

Intra-rater and inter-rater reliability of a handheld myotonometer measuring mechanical properties of lower lumbar miofascial tissue in healthy adults

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Background. Biomechanical muscle properties, such as stiffness, can be valuable indicators of tissue health and hold promise as diagnostic and treatment benchmarks for Chronic Low Back Pain (CLBP). The development of accessible assessment technologies, such as the MyotonPRO® portable device, allows for quantifying changes in muscle tone and stiffness conveniently. This study aims to evaluate the reliability of lumbar erector spinae muscle stiffness using MyotonPRO in healthy adults and to compare stiffness changes between prone and seated positions.

Methods. Thirty asymptomatic participants (N= 15 women and N= 15 men) ages between 18 and 65 years were recruited to participate in this study. Two examiners tested muscle stiffness on the palpable muscle belly, one finger from the spinous process at the level of the L4 vertebra, first from the left side and then from the right side, both in the prone position and after in the sitting position. For inter-rater reliability, all participants were tested by two examiners on the same day, and intra-rater reliability was calculated from the assessment results of the same examiner with an exact 24-hour interval. In order to assess the reliability, intraclass correlation coefficients (ICC), standard error measurements (SEM) and minimal detectable change (MDC) at the 95% confidence interval were calculated.

Results. Statistical data analysis revealed good intra-rater reliability, with an ICC of 0.88 (95% CI: 0.76–0.94), for the left erector spinae muscles stiffness and excellent intra-rater reliability, with an ICC of 0.91 (95% CI: 0.82–0.95) for the right erector spinae muscle respectively in prone position. The intra-rater reliability in sitting position was excellent, with an ICC of 0.91 (95% CI: 0.82-0.96) for the left side and good to excellent, with an ICC of 0.89 (95% CI: 0.78-0.95) for the right side. Results testing the left-side prone position demonstrated good inter-rater reliability with an ICC of 0.87 (95% CI: 0.73-0.94). Similarly, the right-side prone position demonstrated good inter-rater reliability with an ICC of 0.84 (95% CI: 0.68-0.92). The inter-rater reliability for the left and right side in the sitting position was excellent, with an ICC of 0.96 (95% CI: 0.92–0.98) for the left side and an ICC of 0.95 (95% CI: 0.90–0.97) for the right side, respectively.

Conclusion. This study demonstrated high intra- and inter-rater reliability for measuring the muscle stiffness of lumbar erector with the MyotonPRO in healthy adults and showed the device's ability to detect even small changes in erector spine stiffness, testing both right and left sides and measuring in both prone and sitting positions. The use of the seated position to assess

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Due to the fact that in this research only myofascial stiffness was investigated, it is suggested to replace the myofascial stiffness with mechanical properties.

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lumbar tissue tension in patients with CLBP could be a useful alternative to the prone position, especially for patients who are uncomfortable with prone position. This may have further practical implications for clinical practice.

INTRODUCTION

Musculoskeletal disorders are a major public health problem worldwide and one of the main causes of disability (World Health Organisation (WHO), 2023). According to a recent Global Burden of Disease study, around 1.71 billion people worldwide are affected by musculoskeletal disorders (Cieza et al., 2019). Non-communicable diseases account for almost a fifth of all cases in Slovenia and contribute to 15.6% of the total burden in terms of years lived with disability (YLDs). (Institute for Health Metrics & Evaluation (IHME), 2022).

Low back pain (LBP) is a widespread musculoskeletal disorder affecting up to 90% of individuals at some point, with over 50% experiencing recurrent episodes (Arya, 2014). Slovenia reports the highest LBP prevalence in Europe at 40.7% (eumusc.net, 2013), while LBP ranks as the second most common cause of work absenteeism (Sirbu et al., 2023). LBP prevalence increases with age, sedentary lifestyle, and social status, highlighting the need for targeted interventions and preventive measures.

