Abstract
Purpose: To use a novel, non-invasive hand held device (MyotonPRO) to quantify ratios of relative non-neural tone and mechanical properties of the rectus femoris (RF) and biceps femoris (BF) muscles, and to assess reliability of a novice user. Relevance: The device offers rapid, objective testing of mechanical parameters of muscle in clinical or sports settings. Participants: 21 healthy males (20-35 years)
Methods: Relaxed muscle parameters of RF and BF were obtained using the MyotonPRO. The device applies a brief mechanical impact, producing muscle oscillations from which tone (state of intrinsic tension, indicated by frequency [Hz]) and mechanical properties of elasticity (logarithmic decrement) and stiffness (N/m) are measured. Data were collected on two days, one week apart. Two series of 10 single measurements on each muscle were used to test within-day reliability. The mean of the two sets was used for between-day reliability.
Analysis: The relative parameters between RF and BF were expressed as a ratio. Reliability was assessed using intra-class correlation coefficients (ICCs).
Results: The mean (±SD) RF:BF ratios for resting muscle were: frequency 1:0.96 (±0.05), decrement 1:1.10 (±0.17) and stiffness 1:0.95 (±0.07). Reliability of all three parameters was excellent within-sessions (ICCs 3,2 >0.99) and good between-days (ICCs 3,1 0.72-0.87).
Conclusions: The relative resting tone and mechanical properties of RF and BF has been characterised in young males, with ratios close to 1:1. Measures made by a novice user were reliable, indicating that the MyotonPRO has the potential for assessing changes in muscle properties objectively over time. Studies are needed in large healthy cohorts of different ages, activity levels and genders to produce reference data for assessing patients.
Implications: The relative tone and mechanical properties of RF and BF could potentially be used as a rapid method for assessing risk of injury in sporting populations and presence of abnormality in musculoskeletal and neurological conditions, once normal values have been established in relevant groups.
Keywords: Myotonometry, Mechanical properties, Reliability, Quadriceps, Hamstrings

Acknowledgements
The authors thank the participants for their time, Dr Peter Nicholls for statistical support and the following students on related projects who collaborated on the standardisation of protocols between studies of other muscles and participant groups: PhD student Sandra Agyapong-Badu; and MSc students Kate Mooney, Lucy Aird and Louise Bailey. The authors also thank Myoton AS (Estonia) and Myoton Ltd (London) for providing the MyotonPRO device and training by Aleko Peipsi (CEO Myoton). There were no conflicts of interest within the present study, which was conducted independently of the company providing the equipment.

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Testing muscle tone and mechanical properties of rectus femoris and biceps femoris using a novel hand held MyotonPRO device: relative ratios and reliability
James Mullix, Martin Warner, Maria Stokes
INTRODUCTION

Objective measurement of muscle tone or state of tension (Masi et al. 2010) and mechanical properties, such as elasticity and stiffness, in clinical or sports settings would greatly enhance detection of risk of injury and monitoring of the effects of interventions, such as treatment or training. Various devices are available for testing the tone and mechanical properties of skeletal muscle but some are more clinically feasible to use than others (Chen et al. 2005; Gavronski et al. 2007; Gennisson et al. 2005; Leonard et al. 2004; Tous-Fajardo et al. 2010; Vir et al. 2006). These devices elicit mechanically or electrically evoked (Tous-Fajardo et al., 2010) muscle activity allowing measurement of tone, contractile and mechanical properties. Validity of some devices has been demonstrated, e.g. by correlation with established diagnostic methods for quantifying muscle tone, such as electromyography (EMG) and intramuscular pressure (e.g. Ditroilo et al. 2011; Khorhonen et al. 2005; Leonard et al. 2004).

