reported psychometric properties of the Myoton technique, a systematic investigation of the influence of various experimental and muscle conditions on measurement of muscle tone, stiffness and elasticity has yet to be conducted, to indicate how well standardised the conditions need to be for recording consistent data. For instance, it is well known that other muscle testing techniques, such as electromyography (EMG) and strength testing need to be standardised and certain conditions affect recordings. For example, EMG recordings are optimal at the motor point and locations for various muscles have been recommended by the SENIAM guidelines [14] . It is unknown how far an operator can move away from the middle of the muscle belly of interest and still record consistent and true readings. The EMG signal is also known to increase during contraction and the effect of contraction has also been shown for Myoton recordings, with increase in stiffness, tone and elasticity [12]. Changes in muscle length are known to affect muscle force (length-tension relationship; [7], so muscle length and joint position may also be important to standardise for Myoton testing. Muscle testing techniques are usually performed after a period of rest but the effect of prior physical activity on Myoton recordings is not known. The factors mentioned above therefore require systematic investigation to examine their effects on recordings using the Myoton technology.

Although changes in muscle properties induced by joint position, activity, voluntary contraction or disease condition can be perceived by the individual and/or the therapist, they are difficult to measure objectively. Investigation of factors that may affect the reliability or validity of a measure is vital for a device such as the MyotonPRO, which is potentially useful in a clinical or field setting. Previous reliability studies [2, 4] have raised questions regarding factors such as consistency of the location of the recording site over the muscle, accurate placement of the device’s probe on muscle, joint angles (and hence muscle length) and level of contraction, as well the influence of physical activity in measuring muscle mechanical properties. Considering such factors when standardising test protocols would aid the validity of data from different populations to enable accurate discrimination of findings between healthy and clinical groups. Specifically, factors affecting the assessment of muscle groups in both upper and lower extremities, which are major determinants of most activities of daily living such as walking, stair climbing and transfer need to be quantified objectively. This paper presents four hypothesis-driven experiments that focus on identifying muscle conditions and experimental factors that may influence reproducibility of Myoton recordings, including different recording sites, muscle length (joint angles), level of contraction (resting or contracted) and prior activity. Previous study by the research group reported ageing effects for stiffness, elasticity and tone in both upper and lower limb muscles [3] , hence one of the experiments explored the difference in probe locations over young and older muscles to identify age-specific muscle response to probe relocation. The experiments were designed to improve the robustness of existing protocols for assessing muscle mechanical properties using the MyotonPRO device.

Aims

1) To determine the effect of changing the location of the MyotonPRO testing site on mechanical tone, elasticity and stiffness in biceps brachii (BB) and rectus femoris (RF) muscles in healthy young and older participants.
2) To determine the effect of change in muscle length on Myoton parameters in the rectus femoris muscle in healthy young participants.
3) To determine the effect of a change in rectus femoris contractile state on recordings of MyotonPRO parameters in healthy young participants.
4) To determine the effect of prior physical activity on Myoton parameters of quadriceps muscle in healthy young participants.

Null Hypotheses

There will be no statistically significant difference in mechanical tone, elasticity or stiffness of biceps brachii or rectus femoris muscles following: 1) change in the MyotonPRO probe position on the skin; 2) change in muscle length; 3) change in level of contraction; 4) physical activity just prior to testing.

2. Methods

This study used a cross-sectional, observational design to examine an experimental factor (location of testing site) and three muscle conditions (length, contractile state, prior activity) for their influence on Myoton parameters.

2.1. Participants

Fifty-three healthy adults (26 young, 27 older) were studied (Table 1). There were variations in the number of participants involved in the four experiments, as detailed in the sections describing each experimental protocol. Young participants aged 18-35 years were recruited from the University and older participants aged 65-90 years were recruited from the local community. Young participants were included if they did not participate in sports or exercise more than three times per week, or competitively at university level or above. For older participants, activity was assessed using the Physical Activity Scale for the elderly (PASE) to ensure only sedentary or moderately active participants were included [29]. These criteria were for a larger study of the effects of ageing on Myoton parameters [3]. Exclusion criteria for both age groups were: conditions known to affect muscle and function; upper and lower limb pathology (fracture, surgery, neoplasm), skin disorders, neurological conditions and musculoskeletal conditions/injuries severe enough to require treatment or prevent activity for more than one week in the previous five years. Those taking medications, such as skeletal muscle relaxants, neuromuscular blocking drugs, and those unable to understand study requirements were excluded. The study was approved by the host institution’s Ethics Committee and was conducted in accordance with the Helsinki Declaration of 1975. All participants gave their written informed consent.

[Insert Table 1 near here]

2.2 Equipment and data acquisition

The MyotonPRO is a hand-held device that elicits oscillations of muscle after a probe applies a brief mechanical impulse with quick release under constant pre-load to the skin over the muscle. From these oscillations, the device quantifies various parameters simultaneously, including non-neural tone, and mechanical properties such as dynamic stiffness and elasticity. The resting tone or state of tension (frequency [Hz]) which is defined as the maximum frequency computed from the signal spectrum by Fast Fourier Transform (FFT) was recorded. The F-parameter is the frequency (f_{max}) that corresponds to the maximum value of the signal spectrum ($F=f_{max}$). The higher the frequency of dampened oscillations (natural oscillation frequency), the greater the muscle tension.

