Lumbar myofascial physical properties in healthy adults: myotonometry vs. shear wave elastography measurements

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ABSTRACT

Objective: The human resting myofascial tone maintains the body tone in a neutral posture, the assessment of this and other muscle physical properties (MPP) is relevant, since, it is altered in many pathological states.
Patients and methods: Seventeen healthy subjects (8 males), between 18-50 years old, were assessed. The MPP of lower lumbar muscles was evaluated on right and left sides during prone resting position using two devices; myotonometry (stiffness, elasticity and tone) and ultrasound-based shear-wave elastography (SWE) (shear modulus). MTM measurements were performed at two anatomic points (ANp), selected by an experienced reader and at an adjacent ultra-sound determined point (USp). Myotonometry measurements of the erector spinae and SWE measurements of multifidus muscles at the L3-4 level were compared between genders and sides. The intra-reader reliability (IRR) for each device and correlations between techniques were analysed. MTM measurements performed at ANp and USp were compared. The intraclass correlation coefficient (ICC) was assessed for both devices. Correlations between stiffness (myotonometry) and shear modulus (SWE) at the respective muscle depths were assessed with Spearman correlation.
Results: Males had greater stiffness and tone than females, particularly on the dominant side. MPP assessed by myotonometry were not different between ANp and USp. Good/Excellent IRR was documented for measurements by MTM (ICC≥0.90) and SWE (ICC≥0.85). No correlation in myotonometry stiffness and SWE shear modulus was found. For myotonometry assessments, the addition of ultrasonography was not different from anatomic localizations. No correlation of measurements was found between devices assessing respective L3-4 level muscles.
Conclusions: Gender and side differences must be considered when assessing MPP in axial muscles. For MTM assessments, the addition of ultrasonography was not different to anatomic references. No correlation was found between devices.

Keywords: Myofascial physical properties; Human resting myofascial tone (HRMT); Young adults; Elastography; Myotonometry.

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INTRODUCTION

The model of human resting myofascial tone (HRMT) is based on the concept that non-contracted (resting) muscle maintains body tone/tension in a relaxed gravity-neutral posture by muscular tonicity transmitted through the connective tissue fascia that covers the muscular tissue, independently from central nervous system signals (electromyography-silent)[1]. This property maintains postural stability in balanced equilibrium positions[2,3]. Insufficiency of paraspinal (axial) human resting myofascial tone is a proposed risk factor for adolescent idiopathic scoliosis and conversely hypertonicity and/or stiffness has been hypothesized as a risk factor for development of ankylosing spondylitis (AS)[1,4,5].

Furthermore, the reported association between myofascial lumbar stiffness and AS4 needs confirmation[6]. Excessive axial myofascial stiffness might contribute to spinal microinjury at the site of ligamentous entheses (enthesopathy)[7,8], a potential mechanism relevant to AS susceptibility and progression[9]. It is of interest to evaluate and compare different approaches to assess these properties.

Two approaches that use external mechanical impulse have been proposed to assess muscle stiffness and other myofascial physical properties (MPP): the myotonometry (MTM) device (MyotonPRO®)[10-12] and the ultrasound-based shear wave elastography (SWE) measurement of shear elastic (Young's) modulus[13].

The MyotonPRO® is a hand-held, non-invasive device which applies a precise mechanical impulse (0.18N for 15ms) that causes underlying tissue oscillations and allows measurement of tissue stiffness, elasticity (reverse of decrement) and tension/tone, to a depth of 2cm [10, 14]. Reliability of MTM for extremity[15] and axial muscles measured at the lumbar (L3-L4) level[16] have been reported. The SWE is based on the propagation of shear waves in tissues and can be coupled to 2-dimensional ultrasound imaging, allowing deeper depth measurements [17]. Studies on extremity and axial muscles have shown that SWE provides a reliable measurement of resting muscle shear elastic modulus[9, 13], including axial muscle stiffness at rest and at different contraction levels[17,18].