Biomechanical muscle properties, such as stiffness, are objective parameters that indicate the condition of the tissue and could become a reference for the diagnosis and treatment of CLBP (Lohr et al., 2017). The modulation of the stiffness of the erector spinae muscle of the lumbar spine remains unclear. Further research into the biomechanical properties of the lumbar muscles is needed to identify the mechanism of activation in CLBP. New technologies such as magnetic resonance elastography (MRE) and shear wave elastography (SWE) have been used to quantify the biomechanical properties of the muscles (Hong et al., 2016; Koppenhaver et al., 2019). Therefore, all of these technologies have inherent limitations due to complex methodological procedures, image readout duration, transportability, accessibility and cost-effectiveness (Li et al., 2022).

Quantifying changes in paraspinal muscle tone and stiffness in the clinical setting remains a challenge. To measure lumbar muscle stiffness, an affordable and convenient assessment technology such as the MyotonPRO® portable, non-invasive digital handheld device has been developed. This device applies a short (15 ms) low-intensity (0.58 N) mechanical impulse to the skin and records the oscillatory tissue response. The internal software then calculates the resting tension, elasticity and stiffness of the tissue based on an acceleration curve (Bizzini and Mannion, 2003). The reliability and validity of the MyotonPRO® has been studied in various populations to measure the viscoelastic properties of different skeletal muscle groups in both healthy adults and individuals with pathological conditions. This includes assessing the properties of the lumbar and back muscles and myofascial tissue (Hu et al., 2018; Nair et al., 2017). The MyotonPRO® has good to excellent intra- and inter-rater reliability in healthy skeletal muscles (Chen, 2019). It has also been shown to reliably measure changes in superficial lumbar myofascial stiffness to a depth of 2 cm in both healthy individuals and those with lumbalgia (Grześkowiak, 2023). The myotonometer has also shown acceptable reliability when used in a clinical setting in young and

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Comment [Zc10]: According to the previous studies (Li et al. (2020), Feng et al. (2019), Lohr et al (2018)) that have been done in this field on healthy people and LBP patients, the novelty of the present research is not well stated.

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Comment [Zc12]: In the study of Li et al. (2020), the myofascial stiffness of LBP patients in the sitting position was investigated. Your current study is on healthy subjects, we cannot generalize the results to LBP patients.

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older adults with CLBP (XU et al., 2018; Wu et al., 2020). Li et al. (2020) demonstrated an excellent intra- and inter-rater reliability in quantifying lumbar erector spinae muscle stiffness in patients with CLBP in both prone and seated positions. Feng and Zhang (2019) also investigated changes in muscle stiffness in healthy volunteers in the static prone position, sitting and upright standing. These results suggest that it is useful to explore the modulation of the stiffness of the erector spinae muscle of the lumbar spine in different postures to prevent CLBP.

Currently, there are few studies evaluating the reliability of an instrument to measure lumbar muscle stiffness in pathological postures, either in prone or sitting position. Therefore, the aim of this study was to evaluate the intra- and inter-reliability of lumbar erector spinae muscle stiffness with the MyotonPRO in healthy adults and to compare the changes in lumbar spine stiffness between prone and seating position for the right and left side. This could provide therapists with a method to program and optimize all rehabilitation processes. The use of the seated position to assess lumbar tissue tension in patients with CLBP could be a useful alternative to the prone position, especially for patients who are uncomfortable in the prone position. This could have further practical implications for clinical practice.

MATERIALS & METHODS

Study design

The aim of this study was to evaluate the inter-rater and intra-rater reliability of the assessment of lumbar muscle stiffness of the erector spinae muscle in prone and seated positions using a MyotonPro® handheld myotonometer in healthy subjects.

Ethics statements

The current study was carried out in accordance with the Declaration of Helsinki and was authorized by the Republic of Slovenia National Medical Ethics Committee (No. 0120-520/2022/3). The research was conducted at the University Medical Centre of Ljubljana's Institute for Medical Rehabilitation and Clinical Department of Neurosurgery. Prior to participation, all volunteers have read an information about the purpose of the study and have provided written informed consent.

Sample

Thirty asymptomatic participants (N= 15 women and N= 15 men) between the ages of 18 and 65 years were recruited to participate in this study. A convenience sampling method was used. All participants (N= 30; mean age: 39,8 years, SD= 10,0 years; mean height: 174,5 cm, SD= 10,5 cm; mean body mass: 72,3 kg, SD= 14,7 kg; mean body mass index: 23,5, SD= 2,9) completed the experimental protocol with no reports of medical problems, discomfort or adverse reactions related to the study. Descriptive statistics of the participants is presented in (Table 1).