Stiffness (N/m) is a measure of the muscle’s ability to resist an external force that modifies its shape and is calculated using the formula (Fig. 1):

$$S = \frac{a_{\max} \cdot m_{probe}}{\Delta l}$$

Where ^{a}max is the maximum acceleration, ^{m}probe is the mass of measurement mechanism, and Δl is the maximum displacement of the tissue. The higher the N/m value, the stiffer the muscle [13].

Elasticity describes the tissue’s ability to restore its shape after being deformed. Elasticity is represented by logarithmic decrement (D), which describes the dampening of the oscillation i.e. dissipation of mechanical energy during an oscillation cycle (Fig. 1). The smaller the decrement value, the smaller the subsequent dissipation of mechanical energy and the higher the elasticity [13]. Elasticity (logarithmic decrement) is expressed in arbitrary units as:

$$[D = ln \left\{\frac{a_1}{a_3}\right\}]$$

a_1= maximum displacement; maximum tissue resistance measured in mG, a_3= maximum displacement of the second period of oscillation which takes place due to the recuperation of stored residual mechanical energy in the tissue.

[Insert Figure 1 near here]

Recordings of muscle parameters were obtained by placing the probe of the device (3mm diameter) perpendicular on the skin over the muscle of interest (Fig. 2), with constant preload (0.18N) independently of the investigator, to pre-compress subcutaneous tissues. The authors have examined relationships between subcutaneous fat (measured using ultrasound) and muscle parameters in an earlier publication and found it to be inconsistent between ageing and gender differences to allow a conclusion to be drawn about any direct effects of fat on muscle parameters [3]. The device delivered a brief (15ms), low force (0.4N) mechanical impulse, inducing the damped natural oscillations of the underlying tissues described below. These oscillations were recorded by an accelerometer within the device. Two sets of 10 mechanical impulses (one second apart) were delivered, with a one-minute rest between the two sets.

[Insert Figure 2 near here]

2.3 Experimental Procedures

Data were collected by one investigator (SAB) under five recording conditions, which were a control condition and four experimental conditions. Two sets of 10 impulses were recorded from the relevant muscle in each condition.

2.3.1 Condition 1: Baseline control recordings in middle of resting muscle

Prior to testing, each of the 53 participants rested in supine lying for 10 minutes and had been asked to travel by car, bus or taxi and not to cycle, run or walk briskly to the laboratory. For BB, the participant lay supine with the shoulder externally rotated and elbows extended and wrist supinated. A rolled towel placed under the wrist to flex the elbow approximately 15^0 from the horizontal to take the stretch off the muscle and enable relaxation [1]. Measurements were taken at a point midway between the most anterior aspect of the lateral tip of the acromion and the mid cubital fossa, following a gentle, resisted isometric muscle contraction to identify the middle of the muscle belly. The probe was placed on the big head of the biceps brachii and the probe remained on the same head of the BB during the 1 cm movements in *Condition 2* below. Rectus femoris was tested with the knee extended and hip in neutral, with sandbags placed either side of the ankle to maintain this position. Measurements were taken at two thirds of the distance between the anterior superior iliac spine (ASIS) and the superior pole of the patella, to locate a reproducible site over the muscle [22]. Participants were instructed to relax as much as possible during Myoton recordings on BB and RF.

2.3.2 Condition 2: Four additional recording sites

The effect of recording site on Myoton parameters was examined in both BB and RF muscles, in all 53 participants. In addition to the middle of the muscle along the sagittal plane on the dominant side, recordings were taken from four additional points: 1cm medial, lateral, superior and inferior to the midpoint in BB and RF. This was to assess the effect of probe movement in between measurements on quantification of muscle properties. The recordings from a total of 100 impulses were made (5x2 sets of 10 impulses) from each muscle on the dominant side.

2.3.3 Condition 3: Increased muscle length

Rectus femoris was tested in the 26 young participants, with the knee at 90 degrees of flexion. Participants sat on a purpose built chair, with their knee and hip joints flexed at 90^0. Two sets of 10 measurements were recorded over the middle of the muscle belly.

2.3.4 Condition 4: Contracted muscle

In the young participants, RF was tested in the same sitting position as in *Condition 3* but during isometric contractions of quadriceps. The participant was secured with a pelvic seat belt and a strap was placed just above the malleoli of the dominant leg and connected to a strain gauge bar behind the ankle. The participant then performed three isometric maximal voluntary contractions (MVC) of the quadriceps muscle lasting three seconds each, with a three-minute rest between each contraction. The maximum value from three trials was recorded and then 30% of this value calculated as the target force for Myoton recordings. RF thickness was reported to increase at 30% MVC in young males [11], hence this level was identified in the pilot testing as a suitable force, as it was easier for participants to maintain throughout the testing period than a maximal contraction. The participant was then instructed to maintain this 30% MVC contraction for 15 seconds (to avoid possible movement artefact on either side of the 10 seconds MyotonPRO recording) whilst MyotonPRO recordings were made over the middle of the muscle belly.