Previous studies indicated significant correlations between MTM and SWE in measurements of the homogeneous tissues of the medial and lateral gastrocnemius muscle and Achille's tendon[19]. Correlation was also found in measurements of both devices across different levels of contractility (zero resting, 40 percent, and 80 percent contractility) of infraspinatus, erector spinae, and gastrocnemius muscle[20]. However, to our knowledge, no study has yet compared results of MTM measurements of resting lower lumbar myofascia performed in areas selected by anatomic reference *versus* ultrasound guided. Although SWE is a valid and reliable tool for quantifying the stiffness of muscle and tendons, access to this technique is not easy and requires advanced technical expertise. The associated high maintenance costs limit wider clinical use. It would be of utmost importance to have an alternative portable, easy, less costly device than SWE to perform these MPP measurements in clinical practice.

This preliminary study in healthy young adults aims primarily to:

(i) Provide objective data on resting lower lumbar myofascial MPP namely, stiffness, tone and elasticity by MyotonPRO and shear modulus by SWE, stratified by gender and side dominance.

And as secondary objectives:

(ii) Determine if MTM measurements differ according to the method used to localize an area of interest (anatomic reference versus ultrasound guidance).

(iii) Assess intra-reader reliability of the MTM and SWE measurements at the lower lumbar (L3-L4) myofascial level;

(iv) Assess the correlation between measurements of stiffness assessed by MTM (easy and quick to perform) and shear modulus assessed by SWE (requires the acquisition of competence to perform and interpret) in lower lumbar myofascial area.

METHODS

PATIENTS AND STUDY DESIGN

In the context of the MyoSpA study, an ongoing project assessing the role of muscle physical properties in Spondyloarthritis susceptibility/progression, an observational cross-sectional study was conducted involving a convenience sample of 17 young self-reported health university students (8 males, 9 females). The following inclusion criteria were used in subject recruitment: i) age between 18 and 50 years; ii) right side dominance; iii) capacity to understand and sign the informed consent. The exclusion criteria were: i) low back pain or history of low back surgery; ii) any known rheumatic or neuromuscular disorder; iii) intake of muscle relaxants or neuromuscular blocking drugs, and iv) body mass index (BMI) higher than 30 kg/m2 (to avoid subcutaneous tissue over 20 mm thick over the muscle of interest, that compromises MTM assessment). The study was performed in accordance to the Helsinki declaration principles and was approved by the local ethics committee. All study participants signed an informed consent form before inclusion in the study.

DATA COLLECTION AND FLOWCHART OF MEASUREMENTS

Demographic and clinical data were obtained with a standardized questionnaire that included age, gender, height (cm), weight (kg), occupation and exercise (minutes of exercise per week) in subjects who reported being physically active. The MPP of axial lumbar myofascia was assessed using the MTM device, MyotonPRO® (Myoton AS, Tallinn, Estonia) and an ultrasound scanner with SWE (Aixplorer, v10, Supersonic Imagine, Aix-en-Provence, France). The MTM device provides information on dynamic stiffness (N/m), tone (Hz) and decrement (D), which is an inverse of elasticity. The SWE provides data regarding elastic stiffness assessed as shear (Young's) modulus (kPa).

Subjects were positioned prone on a padded, full-length examination table with skin of the lumbar area exposed, placing arms at their sides to achieve complete torso and lumbar relaxation (Figure 1). The localization for lumbar myofascia MTM measurements was identified by two techniques: (i) defined anatomic point (ANp), solely selected by an experienced rheumatologist (FPS) mid-way between L3-L4 spinous processes at the respective left and right extensor muscle bulk prominences (circa 2.5 cm lateral to the spinous processes on each side, correspondent to the localization of Multifidus muscle), and (ii) ultra-sound guided point (USp), selected by ultra-sound focusing on the mid-bulk multifidus muscle at the L3-4 level. Measurements by MTM and SWE were performed consecutively in the prone position after 10 min of rest (Moment 1). That first MTM measurement at the ANp, is coded as "ANp_MTM_M1", and the SWE measurement as "USp_SWE".