A new hand held device (MyotonPRO; Myoton AS, Estonia and Myoton Ltd, London), offers in-vivo, non-invasive measurement of non-neural tone and mechanical properties, using accelerometer based sensing of damped muscle oscillations. The MyotonPRO is hand held and relatively easy to use, making it suitable for testing outside the laboratory in clinical and sports environments. The device elicits secondary oscillations of muscle after a brief mechanical impact (i.e. tap) with quick release under constant preload, which is pre-set within the device. Reliability studies conducted with earlier Myoton prototypes (e.g. Myoton-2) found excellent intra-rater reliability (intraclass correlation coefficients, ICCs, 0.98-0.99), and inter-rater reliability (ICCs 0.97-0.99) in the trapezius muscles in one testing session (Vir et al. 2006). Test-retest reliability of stiffness on two days found good to excellent reliability (ICCs 0.80-0.93) for all muscles assessed (rectus femoris, RF, long head of biceps femoris, BF, vastus lateralis and gastrocnemius) except for the vastus lateralis (ICC 0.40) (Bizzini and Mannion 2003). No data were reported for tone or elasticity.

The technology of the MyotonPRO differs from its predecessors by using a triaxial accelerometer and a system which allows measurements to be taken with the device held in any direction, as it is not affected by gravity. Previous devices required the probe to be held vertical, limiting the available muscles and postures in which testing could be conducted. The MyotonPRO therefore enables access to more muscles, as the probe can be applied at different angles (remaining perpendicular to muscle surface).

Studies have measured RF and/or BF characteristics (Bizzini and Mannion 2003; Ditroilo et al. 2011; Gavronski et al. 2007; Hein and Vain 1998; Rihvk et al. 2010; Vain 2002). However, sample sizes were generally too small and limited to specific participant groups, e.g. two male cross country skiers (Vain 2002) and five male triathletes (Gavronski et al. 2007). Cross-joint muscle imbalance between quadriceps and hamstrings is associated with muscle injuries and lower limb joint dysfunction, and relative measures of muscle characteristics can indicate imbalance e.g. strength/power (Croce and Miller 2006). The relative mechanical properties between antagonistic muscles have not been characterised in healthy individuals and may provide useful reference data for assessing muscle imbalance.

The present study aimed to quantify the relationship between the properties of RF and BF in moderately active, healthy young males, by expressing the data as ratios for assessing muscle imbalance. A further aim was to examine intra-rater reliability of a novice operator in the use of the new MyotonPRO device, both within a testing session and between different days.

METHODS

The MyotonPRO (MyotonAS, Estonia and Myoton Ltd, London) is a wireless hand held device, which is placed perpendicular to the skin over the muscle belly being measured. The device is applied under constant preload (0.18N), to pre-compress subcutaneous tissues, and exerts a brief (15 milliseconds) mechanical tap at a pre-determined force (0.4 Newtons), followed by quick release, causing damped oscillations which are recorded by the testing probe (http://www.myoton.com/en/Technology/Technical-specification). Non-neural tone, or state of tension, is calculated from signal spectrum Fmax (FFT – Fast Fourier Transform) and is characterised by frequency (Hz) of the damped oscillations. Stiffness (N/m) is characterised by the muscle’s ability to resist an external force that modifies its shape (Pisano et al. 2000). Elasticity is characterised by the logarithmic decrement (log) of the damped oscillations (dissipation of mechanical energy during one oscillation cycle), reflecting ability of the tissue to recover its shape after being deformed.

Participants

Twenty-one healthy males (mean age 25.7 years, standard deviation 4.2, range 20-35 years) were recruited among university staff and students. A sample of 20 is recommended as the minimum for reliability testing (Atkinson and Nevill 2001). Participants had no history of neurological or musculoskeletal disorders, back or lower limb injuries such as fractures, or pain that restricted activity for more than one week. They were permitted to undertake vigorous physical activity up to three times per week. Anthropometric details are shown in Table 1. Those with BMI >30 kg/m² were excluded, as muscle properties may be masked by excessive subcutaneous tissue (Gapayeva and Vain 2008). Participants were right lower limb dominant, assessed by the foot used to kick a ball (Hoffman et al. 1998), and the right side was tested. They were asked to avoid alcohol for 24 hours and strenuous physical activity for at least 48 hours prior to testing.