2.3.5 Condition 5: Prior physical activity

Twenty five of the young participants were then taken outside the lab to a regular stairway with 11 steps, where they were instructed to ascend and descend the steps three times, one step at a time, at their own selected comfortable pace. The location of the probe was marked on the skin before participants performed the stair ascent/decent task. Stair climbing was identified as a regular physical activity of daily living which could be performed easily by participants. They then returned to the lab, where recordings from RF were repeated following the exact same protocol as in condition 1.

2.4 Data management and statistical analyses

Data were downloaded into Microsoft Excel and analysed using SPSS 21(SPSS Inc, Chicago, IL). The data for all three MyotonPRO parameters (tone, elasticity, stiffness) were examined for normality using the Shapiro-Wilk test and found to be normally distributed. The means of 20 measurements (two sets of 10 impulses) taken at each measurement point was used for the analysis. Descriptive statistics were used to summarise the data as means and standard deviations (SD) for each condition. For the variable testing site condition, a repeated measures analysis of variance (ANOVA) was used to compare the means for mechanical tone, elasticity and stiffness from all five testing sites for BB and

RF separately. Paired t-tests and Bonferroni correction factor were conducted to identify which specific means differed. Greenhouse-Geisser corrections were reported when data violated assumption of sphericity. Paired t-tests were used for the three remaining scenarios to compare the control and experimental conditions, i.e. resting RF muscle versus altered length, contraction and prior activity.

3. Results

3.1 Effect of testing site on muscle parameters recorded over biceps brachii and rectus femoris in young and older participants

3.1.1 Elasticity

For RF in young and older participants, there was no overall significant difference observed between midpoint values and those recorded following a change in probe location for young ($p=0.09$) and older (0.18) adults (Table 2). For BB, elasticity differed significantly following a change in probe location for both the young ($p=0.02$) and older ($p<0.001$) groups.

[Insert Table 2 near here]

3.1.2. Tone (frequency)

Overall, changing the testing site significantly affected tone recordings in both the RF and BB muscles in both age groups ($p<0.001$), as seen in Table 2. T-tests analysis between individual sites and midpoint showed differences in tone for both young and older adults. Tone readings at the medial and superior sites were significantly lower than those at muscle midpoint. For older adults, tone at the inferior site of RF was significantly higher than at the midpoint. A similar trend was observed for BB, where tone at the medial and lateral positions in both young and older adults differed significantly from the muscle midpoint, and for the inferior point in young adults.

3.1.3 Stiffness

Overall, changing the probe location significantly affected RF and BB stiffness just as was observed for tone in both age groups (p range; <0.001-0.02). The t-test analysis between means at the midpoint of the muscle and the four other sites showed that BB stiffness at the lateral positions were significantly higher in both young and older adults. Stiffness decreased when the probe was positioned on the medial aspect but this change was only significant in the young adults. For RF, only the medial site produced a significant difference from midpoint in young adults, whereas significant differences resulted for all four positions in older adults.

3.2 Effect of muscle length on muscle parameters in rectus femoris

There were statistically significant differences in all three muscle parameters following a change in RF muscle length from knee extension in lying to sitting with the knee at 90 degrees of flexion (all $p<0.001$). RF became more elastic (knee extended 1.29±0.2 to flexed 1.1±0.1 log decrement), stiffer (292±46 to 310±49 N/m) with greater tone (16.3±2.0 to 17.0±2.0 Hz), as illustrated in Figure 3.

[Insert Figure 3 near here]

3.3 Effect of level of contraction on muscle parameters in rectus femoris

There were statistically significant differences in all three muscle parameters ($p<0.001$) with change in RF contractile state recorded in sitting with the knee at 90 degrees flexion. Contracting the RF muscle at 30% MVC resulted in increased elasticity (resting 1.1±0.1 to contracting 0.95±0.1 log decrement), tone (17.0±2.0 to 19.0±2.5 Hz) and stiffness (310±49 to 411±90 N/m) in young adults (Fig. 3).

3.4 Effects of prior physical activity on muscle properties of rectus femoris

Data from 25 of the 26 young participants were used for this experiment, since one participant was unable to complete the physical activity due to a feeling of dizziness. There were statistically significant differences in RF tone (pre-activity 16.3±2.0 to post-activity 16.8±2.1; Hz) and stiffness

(292±46 to 304±50; N/m) following stair ascent and descent ($p<0.001$ for both). Elasticity was, however, not affected by physical activity (1.29±0.2 to 1.31±0.2 (log decrement); $p=0.32$), as seen in Figure 3.

4. Discussion

This study was the first systematic investigation that used manipulation of the testing protocol to examine the effect of factors that may affect the consistency of Myoton recordings of mechanical tone, stiffness and elasticity. Significant differences were found for all four experimental conditions compared with the control condition, hence the null hypothesis was rejected, as moving the probe away from the muscle midpoint, altering the length, level of muscle contraction, and prior physical activity produced statistically significant changes in muscle parameters of BB and RF in healthy adults. However, the only changes that were clinically important were those during contraction of RF, with values changing greater than the minimal detectable change (MDC) values reported in reliability studies of healthy young (aged 18-35 years; tone=1.25 Hz; stiffness=34 N/m; elasticity=0.31 log decrement) and older participants (tone=1.49; stiffness=35; elasticity=0.34; [3]. This highlights the importance of ensuring that individual muscles are in a fully relaxed state during recordings of muscle at rest.