Two additional consecutive measurements were performed by MTM (Moment 2), after 20 min of rest (10 min following the previous assessment), at the ANp, "ANp_MTM_M2", and at the USp, "USp_MTM_M2 point".

All measurements were performed on both sides. Each MTM measurement, at each side and timepoint, represents two consecutives individual MTM readings, where the average for each parameter (stiffness, tone and decrement) was considered. Each SWE measurement, at each side, represents three consecutive measurements, where the average shear module was averaged in the results. All, MTM and SWE measurements, were performed by a single trained reader (FPS and SF, respectively).

STATISTICAL ANALYSIS

Descriptive data are presented as medians and interquartile ranges (IQR).

DESCRIPTIVE BIOMECHANICAL DATA AND COMPARISON BETWEEN GENDERS AND SIDES

MTM and SWE were compared between genders and sides, considering the results of ANp_MTM_M1 *vs* USp_SWE_M1, assessments. MTM parameters at ANp_MTM_M1 vs ANp_MTM_M2, (both in ANp, 10 minutes apart to analyse the effect of rest duration in measurements) and between ANp_MTM_M2 *vs* USp_MTM_M2 (consecutive measures performed after 20 minutes rest to compare the influence of different methodologies, ANp/blinded vs US guided identification used, to identify the point of interest) were compared. Mann-Whitney U test (for continuous independent non-normal data), Fischer's exact test (for nominal independent data) and Wilcoxon signed rank test (comparing two groups of dependent non-normally distributed data) were used.

INTRA-READER RELIABILITY

In order to test the intra-reader reliability (IRR), the intraclass correlation coefficient (ICC) (mixed individual definition) was assessed for (i) MTM parameters (stiffness, elasticity and tone) for two individual sets of measurements, per location (ANp *vs* USp) and side (ANp_MTM_M1, ANp_MTM_M2, USp_MTM_M2); (ii) SWE (shear modulus) in three individual measurements by side (USp_SWE)21.

CORRELATION BETWEEN DEVICES

Correlation for stiffness measured by MTM (USp_MTM_M2) and shear modulus measured by SWE (US_SWE) were assessed (pairwise by the same side) using the Spearman correlation test and considering a **r** of <0.90 to ≤0.7 as a good correlation and r ≥0.9 a high correlation[22].

Statistical analyses were performed using R version 3.4. Figures were produced using ggplot R package and default packages from R software.

RESULTS

PARTICIPANTS

Seventeen healthy subjects (8 males) with a median (IQR) age of 26.5 [23.5;32.5] years were included. Men were taller (174 cm [171;177] vs 161m [160;163], p<0.01) and heavier (71.5 kg [68.6;74.1] vs 59.9 kg [51.1;60.8], p<0.01) than women. Only 9 subjects exercised regularly, both genders having similar activity level (Table I).

DESCRIPTIVE BIOMECHANICAL DATA AND COMPARISON BETWEEN GENDERS AND SIDES

TABLE I. DEMOGRAPHIC DATA, STRATIFIED BY GENDER

Variable	Total (n=17)	Male (n=8)	Female (n=9)	p value*
Age (years)	26.5 (23.5;32.5)	27 (23;33)	26.5 (23.5;32.5)	0.92
Height (cm)	167 (161;174)	174 (171;177)	161 (160;163)	<0.01
Weight (kg)	62.9 (59.9;70.7)	71.5 (68.6;74.1)	59.9 (51.1;60.8)	<0.01
BMI (kg/m2)	22.5 (21.6;24.2)	23.90 (22.8;24.8)	21.3 (21.3;25.9)	0.18
Exercise duration (minutes/week)	240 (135;420) (n=9)	225 (120;420) (n=6)	240 (135;600) (n=3)	0.80

Median (interquartile ranges) values are reported *Mann-Whitney U test for continuous independent non-normal samples between male and female subject data; Fischer's exact test for nominal independent non-normal samples; BMI: body mass index