121 ***Inclusion exclusion criteria***

122 Participants were recruited if they were in good health with no musculoskeletal pain in the lower
123 back region during the preceeding week and on the day of testing. Exclusion criteria included a
124 history of previous spinal surgery, spinal deformity (such as scoliosis or kyphosis), osteoporosis,
125 lumbar disc protrusion, pregnancy, and a body mass index (BMI) of 30 kg/m² or higher (Nair et
126 al., 2016). Participants with significant spinal or other pathological conditions, malignant and
127 systemic diseases, or any neurological, respiratory, cardiovascular, and orthopedic disorder that
128 could have an impact on the test results were excluded from the study. Furthermore, the
129 consumption of alcohol and involvement in excessive physical activity within the 24 hours before
130 the test, which could result in dehydration and increased stiffness in the fascial tissue, were
131 regarded as grounds for exclusion.

132 ***Study instruments***

133 The stiffness of lumbar erector spinae muscles was measured using a noninvasive handheld
134 MyotonPro® device (Myoton Ltd., Estonia). The device recorded damped natural oscillation of
135 soft tissue in the form of an acceleration signal to assess the biomechanical properties of stiffness,
136 frequency, decrement, creep, and SRT from the acceleration and displacement signals. The
137 stiffness measurements were performed with the MyotonPro® device by Examiner 1, an
138 experienced physician who has been using the device in daily scientific research for 2 years, and
139 Examiner 2, an experienced physical therapist with over 20 years of practice in musculoskeletal,
140 fascial, and neurological rehabilitation. Prior to the start of the research, both examiners practiced
141 using the device on the lumbar erector spinae muscle. Prior to the main data collection, a pilot
142 test was conducted on a sample of ten individuals using the MyotonPro® device. This was done
143 to improve the examiners testing skills and to refine the protocol. Both examiners are right-
144 handed. The inter-rater reliability was considered as the assessment results from two examiner in
145 the same day, and the intra-rater reliability was calculated by the assessment results from the
146 same examiner with a exact 24 hour interval for all participants.

147 Measurements were obtained initially in resting prone position and then in sitting position
148 bilaterally (on the left side first and then on the right erector muscle belly at the level of L4
149 vertebrae. The location for the measurements was identified as the palpable belly of the muscle,
150 one finger's breadth away from the spinous process at the L4 level (Lorh et al., 2018). The exact
151 location was confirmed and marked with a permanent marker. All measurements were performed
152 in a designated room, where the temperature was maintained at a stable level of approximately
153 25°C for all participants.

154 ***Measurement of the erector spinae stiffnes in different positions***

155 When the subjects arrived, they were asked to rest and relax at room temperature for 10 min to
156 normalize body conditions. Demographic characteristics were measured before the start of the
157 experiment. The protocol procedure was shown to all participants one by one. The first to
158 conduct the measurements were the experienced physician (Examiner 1) and then experienced

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physical therapist (Examiner 2) performed the testing. Each of the subjects was asked to lie in a prone position and to relax on an examination table with their arms resting at their sides for 5 minutes before starting the procedure. To ensure greater relaxation, a foam pad was placed under the ankles to ensure a neutral, relaxed foot position. During the procedure, all participants were asked to hold their breath for 5 seconds at the end of inhalation to minimize the confounding factor resulting from the intra-abdominal pressure changes that occur with natural breathing cycles. Muscle stiffness was measured on the palpable muscle belly, one finger wide from the spinous process at the level of L4 vertebrae bilaterally both in prone position and sitting position. Left erector spinae belly muscle were measured from the participant's left side, and the right erector spinae belly muscle were measured from the right side. Due to a rather large sample size, and the fact that both sides were being measured, it was impossible for the examiners to memorise the results. The measurements were collected on a device separately, so the examiners were blinded to each other's measurements. For the second measurement, the subject was asked to sit in a neutral position on an examination table facing forward, with a head in a neutral position and feet in contact with the floor. To ensure inter-rater reliability, (Examiner 1 and Examiner 2) performed repeated measurements using the prone and sitting protocols 30 minutes apart. In addition, Examiner 2 repeated the test protocol after a 24-hour interval to confirm intra-rater reliability.