The present findings have shown that statistically significant changes, although not all clinically significant, occur in Myoton parameters at different recording sites. The findings also highlight the influence of these three muscle conditions; muscle length, level of contraction, and prior physical activity on muscle mechanical properties. All three muscle parameters were influenced by muscle length and level of contraction. RF became more elastic and had increased tone and stiffness following an increase in the muscle-tendon unit length from lying with the knee extended to sitting up (knee 90^0 flexion) in young adults. A similar observation was noted when participants progressed from relaxed to contracting the quadriceps at 30% MVC. During the contractions, RF became more elastic with increased tone and stiffness. Overall, stiffness was more responsive to the change in muscle state by isometric contraction (32% difference) compared to elasticity (14%) and mechanical tone (12%).

No particular trend was observed in terms of direction of change in mechanical properties when the probe was relocated on the muscles, although it appeared that the further the probe moved laterally from the muscle midpoint, the higher the tone and stiffness values recorded. This was possibly due to measurements being recorded over fascia or tendons rather than muscle. Apart from elasticity of BB in young adults, which did not change with probe re-location, all other parameters recorded significant changes in at least one of the four testing sites away from the midpoint. Decrement values on the other hand decreased indicating greater elasticity as measurements were taken closer to the muscle-tendon unit which is in keeping with published findings about the high elasticity of tendons [5, 26]. It is recommended that accurate and consistent location of the probe is worth considering in order to avoid recording statistically significant differences when using the device particularly for research, as well as clinical purposes. The variation in probe location was the only experiment involving older participants due to the reported changes in muscle architecture with ageing [8, 15]. These results have provided data to inform how far an operator could move from the muscle midpoint of a relatively smaller muscle such as the biceps brachii to minimise errors in recording muscle mechanical properties in older adults.

The high responsiveness to change in RF stiffness across the three muscle conditions, particularly during an increase in force observed in the present study, has been reported previously in studies of the knee extensors [6, 23] and other muscles in healthy participants [13] and in sports players [24]. RF stiffness was more responsive to change than tone and elasticity after rehabilitation in stroke patients [9]. Other studies in people with Parkinson’s disease, reported validity of the Myoton technique in terms of responsiveness to change, as there was reduced muscle stiffness after brain stimulation [25], medication [19] and exercise [21]. Validity in terms of sensitivity and specificity for detecting abnormalities requires further investigation for various clinical applications.

Decrement reflects the elasticity of a muscle and is associated with the ability of the muscle to return to its original shape following deformation [17, 28]. In the present study, elasticity of RF measured before and immediately after physical activity did not change, but was increased following a change in muscle length and level of contraction. Jarocka et al (2012) made a similar observation in the brachioradialis muscle and reported that the decrement of damped oscillations did not change significantly during different levels of activity in 17 young males (mean age and SD; 21 ±1 years). The authors asserted that elasticity was a more stable objective measure compared to frequency and stiffness [16]. However, findings from the present study have demonstrated that this assertion may only be true following physical activity but not other changes in muscle condition.

The frequency of damped oscillations, a reflection of muscle tone, was influenced by changes in all three muscle conditions. Korhonen et al (2005) stated that during isometric contraction, the muscle length remains the same, although an increase in intramuscular pressure could produce tension in the muscle fascia similar to that observed in a load-dependent relationship. This proposition would explain the increased mean for RF muscle tone (state of tension) during 30%MVC in the present study. This is in keeping with findings reported in a recent study where a linear increase in all three mechanical properties of RF with increasing force levels in 20 healthy participants with a mean age of 39.9 years was recorded [23]. Unlike the present study where only one level of MVC was assessed, the above study measured the change in RF properties with increasing force levels (20, 40, 60 and 80% MVC). They reported similar tone to that in the present study (relaxed=16.82±1.9; contracted 19.01±2.5 Hz at 30% MVC) at 40% MVC (relaxed=15.3±2.0; contracted=19.0 ± 4.2Hz), despite the difference in percentage MVC level for their participants. Apart from the above-mentioned study, the RF muscle has been less reported in the literature compared to gastrocnemius and biceps brachii muscles, so findings from the present study are useful for understanding changes in the RF muscle which is important for functional mobility.

Some limitations of the study include the lack of objective confirmation of relaxation using electromyography, as relying on participants to maintain muscles in a relaxed state could be a source of error. More levels of force could have been included, to determine the nature of the relationship between force and Myoton parameters. Also the time course of the effect of activity could have been examined by extending the follow-up period until Myoton parameters had returned to pre-activity levels. The effect of changes in muscle conditions on mechanical properties could have been assessed in other muscles and older adults to identify possible causes of error when using the Myoton technology in this age group. The reliability of the device has been reported by the research group in previous studies [2, 4, 22], but reliability for testing muscle parameters for the different setups in the current study was not examined. The precise change in muscle length from lying to sitting was not recorded in the present study but results of this experiment have highlighted the sensitivity and potential use of the MyotonPRO device with ultrasonography to objectively measure length-tension properties of skeletal muscles. These data provide the basis for more studies in other muscles and the robust nature of the device makes it suitable for measuring both large and smaller muscles in human studies.