<table>
<thead>
<tr>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.1</td>
<td>1.65 - 1.93</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.2 ± 10.9</td>
<td>60.0 - 99.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.86 ± 2.5</td>
<td>19.2 - 29.4</td>
</tr>
</tbody>
</table>

Table 1. Anthropometric details for height (metres), weight (kg) and body mass index (BMI; kg/m²) in 21 healthy young males.


Ethical approval was obtained from the Faculty of Health Sciences Ethics Committee, University of Southampton (Ethics No: FoHS-ETHICS 2011-049) and all participants gave their written informed consent.

**Procedures**

Testing took place on two days, a week apart and at the same time of day. Participants initially lay supine on a plinth and rested for 10 minutes. Room and skin temperature on the thigh were recorded using a digital thermometer. Testing sites on the muscle belly were located using a tape measure and marked using a skin-safe pen. The RF site was two thirds between the anterior superior iliac spine and the superior pole of the patella. The hip was held in neutral by sandbags placed lateral and medial to the ankle. In the prone position, the BF site was midway between the ischial tuberosity and head of the fibula. A pillow was placed under the lower leg to aid relaxation.

The MyotonPRO device was set in multiscan mode to produce 10 consecutive single measurements (one second apart). The probe was placed perpendicular to the skin at the test site over the muscle being measured, as illustrated in Figure 1. Starting with the RF, two sets of 10 recordings were made and this was repeated for BF. The coefficient of variation (CV) of the 10 measurements is displayed and any set of parameters (frequency, decrement, stiffness) with a CV >3% was discarded and the test repeated. The mean of each set of 10 was used for analysis. On the second day, testing sites were relocated and tests repeated.

**Data Analysis**

Data were managed using Microsoft Excel 2007 and analysed using SPSS (Version 17.0). Data were tested for normality using the Shapiro-Wilk's test. A ratio of the relative values for each parameter was produced by dividing the RF value by the BF value (RF:BF).

Reliability between trials (within session) and between days was tested using ICC model 3, 2 (two-way mixed model) (Shrout and Fleiss 1979). Standard error of measurement (SEM) was calculated as $SEM=SD\sqrt{1-ICC}$, where SD is the standard deviation of the pooled data from both measurement sets and ICC is the reliability coefficient (Fleiss 2007; Portney and Watkins 2000). Various classification scales exist for interpreting ICCs and two are reported here: Excellent > 0.75; good to fair = 0.74 – 0.4; poor < 0.4 (Fleiss 2007); very high =1.00– 0.90; high = 0.89–0.70; moderate = 0.69–0.50; low = 0.49– 0.26 (Domholdt 1993). Minimal detectable difference (MDD) was calculated as $MDC=SEM(V2)\times 1.96$ to estimate meaningful differences (Fletcher and Bandy 2008). Bland and Altman analysis was used to calculate mean difference, 95% confidence intervals (95% CI) and graphically to assess bias (Bland and Altman 1986).

**RESULTS**

Data from Day 1 were used to calculate mean ratios and the group means and SDs were: frequency 1:0.96 (±0.05), decrement 1:1.10 (±0.17) and stiffness 1:0.95 (±0.07), with frequency being closest to 1:1 and having the lowest standard deviation, as seen in Figure 2.

Reliability for within day tests was excellent/very high for both muscles (all ICCs >0.990, see Table 2), according ICC classification (Domholdt 1993; Fleiss 2007). Between day reliability was good/high, with ICCs for RF 0.81-0.87 and for BF 0.72-0.86. The SEM and MDD values were similar within and between days (Table 2).

Example Bland and Altman plots of BF stiffness (N/m) displays both within (Figure 3) and between day (Figure 4).
variability. Mean difference for within day measures of BF stiffness was -0.07 and for between days was -2.54. The ranges for limits of agreement were -10.1 to 8.6 for within day and -15.9 to 10.8 for between days, further supporting the ICC results where lower variability was found within session (ICC 0.99) than between days (ICC 0.72). Within day and between day Bland and Altman results for the three variables in both muscles are shown in Table 2.