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Statistical procedures

Data analysis was performed using an Excel program (Microsoft Corporation, Washington, USA), and IBM SPSS Statistics 22 (IBM, New York, USA). Descriptive statistics were applied to condense the demographic dataset, representing central tendencies and variability through means and standard deviations. In evaluating reliability, intraclass correlation coefficients (ICCs) were utilized alongside 95% confidence intervals (CI). This allowed for the assessment of both intra-rater within-session reliability, employing a one-way random model, and inter-rater reliability, utilizing a two-way random model. The degree of reliability of the test, measured by ICC, was determined according to the classification of [Portney, Watkins et al. \(2009\)](#); [Domholdt \(1993\)](#) using the following criteria: excellent (0.90-1.00), good (0.70-0.89), moderate (0.50-0.69), and poor (<0.49). The standard error of measurement (SEM) was calculated using the formula $SEM = standard\ deviation \times \sqrt{1-ICC}$. The minimum detectable change (MDC) was calculated using the formula $MDC = 1.96 \times SEM \times \sqrt{2}$. SEM% was defined as $SEM\% = (SEM/mean) \times 100$ and MDC% as $MDC\% = (MDC/mean) \times 100$. Bland-Altman plots were used to visually represent the degree of agreement. Separate independent t tests were conducted to compare the erector spinae stiffness between the left and right side in different position, prone and sitting, respectively.

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RESULTS

Intra-rater reliability was assessed using a one-way random model consistency. The measurements of the left erector spinae muscles' stiffness in the prone position demonstrated good intra-rater reliability, with an ICC of 0.88 (95% CI: 0.76–0.94), an SEM of 28.67 N/m, and an MDC of 79.47 N/m. Similarly, the right side in the prone position showed excellent intra-rater reliability, with an ICC of 0.91 (95% CI: 0.82–0.95), an SEM of 24.13 N/m, and an MDC of 66.88 N/m. All SEM and MDC values for intra-rater reliability in the lying position were less than 8.87% and 24.61%, for the left and right respectively.

The intra-rater reliability for the left-side sitting position was excellent, with an ICC of 0.91 (95% CI: 0.82–0.96), SEM of 43.30 N/m, and MDC of 120.03 N/m. On the right side, the intra-rater reliability ranged from good to excellent, with an ICC of 0.89 (95% CI: 0.78–0.95), SEM of 49.16 N/m, and MDC of 136.27 N/m. The SEM% and MDC% for intra-rater reliability in the sitting position were both less than 9.35% and 25.9%, for the right and left side respectively.

Inter-rater reliability was analyzed using a two-way random model consistency. In the left-side lying position, the intra-rater reliability was good with an ICC of 0.87 (95% CI: 0.73–0.94), SEM of 26.11 N/m, and MDC of 72.37 N/m. Similarly, in the right-side lying position, the intra-rater reliability was good with an ICC of 0.84 (95% CI: 0.68–0.92), SEM of 36.43 N/m, and MDC of 100.96 N/m. The inter-rater reliability percentages for SEM and MDC in the lying position were both less than 10.94% and 30.32%, for the right and left side respectively.

In terms of intra-rater reliability for the left sitting position, the ICC with 95% CI exhibited excellent values of 0.96 (0.92–0.98), SEM of 32.55 N/m, and MDC of 90.24 N/m. Similarly, for the right sitting position, the ICC with 95% CI was also excellent at 0.95 (0.90–0.97), with SEM of 35.39 N/m, and MDC of 98.11 N/m. The inter-rater reliability percentages for SEM and MDC in the sitting position were both less than 6.58% and 18.24%, for the left and right respectively.

The (Table 2) summarises the results for intra- and inter-rater reliability of erector spinae stiffness using the MyotonPRO. Additionally, (Figure 1) displays the Bland-Altman plots to visually represent the degree of agreement. (Figure 2) represent bias of measurement between different Examiner (\square and \square).