The present findings have added to published work on the validity of the Myoton technique, in terms of responsiveness to change, for assessing muscle properties in healthy groups. Clinicians and researchers need to be mindful of factors such as the exact location of the probe, length of the muscle being assessed, the level of muscle contraction and prior activity to ensure accurate measurements are made. The present finding that contraction altered Myoton parameter values to a greater extent than the MDC, demonstrated contractile state is the most important of the four factors studied for a robust recording. This highlights the importance of ensuring participants are relaxed during testing at rest or maintain constant force during contraction. The portability of the MyotonPRO device makes it a valuable tool to assess muscle mechanical properties outside laboratory settings [27].

In conclusion, moving the Myoton probe away from the muscle midpoint, altering muscle length and level of contraction, and prior physical activity significantly altered mechanical tone and stiffness of BB and RF in healthy adults. Clinically relevant changes in Myoton parameters only occurred with altered level of contraction. The present findings have provided evidence that ensuring muscles are relaxed or under a constant force of contraction when using the Myoton technology to assess muscle properties is crucial. These findings will improve the robustness of existing protocols for assessing muscle mechanical properties using the MyotonPRO device.

Acknowledgement: The authors thank the Ghana Education Trust Fund and Faculty of Health Sciences, University of Southampton for funding a PhD studentship for SA-B; Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis for supporting MW [Grant ref: 20194]; and the manufacturer and supplier of the device, Myoton AS (Estonia) for loaning us the equipment and providing training.

Submission Statement

“We represent that this submission is original work, and is not under consideration for publication with any other journal”.

References

1. Aarrestad DD, Williams MD, Fehrer SC, Mikhailenok E, Leonard CT. Intra- and interrater reliabilities of the Myotonometer when assessing the spastic condition of children with cerebral palsy. J Child Neurol. 2004;19(11):894-901.
2. Agyapong-Badu S, Aird L, Bailey L, Mooney K, Mullix J, Warner M, et al. Interrater reliability of muscle tone, stiffness and elasticity measurements of rectus femoris and biceps brachii in healthy young and older males. WPHS. 2013;1(4):1-11.
3. Agyapong-Badu S, Warner M, Samuel D, Stokes M. Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device. Arch Gerontol Geriat. 2016;62:59-67.
4. Aird L, Samuel D, Stokes M. Quadriceps muscle tone, elasticity and stiffness in older males: Reliability and symmetry using the MyotonPRO. Archives of Gerontology and Geriatrics. 2012;55(2):e31-e9. doi: 10.1016/j.archger.2012.03.005.
5. Alexander RM. Tendon elasticity and muscle function. Comparative Biochemistry and Physiology. 2002;Part A(133):1001-11.
6. Bizzini M, Mannion AF. Reliability of a new, hand-held device for assessing skeletal muscle stiffness. Clin Biomech. 2003;18(5):459-61. Epub 2003/05/24. doi: S0268003303000421 [pii]. PubMed PMID: 12763442.
7. Brughelli M, Cronin J. Altering the length-tension relationship with eccentric exercise : implications for performance and injury. Sports Med. 2007;37(9):807-26.
8. Buford T, Lott D, Marzetti E, Wohlgemuth S, Vandenborne K, Pahor M, et al. Age-related differences in lower extremity tissue compartments and associations with physical function in older adults. Experimental Gerontology. 2012;47(1):38-44.
9. Chuang LL, Wu CY, Lin KC. Reliability, validity and responsiveness of myotonometric measurement of muscle tone, elasticity and stiffness in patients with stroke. Archives of physical medicine and rehabilitation. 2012;93(3):532-40. Epub 2012/01/10. doi: 10.1016/j.apmr.2011.09.014. PubMed PMID: 22222143.
10. Davidson MJ, Bryant AL, Bower WF, Frawley HC. Myotonometry Reliably Measures Muscle Stiffness in the Thenar and Perineal Muscles. Physiotherapy Canada. 2017;69(2):104-12. doi: 10.3138/ptc.2015-85.
11. Delaney S, Worsley P, Warner M, Taylor M, Stokes M. Assessing contractile ability of the quadriceps muscle using ultrasound imaging. Muscle & Nerve. 2010;42(4):530-8. doi: 10.1002/mus.21725.
12. Ditroilo M, Hunter A, Haslam S, De Vito G. The effectiveness of two novel techniques in establishing the mechanical and contractile responses of the biceps femoris. Physiol Meas. 2011;32:1315-26.
13. Gavronski G, Veraksits A, Vasar E, Maaroos J. Evaluation of viscoelastic parameters of the skeletal muscles in junior triathletes. Physiol Meas. 2007;28(6):625-37.
14. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. Journal of Electromyography and Kinesiology. 2000;10(5):361-74. doi: 10.1016/s1050-6411(00)00027-4.
15. Ikezoe T, Mori N, Nakamura M, Ichihashi N. Atrophy of the lower limbs in elderly women: is it related to walking ability? Eur J Appl Physiol. 2011;111(6):989-95.
16. Jarocka E, Marusiak J, Kumorek M, Jaskólska A, Jaskólski A. Muscle stiffness at different force levels measured with two myotonometric devices. Physiological Measurement. 2012;33(1):65.
17. Korhonen RK, Vain A, Vanninen E, Viir R, Jurvelin JS. Can mechanical myotonometry or electromyography be used for the prediction of intramuscular pressure? Physiological Measurement. 2005;26(6):951-63. Epub 2005/11/29. doi: S0967-3334(05)90975-1 [pii]