Example Bland and Altman plots of BF stiffness (N/m) displays both within (Figure 3) and between day (Figure 4) variability. Mean difference for within day measures of BF stiffness was -0.07 and for between days was -2.54. The ranges for limits of agreement were -10.1 to 8.6 for within day and -15.9 to 10.8 for between days, further supporting the ICC results where lower variability was found within session (ICC 0.99) than between days (ICC 0.72). Within day and between day Bland and Altman results for the three variables in both muscles are shown in Table 2.

Reliability of the ratios between days was good, with ICCs of 0.79 for frequency (Hz), 0.72 for decrement (Log), and 0.80 for stiffness (N/m).

<table>
<thead>
<tr>
<th>Bland and Altman 95% CI</th>
<th>WITHIN SESSION</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>LOWER</th>
<th>UPPER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>Set 2</td>
<td>ICC</td>
<td>SEM</td>
<td>MDD</td>
<td>0.99</td>
<td>1.34</td>
<td>0.04</td>
<td>-0.28</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>RF 15.5 ± 1.06</td>
<td>15.4 ± 1.09</td>
<td>0.99</td>
<td>0.49</td>
<td>1.34</td>
<td>0.04</td>
<td>-0.28</td>
<td>0.36</td>
</tr>
<tr>
<td>BF 16.2 ± 1.02</td>
<td>16.2 ± 1.10</td>
<td>0.99</td>
<td>0.46</td>
<td>1.21</td>
<td>0.04</td>
<td>-0.34</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Decrement (Log)</td>
<td>RF 1.34 ± 0.19</td>
<td>1.31 ± 0.19</td>
<td>0.99</td>
<td>0.07</td>
<td>0.19</td>
<td>0.02</td>
<td>-0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>BF 1.22 ± 0.15</td>
<td>1.21 ± 0.15</td>
<td>0.99</td>
<td>0.09</td>
<td>0.24</td>
<td>0.01</td>
<td>-0.04</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Stiffness (N/m)</td>
<td>RF 276 ± 22.0</td>
<td>275 ± 21.6</td>
<td>0.99</td>
<td>10.4</td>
<td>28.8</td>
<td>1.05</td>
<td>-5.55</td>
<td>7.64</td>
</tr>
<tr>
<td>BF 290 ± 26.3</td>
<td>290 ± 26.8</td>
<td>0.99</td>
<td>13.5</td>
<td>37.4</td>
<td>-0.07</td>
<td>10.1</td>
<td>8.57</td>
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<tr>
<td>BETWEEN DAYS</td>
<td>Day 1</td>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>RF 15.5 ± 1.07</td>
<td>15.4 ± 1.06</td>
<td>0.81</td>
<td>0.47</td>
<td>1.29</td>
<td>0.11</td>
<td>-1.23</td>
<td>1.44</td>
</tr>
<tr>
<td>BF 16.2 ± 1.06</td>
<td>16.1 ± 1.15</td>
<td>0.86</td>
<td>1.1</td>
<td>1.16</td>
<td>0.11</td>
<td>-1.08</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Decrement (Log)</td>
<td>RF 1.32 ± 0.19</td>
<td>1.29 ± 0.21</td>
<td>0.87</td>
<td>0.07</td>
<td>0.21</td>
<td>0.03</td>
<td>-0.18</td>
<td>0.25</td>
</tr>
<tr>
<td>BF 1.21 ± 0.14</td>
<td>1.19 ± 0.11</td>
<td>0.77</td>
<td>0.13</td>
<td>0.18</td>
<td>-0.2</td>
<td>-0.16</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Stiffness (N/m)</td>
<td>RF 276 ± 21.8</td>
<td>275 ± 20.0</td>
<td>0.83</td>
<td>8.57</td>
<td>23.8</td>
<td>1.02</td>
<td>-23.53</td>
<td>25.53</td>
</tr>
<tr>
<td>BF 290 ± 26.4</td>
<td>286 ± 26.3</td>
<td>0.72</td>
<td>26.1</td>
<td>38.7</td>
<td>3.87</td>
<td>-35.93</td>
<td>43.67</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Muscle tone and mechanical properties and reliability of the rectus femoris (RF) and biceps femoris (BF), within session and between days, in a group of healthy young males (mean ± standard deviation)

Mean difference; ICC = intraclass correlation coefficient; SEM = standard error of measurement; MDD = minimal detectable change.
95% CI, 95% confidence intervals, with lower and upper limits of agreement.
Figure 3 Within day Bland and Altman plot for stiffness of biceps femoris on day one. Mean difference = -0.07 N/m.