The data in (Table 3) and (Table 4) summarise the mean muscle stiffness, paired difference analysis and effect sizes between prone and sitting positions and right and left erector spinae muscle stiffness in healthy populations.

The data in (Table 3) show significant differences in tissue tension between the prone and sitting positions, with the sitting position showing a higher level of muscle stiffness: the mean muscle stiffness in the prone position is 328.07 N/m, with a standard deviation of 83.53 N/m. On the other hand, the mean stiffness of the M. erector spinae in the sitting position is significantly

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Please do a general review of how results are reported. In terms of writing, there are many mistakes in this section that confuse the understanding of the concepts. You can present the results by classification.

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Inter-rater reliability or intra-rater reliability?
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higher at 511.17 N/m, with a standard deviation of 151.22 N/m. The paired differences analysis shows that the mean difference in tissue tension between the prone and sitting positions is -183.10 N/m with a standard deviation of 99.03 N/m. This difference is statistically significant with a t-value of -10.12 and a two-tailed p-value of less than 0.001. In addition, effect size analysis using Cohen's d and Hedges' correction indicates a significant difference in tissue stiffness between the two positions. Cohen's d is calculated as -1.84, while Hedges' correction gives a value of -1.80. Both effect size measures indicate a large effect, highlighting the significant difference in tissue stiffness between the prone and sitting positions.

Paired sample analysis revealed some notable differences in M. erector spinae stiffness between the left and right sides. On the left side, the mean M. erector spinae stiffness was 405.32 N/m with a standard deviation of 112.65 N/m, while on the right side it was slightly higher at 433.92 N/m with a standard deviation of 115.47 N/m. A strong positive correlation of 0.91 was observed between the tissue tension values on both sides, which was statistically significant ($p < 0.001$). Analysis using the paired samples test showed a mean difference in tissue tension between the left and right sides of -28.60 N/m with a standard deviation of 46.51 N/m. This difference was found to be statistically significant, supported by a t-value of -3.36 and a two-tailed p-value of 0.002. Effect size analysis using Cohen's d and Hedges' correction indicated a moderate effect size. Cohen's d was calculated to be -0.61, while Hedges' correction yielded a value of -0.59. These effect sizes suggest a moderate difference in erector muscle stiffness between the left and right sides. In summary, these results show statistically significant differences in tissue tension between the left and right sides, with the right side showing slightly higher tension levels on average.

DISCUSSION

The primary objective of this study was to determine the intra- and inter-reliability of lumbar M. erector spinae stiffness using MyotonPRO in healthy adults in both prone and sitting position. We were also able to compare M. erector spinae stiffness between the right and left sides and the changes in stiffness from the prone to the sitting positions in a healthy population. The study found good to excellent intra-rater reliability for both the left (ICC=0.76–0.94) and right (ICC=0.82–0.95) M. erector spinae in the supine position. Similarly, in the sitting position, excellent intra-rater reliability was observed for the left side, (ICC=0.82–0.96) and good to excellent reliability was observed for the right side (ICC=0.78–0.95). Inter-rater reliability showed strong agreement between raters for both the supine and sitting positions. The ICC values demonstrate a high degree of consistency between measurements taken by different raters. Within the range of highly reliable values, some values may deviate from the average due to variations in muscle stiffness between measurements or from one day to the next due to muscle activity. Our reliability results are comparable to those of previous studies using MyotonPRO in healthy populations, showing high ICC values for test-retest intervals testing in prone position ranging from (ICC=0.75 to 0.99), indicating good to excellent relative reliability (Lohr et al., 2018). In addition, analysis of the biomechanical properties of the lumbar extensor myofascia in healthy individuals and elderly patients with CLBP also showed high reliability between different examiners, with measurements of muscle tone, stiffness and elasticity of the left and right