Regarding MTM measurements, males had greater stiffness (Supplementary Table S1) than females at all available timepoints considering average of both sides (for ANp_MTM_M1; 291 [215;377] N/m vs 204 [197;265] N/m, p=0.020) and for dominant (right) side (for ANp_MTM_M1; 298 [230;348] vs 211 [195;252], p=0.010). Gender difference was not quite significant for non-dominant (left) side (for ANp_MTM_M1; 285 [201;345] vs 210[187;280], p=0.08). Males also had a greater tone than females at all available timepoints (Supplementary Table S2) considering average of both sides (for ANp_MTM_M1; 14.6 [13.6;17.0] Hz vs 13.2 [12.7;14.5] Hz, p<0.010), and for the dominant (right) side (for ANp_MTM_M1; 15.1 [13.7;16.9] vs 13.2 [12.8;14.0], p<0.010) and for the non-dominant (left) side (for ANp_MTM_M1; 14.5 [13.6;15.2] vs 12.9 [12.6;15.2], p=0.020).

No difference between genders or sides was found for elasticity (inverse of decrement) using MTM (Supplementary Table S3) in any of the timepoints. Males had an average decrement (ANp_MTM_M1) of 1.14 [0.96;1.19] and female 1.10 [0.90;1.21] (p=0.730).

Regarding SWE measurements, median shear modulus was 12.9 [11.9;16.5] kPa, for male mean value was 13.3 [12.5;14.7] and for female 15.6 [13.7;19.4], with no significant difference between gender (p>0.05). No relevant difference in shear modulus, was found between sides, 13.7 [12.0;17.4] kPa for left and 14.2 [13.0;18.0] kPa for right side (p>0.05) (Supplementary Table S4).

COMPARISON BETWEEN SHORT/LONG PERIOD OF REST AND ANATOMICAL VS ULTRASOUND GUIDED MTM MEASUREMENTS

Comparison between MTM measurements made at ANp_MTM_M1 (10 minutes rest) and ANp_MTM_M2 (20 minutes rest), which means, at the same location (ANp), 10 minutes apart did not show any significant difference.

Additionally, comparison between MTM measurements made at the same moment (M2, after 20 minutes rest), at ANp_MTM_M2 and USp_MTM_M2, which means at points, identified by anatomical reference (ANp) and ultrasound guided (USp), did not show any significant difference for stiffness, tone or elasticity (Table II).

INTRA-READER RELIABILITY

Good to excellent IRR was observed for stiffness (ICC ≥0.90) and tone (ICC ≥0.96) measured by MTM and for shear modulus measured by SWE (ICC ≥0.85), when assessing multiple consecutive measurements at the same location, independently of the side or timepoint, but less so for decrement (ICC ≥0.59). This value was obtained at ANp_MTM_M2 at right side. The others values of ICC were higher (ICC>0.88) (Supplementary Table S5).

CORRELATION BETWEEN DEVICES

No correlation was found between stiffness measured by MTM (USp_MTM_M2) and shear modulus measured by SWE (USp_SWE) of the lower lumbar myofascia (-0.31≤Pearson ≤-0.28), which represents measurements at the same point with different devices. (Table III).

DISCUSSION

In previous studies, two approaches have been proposed to assess MPP, one is an external mechanical impulse, MTM, and the other is the ultrasound-based SWE[9-12]. We utilized both methods to evaluate axial

TABLE II. MUSCLE PHYSICAL PROPERTIES (MPP) BY GENDERS AND COMPARISON OF ANATOMICAL AND ULTRASOUND GUIDED MPP ASSESSED BY MYOTONOMETRY