Figure 4 Between day Bland and Altman plot for stiffness of biceps femoris. Mean difference = 3.86 N/m.
DISCUSSION
This study has indicated that the relative tone and mechanical properties of rectus femoris and biceps femoris in healthy young males, with low to moderate habitual physical activity levels, are approximately 1:1 and this ratio could be used to assess muscle imbalance in clinical and sporting settings. Measurements obtained by a novice user were reliable on different days using a new hand held device, the MyotonPRO.

The across joint ratios, for each parameter, were within 0.9 to 1.1 for a healthy group of young males. This demonstrates the potential for using these ratios for detecting risk of muscle injury from a subclinical muscle imbalance. Effects of treatments could also be monitored over time, such as stretching and exercise, and guide return to activity after injury. Larger numbers would, however, be needed to determine the reference data in relevant healthy populations of different ages, genders and activity levels, including specific sports.

Preliminary data from current studies on elite sporting populations by the authors’ research group indicate that the balance between RF and BF is not always as close to 1:1 as in the non-elite. These observations stress the importance of sport specific studies to characterise the relationship and thus enable accurate screening for risk of injury or assessment of existing injury. Between-side comparison is often used clinically to detect abnormalities but it is necessary to know how symmetrical a given characteristic is between the two sides of the body. For example, strength asymmetry of the hamstring has been identified as a predisposing factor to injuries in footballers, as a weaker hamstring may increase the risk of injury (Zakas 2006). Asymmetry of 15% has been shown to increase the risk of injury (Croisier et al. 2008).

Symmetry of MyotonPRO measurements for quadriceps in healthy older males was recently found to be very high, with differences between the dominant and non-dominant sides being less than 2.5% for all three parameters (Aird et al. 2012). Studies are needed to determine the level of symmetry of the mechanical properties of various muscles, in different populations, to enable accurate assessment of asymmetry using the MyotonPRO.

The MyotonPRO demonstrated excellent intra-rater reliability within sessions and good reliability between days for RF and BF measurements obtained by a novice user. The lower reliability observed between days than within the same session could be due to a combination of several factors, including: skill of the operator in finding the same testing site on different days; different state of the muscle due to e.g. different length from slightly altered joint angle; different activity prior to testing, or change in temperature etc. Differences in recordings were unlikely to be due to the devices, which are calibrated by the manufacturer do not vary on different days when tested on phantom materials. Between-day reliability is necessary to enable monitoring of changes over time in response to an intervention e.g. treatment or sports training. The SEM and MDD values between-days (Table 2) provide guidance for the expected error, beyond which differences between groups or change over time would indicate real difference in mechanical properties (Portney and Watkins 2000). The present between-day findings are similar to those found for stiffness using an earlier prototype, the Myoton-2 (Bizzini and Mannion 2003), with between day ICCs for RF and BF of 0.85 and 0.80 respectively. As with the present study, reliability was higher for RF than BF, possibly due to relocation of the testing site on different days; different skill of the operator in finding the same session could be due to a combination of several factors, including: age bands, males and females, from sedentary to specific elite sports populations.

Comparisons of absolute values with previous studies are made in Table 3. Values for BF closely matched those from a study using the Myoton-3 device (Rihv et al. 2010). Larger differences seen for other studies (Ditroilo et al. 2011;Gavronski et al. 2007;Hein and Vain 1998; Hein & Vain 1998; Vain 2002) may be due to different methodological approaches, as well as participant characteristics and activity levels. For example, in studies of cross country skiers (Vain 2002) and triathletes (Gavronski et al. 2007), the athletes had lower tone (frequency values) and less stiffness (N/m values) than in the present study for both RF and BF (Table 3), although those studies only included two and five participants respectively. Further research into both specific sporting groups and the general population (ages, genders) is therefore needed to provide relevant reference data sets.