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extensor myofascial tissues tested also in prone position ranging from ICC=0.90 to ICC=0.95 (Zugui et al., 2020). Currently, only a few studies have evaluated the reliability of MyotonPRO for measuring lumbar muscle stiffness in pathological state, whether lying and seated. However, there are no studies evaluating reliability in healthy populations only in sitting position. These methodological differences underscore the importance of careful interpretation when synthesizing findings across studies. Such variations in measurement techniques may influence the overall understanding of back muscle stiffness in individuals with and without low back pain. In their systematic review, Vatovec and Voglar (2022) compared back muscle stiffness, assessed using myotometry, among individuals with varying states of low back pain (LBP) and those without. Their analysis of pooled data highlighted notable differences in research methodologies. For instance, Wu et al. (2022) examined muscle tone (measured in Hz) and stiffness (measured in N/m) in paravertebral muscles at each level from L1 to L5, with participants positioned prone. In contrast, Iliahi et al. (2020) investigated five biomechanical properties of stiffness—frequency, decrement, creep, and stress relaxation time—in the L3-L4 myofascial tissue. Their study focused on individuals with chronic low back pain (CLBP) and matched normal controls, evaluating both left and right sides in a prone position. Furthermore, Alcaraz-Clariana et al. (2021) contributed to the diversity of methodologies by examining stiffness and tone specifically in the erector spinae muscles at the L5 level, also with participants in a prone position. However, all these methodological differences our results expose that myotonometry can reliably detect changes in M. erector spinae stiffness when measured in both prone and sitting positions in healthy population, as Zhang et al. (2022) also demonstrated excellent intra- and inter-rater reliability (ICC = 0.88-0.99) tested in patients with (CLBP). McGowen et al (2023) also reported good to excellent test-retest reliability of stiffness measures in Baylor University Army Cadets, measuring the lumbar multifidi and longissimus thoracis muscles in standing (ICC = 0.81-0.98) and squatting (ICC = 0.93-0.96) functional posture. Hu et al., (2018) explains that the reliability of the MyotonPro is also due to the lumbar level at which the measurements are taken. In the upper lumbar levels (L1-L2), measurements are less reliable than in the lower lumbar levels (L4) due to the crural attachment of the diaphragm at L1 and L2, which may affect the tone and stiffness of the paraspinal muscles during the respiratory cycle. This study used the SEM to estimate the distribution of repeated measures around the 'true' score, while the MDC reflects the smallest amount of true change rather than the measurement error inherent in the score (Lin et al., 2009). Intra-rater reliability SEM and MDC values were less than 8.87% and 24.6%, respectively, for the lying prone position, and less than 9.35% and 25.9%, respectively, for the sitting position. For the assessment of inter-rater reliability, both the standard error of measurement (SEM) and minimal detectable change (MDC) percentages were less than 10.94% and 30.32% in the prone position, and less than 6.58% and 18.24% in the seated position. The values reported indicate that the measurements were reliable with minimal inherent error. This suggests that the measurements obtained in both the prone and sitting positions were consistent and accurate. Bland-Altman analyses were conducted to identify systematic bias and compare the 95% limits of agreement between the testing sessions when using the MyotonPRO to measure lumbar erector spinae stiffness in healthy participants in both laying prone and sitting position. Bland-Altman analyses