   10.1088/0967-3334/26/6/006. PubMed PMID: 16311444.
18. Little P, Stuart B, Stokes M, Nicholls C, Roberts L, Preece S, et al. Alexander Technique and Supervised Physiotherapy Exercises in back paiN (ASPEN): a four-group randomised feasibility trial. NIHR Efficacy Mech Eval. 2014;1(2):1-81.
19. Marusiak J, Jaskólska A, Koszewicz M, Budrewicz S, Jaskólski A. Myometry revealed medication-induced decrease in resting skeletal muscle stiffness in Parkinson's disease patients. Clin Biomech. 2012;27(6):632-5.

20. Marusiak J, Kisiel-Sajewicz K, Jaskolska A, Jaskolski A. Higher muscle passive stiffness in Parkinson's disease patients than in controls measured by myotonometry. Archives of physical medicine and rehabilitation. 2010;91(5):800-2.

21. Marusiak J, Żeligowska E, Mencel J, Kisiel-Sajewicz K, Majerczak J, Zoladz JA, et al. Interval training-induced alleviation of rigidity and hypertonia in patients with Parkinson's disease is accompanied by increased basal serum brain-derived neurotrophic factor. J Rehabil Med. 2015;47(4):372-5.

22. Mullix J, Warner M, Stokes M. Testing muscle tone and mechanical properties of rectus femoris and biceps femoris using a novel hand held MyotonPRO device: relative ratios and reliability. WPHS. 2012;1(1):1-8.

23. Mustalampi S, Hakkinen A, Kautiainen H, Weir A, Ylinen J. Responsiveness of Muscle Tone Characteristics to Progressive Force Production. J Strength Cond Res. 2013;27(1):159-65.

24. Pruyn EC, Watsford ML, Murphy AJ. Validity and reliability of three methods of stiffness assessment. JSHS. 2015:1-8. doi: http://dx.doi.org/10.1016/j.jshs.2015.12.001.

25. Rätsep T, Asser T. Changes in viscoelastic properties of skeletal muscles induced by subthalamic stimulation in patients with Parkinson's disease. Clin Biomech. 2011;26(2):213-7.

26. Roberts TJ. The integrated function of muscles and tendons during locomotion. Comparative Biochemistry and Physiology 2002;Part A(133):1087-99.

27. Schneider S, Peipsi A, Stokes M, Knicker A, Abeln V. Feasibility of monitoring muscle health in microgravity environments using Myoton technology. Medical & Biological Engineering & Computing. 2015;53(1):57-66. doi: 10.1007/s11517-014-1211-5.

28. Viir R, Laiho K, Kramarenko J, Mikkelsson M. Repeatability of trapezius muscle tone assessment by a myometric method. J Mech Med Biol. 2006;6(2):215-28.

29. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): Development and evaluation. J Clin Epidemiol. 1993;46(2):153-62. Epub 1993/02/01. doi: 0895-4356(93)90053-4 [pii]. PubMed PMID: 8437031.

Tables

Table 1.Participant characteristics

	Mean ± SD			
	Young male (n=21)	Young female (n=5)	Older male (n=10)	Older female (n=17)
Age (years)	24.1 ± 4.7	25.4 ± 3.6	75.8 ± 7.6	78.2 ± 7.9
Height (m)	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Body mass(kg)	73.9 ± 9.3	55.1 ± 5.2	81.8 ± 10.1	62.6 ± 9.4
BMI (kg/m^2)	23.2 ± 2.5	21.2 ± 0.9	27.1 ± 2.8	24.6 ± 2.6

SD; standard deviation, BMI; body mass index

Table 2. Effect of recording site on rectus femoris and biceps brachii elasticity, tone and stiffness using repeated measures (ANOVA) and paired t-tests