A limitation of the present study was that reliability testing was restricted to one rater, so inter-rater reliability of the MyotonPRO needs to be established. Reliability for BF might be improved by more robust relocation of the testing site, e.g. a second measurement around mid-thigh, intersecting the vertical location reference at 90 degrees, could aid consistency for muscle belly testing location in both horizontal and vertical planes. Another possibility, although subjective, is to ask the participant to make a small contraction to enable the investigator to palpate the muscle belly.

Research to determine the tolerance for measurement error is warranted for different muscles. Larger numbers need to be studied in different participant groups including: age bands, males and females, from sedentary to specific elite sports populations.
### Table 3 Comparative data of mechanical properties of relaxed rectus femoris (RF) and biceps femoris (BF) muscles from previous studies in the literature using earlier Myoton prototypes. Percentage difference between the previous studies and the present study are given in parentheses.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Participants</th>
<th>Muscle(s)</th>
<th>Mean values (% difference from present findings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study - Mullix et al (2012)</td>
<td>21 male university staff and students</td>
<td>RF</td>
<td>15.5</td>
</tr>
<tr>
<td>Heiv &amp; Vain (1998)</td>
<td>22 male physical education students</td>
<td>RF</td>
<td>13.21</td>
</tr>
<tr>
<td>Vain (2002)</td>
<td>Two male cross-country skiers</td>
<td>RF</td>
<td>13.54</td>
</tr>
<tr>
<td>Bizzini &amp; Mannion (2003)</td>
<td>Five male and five female volunteers</td>
<td>RF</td>
<td>12.59</td>
</tr>
<tr>
<td>Gavronski et al (2007)</td>
<td>Five male triathletes</td>
<td>RF</td>
<td>12.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BF</td>
<td>14.03</td>
</tr>
</tbody>
</table>

Ratios of the relative tone and mechanical properties have been produced for RF and BF, which were almost 1:1 in healthy young males, indicating that larger studies are warranted to produce reference data for other muscles and cohorts to aid assessment of muscle balance. Intra-rater reliability of the MyotonPRO device were excellent (ICC>0.99) within a session and good between-days for RF (ICC>0.81) and BF (ICC>0.72). Reliability for BF may be improved by more accurate relocation of the testing site. Frequency (Hz) was the most reliable variable, followed by stiffness (N/m) and then decrement (Log). Further research using standardised protocols is needed in larger groups to provide reference data for absolute values of the three parameters studied, as well as the ratios of relative values. Specifically, homogeneous groups of different ages, genders and levels of physical activity, including sporting groups, need to be studied to provide reference data for comparison with patients to assess risk of injury and changes with treatment.

### CONCLUSIONS

Ratios of the relative tone and mechanical properties have been produced for RF and BF, which were almost 1:1 in healthy young males, indicating that larger studies are warranted to produce reference data for other muscles and cohorts to aid assessment of muscle balance. Intra-rater reliability of the MyotonPRO device were excellent (ICC>0.99) within a session and good between-days for RF (ICC>0.81) and BF (ICC>0.72). Reliability for BF may be improved by more accurate relocation of the testing site. Frequency (Hz) was the most reliable variable, followed by stiffness (N/m) and then decrement (Log). Further research using standardised protocols is needed in larger groups to provide reference data for absolute values of the three parameters studied, as well as the ratios of relative values. Specifically, homogeneous groups of different ages, genders and levels of physical activity, including sporting groups, need to be studied to provide reference data for comparison with patients to assess risk of injury and changes with treatment.

### Key points

1. The generation of a ratio of muscle parameters indicates that this could be used as a reference against which to assess muscle imbalance between the RF and BF.

2. The excellent or good between-day and excellent within-session reliability of using the MyotonPRO, indicating its potential use for objective, non-invasive measurement of the non-neural tone and mechanical properties of superficial skeletal muscles.

3. These findings indicate that larger studies are warranted to produce reference data for different populations.
References