310 have an advantage in that scatter plots can be used to visually interpret data from the observations
 311 of any outliers, bias, or relationship between variance in measures, size of the mean, and limits of
 312 agreement (Chang et al., 2013). In our study, the 95% CI of the mean difference included 0,
 313 which confirmed good repeatability. The results of this study showed that healthy participants
 314 had greater M. erector spinae stiffness on the dominant right side (335.6 N/m) than on the non-
 315 dominant left side (320.4 N/m) measured in the prone position. The right side showed 4.73%
 316 more stiffness than the left side. Even in the sitting position, the right side had higher values
 317 (532.2 N/m) than the left side (490.1 N/m). The right side had a higher stiffness of 8.57%. Hu et
 318 al (2018) found no significant differences in paraspinal muscle stiffness between the left and right
 319 sides in young adults with (CLBP) in the prone position (left = 280.9 N/m and right = 289.7
 320 N/m). Therefore, measurements of muscle stiffness in the supine position were not performed.
 321 Becker et al., (2018) reported that the activity of the lumbar erector spinae was significantly
 322 higher in patients with (CLBP) from sit to stand, 30 seconds of standing, and climbing stairs, and
 323 significantly lower during static waist flexion compared with healthy controls. Feng and Zhang
 324 (2018) argue that the human musculoskeletal system is in a state of balance and left-right
 325 symmetry when healthy. However, incorrect postures can cause alterations in muscle tone,
 326 resulting in asymmetry and postural problems. In their study of healthy individuals, the
 327 researchers found no difference in the rigidity of the erector spinae muscles on both sides in
 328 prone, sitting, and standing positions. However, our study revealed that the rigidity of the erector
 329 spinae muscles may vary depending on right-hand dominance. These differences were observed
 330 in both prone and sitting postures. Overall, the results suggest that the use of MyotonPRO
 331 maintains high levels of reliability in different positions, highlighting its practical and portable
 332 utility for assessing muscle stiffness in clinical practice better than assessments based only on
 333 palpation or observation of posture by the physician. After a detailed analysis, we realised that
 334 this study has certain limitations. Firstly, the lumbar erector spinae muscles consist of several
 335 small muscles interspersed between fascial planes, and therefore the muscle stiffness
 336 measurement obtained on the palpable muscle belly, one finger wide from the spinous process at
 337 the L4 level, maybe does not represent the true stiffness of all adjacent and deeper structures.
 338 Secondly, muscle stiffness in the elderly may differ from that of the young, as has been analysed
 339 in previous studies (Ebay et al. 2015; Ikezoe et al. 2012). Third, possible differences between
 340 men and women in the stiffness of the M erector spinae muscle should be tested, as they have
 341 done previously (Taş and Salkın, 2019), by testing the stiffness of the Achilles tendon and the
 342 gastrocnemius muscle at rest and under tension. Fourth, our test protocol procedure was designed
 343 to be easily replicated in clinical settings for patients with LBP who have difficulty lying in the
 344 prone position and are more comfortable in the sitting position. Therefore, further research should
 345 be conducted to understand whether differences in the test protocol procedure, including
 346 measuring the right side first rather than the left side, have an effect on the test results. However,
 347 in our study we only analysed muscle stiffness, so in future research it would be a good idea to
 348 analyse all the parameters obtained from the Myoton PRO, including skin oscillation frequency,
 349 logarithmic decrease, relaxation time and creep, to ensure even greater reliability of the device.

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350 CONCLUSION

356 This study demonstrated high intra- and inter-reliability of lumbar erector muscle stiffness with
357 the MyotonPRO in healthy adults and showed the device's ability to detect even small changes in
358 M erector spine stiffness, testing both right and left sides and measuring in both prone and sitting
359 position. The use of the sitting position to assess lumbar tissue stiffness in patients with CLBP
360 could be a useful alternative to the prone position, especially for patients who are uncomfortable
361 in the prone position. This may have further practical implications for clinical practice.

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Use of the sitting position test in healthy subjects cannot be generalized to LBP patients. It seems that research should be done on LBP patients for this conclusion.

362 ACKNOWLEDGEMENTS

363 We thank to all the participants for their cooperation.

364 ADDITIONAL INFORMATION AND DECLARATIONS

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367 funders had no role in study design, data collection and analysis, the decision to publish, or the
368 preparation of the manuscript.

369 *Grant Disclosures*

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371 0388.

372 *Competing Interests*

373 The authors declare that they have no competing interests.

374 *Author Contributions*

- 375 • Fabio Valenti conceived and designed the experiments, performed the experiments,
376 analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the
377 article.
- 378 • Sara Meden- performed the experiments and analyzed the data.
- 379 • Maja Frangež conceived and designed the experiments, analyzed the data, authored or
380 reviewed drafts of the article, and approved the final draft.
- 381 • Renata Vauhnik conceived and designed the experiments, analyzed the data, authored or
382 reviewed drafts of the article, and approved the final draft.

383 *Human Ethics*

384 The following information was supplied relating to ethical approvals (i.e., approving body and
385 any reference numbers): The current study was carried out in accordance with the Declaration of
386 Helsinki and was authorized by the Republic of Slovenia National Medical Ethics Committee
387 (No. 0120-520/2022/3).

388 **Data Availability**

389 The following information was supplied regarding data availability:

390 The raw measurements are available in the Supplemental File.

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