	Mean ± SD					ANOVA	
	Midpoint	Medial	Lateral	Superior	Inferior	F	P value
			Young n=26				
Rectus Femoris							
Elasticity	1.29±0.2	1.25±0.2	1.26±0.2	1.25±0.2	1.26±0.2	4,100=2.1	0.09
Tone	16.2±2.1	15.6±1.7*	16.2±1.9	16.0±1.8	16.2±1.9	2.4,60.9=8.9	<0.001[1]
Stiffness	290±46	279±43*	291±44	286±40	291±44	2.8,70.1=9.1	<0.001[1]
Biceps Brachii							
Elasticity	1.08±0.2	1.07±0.2	1.11±0.2	1.0±0.2	1.02±0.1*	2.2,54.4=4.1	0.02[1]
Tone	13.7±0.7	13.5±0.7*	14.2±1.1*	13.6±0.7	13.9±0.7*	1.9,46.4=14.9	<0.001[1]
Stiffness	207±23	200±26*	217±28*	203±22	212±22*	2.55,63.8=21.6	<0.001[1]
			Older n=27				
Rectus Femoris							
Elasticity	1.64±0.2	1.64±0.2	1.62±0.3	1.63±0.2	1.55±0.3	1.9,47.9=1.8	0.18[1]
Tone	15.5±2.2	14.6±1.7*	15.9±2	15±1.9	16±2.2*	2.7,66.8=15.9	<0.001[1]
Stiffness	319±43	300±32*	329±43*	308±39	329±47*	4,100=21.2	<0.001
Biceps Brachii							
Elasticity	1.52±0.2	1.52±0.2	1.76±0.4*	1.57±0.3*	1.49±0.2	1.9,50.7=9.2	<0.001[1]
Tone	15.2±1.4	14.8±1.4*	16.3±2.3*	15.4±1.6	15.5±1.5	2.5,63.6=7.5	<0.001[1]
Stiffness	272±29	269±31	291±39*	282±40	279±35	2.1,54.5=4.4	0.02[1]

SD; standard deviation, midpoint; muscle belly, medial;1cm medial to midpoint, lateral;1cm lateral to midpoint, superior; 1cm superior to midpoint, inferior; 1cm inferior to midpoint, elasticity (log decrement), tone (frequency, Hz), stiffness (N/m), *significant difference between midpoint values and four other positions at p<0.05level (2tailed), [1]Greenhouse-Geisser correction

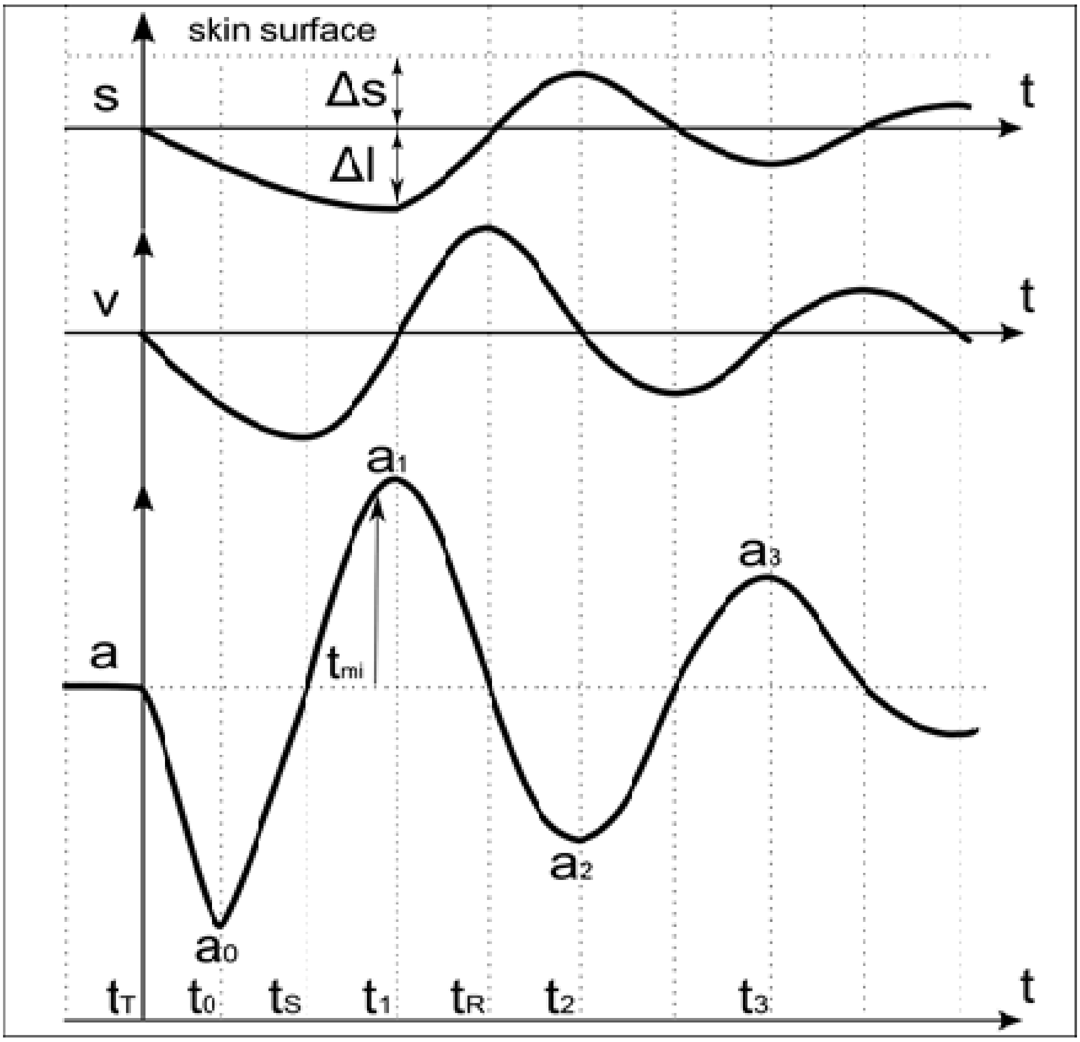

Figure 1. A description of the relationship between displacement oscillation (S) and oscillation velocity (V) in relation to oscillation acceleration (a). a_{max}= maximum acceleration (a_o), m_{probe}= mass of measurement mechanism, Δl= maximum displacement of the tissue, a_1= maximum displacement; maximum tissue resistance measured in mG, a_3= maximum displacement of the second period of oscillation which takes place due to the recuperation of stored residual mechanical energy in the tissue

Figure 2. MyotonPRO technique for measurement of rectus femoris (RF) stiffness, tone and elasticity. The device probe is held perpendicular to the skin for accurate recordings

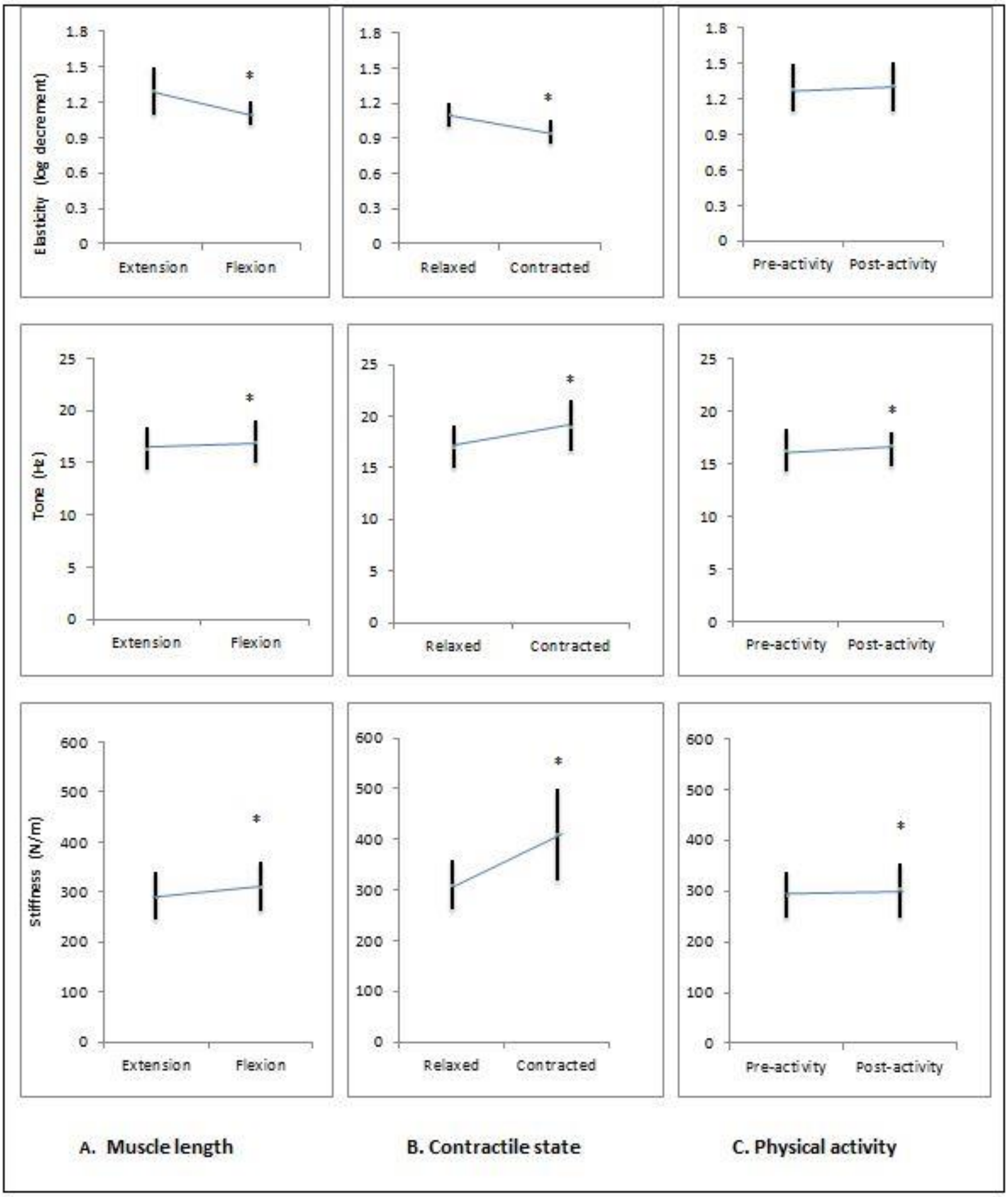

Figure 3**.** Variations in rectus femoris mechanical properties under three conditions of changes in: a)muscle length, b) contractile state, c) prior physical activity (mean and SD values). Significant changes ($p<0.001$; highlighted with *) in Myoton parameters tone and stiffness only were observed under changes in muscle length, level of contraction and prior activity