Measurement point	Total (n=17)	Male (n=8)	Female (n=9)
Stiffness (N/m)			
ANp_MTM_M1 mean both sides	215 (204;377)	291 (215;377)	204 (197;265)
ANp_MTM_M2 mean both sides	298 (215;356)	355 (315;364)	215 (214;218)
USp_MTM_M2 mean both sides	261 (233;314)	305 (271;366)	233 (219;251)
p-value			
ANp_MTM_M1 vs ANp_MTM_M2#	0.79	0.79	0.40
p-value			
ANp_MTM_M2 vs USp_MTM_M2#	0.21	0.42	0.14
Tone (Hz)			
ANp_MTM_M1 mean both sides	13.6 (13.1;15.3)	14.6(13.6;17.0)	13.2(12.7;14.5)
ANp_MTM_M2 mean both sides	14.8 (13.3;16.5)	16.4 (15.0;16.5)	13.3(12.3;13.5)
USp_MTM_M2 mean both sides	14.9 (13.6;15.5)	15.4 (15.0;17.1)	13.6(13.0;14.2)
p-value			
ANp_MTM_M1 vs ANp_MTM_M2#	0.91	1.00	0.73
p-value			
ANp_MTM_M2 vs USp_MTM_M2#	0.26	0.28	0.49
Decrement (D)			
ANp_MTM_M1 median both sides	1.12 (0.95;1.19)	1.14 (0.96;1.19)	1.10 (0.90;1.21)
ANp_MTM_M2 median both sides	1.21 (1.02;1.32)	1.29 (1.21;1.32)	1.02 (1.00;1.22)
USp_MTM_M2 mean both sides	1.08 (1.01;1.38)	1.08 (1.03;1.39)	1.03 (1.00;1.35)
p-value			
ANp_MTM_M1 vs ANp_MTM_M2#	0.39	0.30	0.73
p-value			
ANp_MTM_M2 vs USp_MTM_M2#	0.51	0.96	0.68

Median (interquartile ranges) values are reported *Mann-Whitney U test for continuous independent non-normal samples between male and female subject data; Fischer's exact test for nominal independent non-normal samples. MTM: myotonometry; ANp_MTM_M1: mean value of three consecutive measurements performed by MTM at baseline on a region of the multifidus muscle marked without the help of any imaging technique; ANp_MTM_M2: mean value of three consecutive measurements performed by MTM after 10min on a region of the multifidus muscle marked without the help of any imaging technique; USp_MTM_M2: mean value of three consecutive measurements performed by MTM after 10min on a region of the multifidus muscle marked by US-SWE

TABLE III. CORRELATIONS BETWEEN SHEAR MODULUS (SWE) AND STIFFNESS (MTM) AT DIFFERENT TIME POINTS BY LEFT AND RIGHT SIDES*

Time points and sides	Spearman R	p-value
ANp_MTM_M1 Left side	-0.38	0.19
ANp_MTM_M1 Right side	-0.17	0.56
ANp_MTM_M2 Left side	0.02	0.94
ANp_MTM_M2 Right side	-0.29	0.29
USp_MTM_M2 Left side	-0.31	0.26
USp_MTM_M2 Right side	-0.28	0.43

Correlation between shear modulus measured at baseline (10 min. rest US_SWE) and stiffness measured by myotonometry (MTM), at anatomic defined measurement point (ANp) and ultrasound defined measurement point (USp) at different timepoints (ANp_MTM_M1, ANp_MTM_M2 and USp_MTM_M2). USp_MTM_M2 and USp_SWE measurements were made at the same SWE localization (with different devices).

lumbar MPP in the same group of healthy volunteers, comparing the effect of gender and side dominance, in the results obtained. Several factors may indeed influence the results obtained by these devices.

Our results show that males have higher stiffness and tone than females, particularly on the dominant side. These findings confirm previous studies of lower lumbar muscles of healthy, younger subjects[11], and are consistent with studies of knee extensors muscles using the free oscillation technique[24] and in rectus femoris using MTM[10]. However, in our study, no significant difference was identified between side or gender for shear modulus measured by SWE, although slightly higher values were found in females. Eby *et al.*[22, 23] reported that shear modulus values tended to be higher for females than males and increased with advancing age.

Moreover, we found no significant difference for tone or stiffness assessed by MTM between sides in all subjects and in each gender. Indeed, data regarding the influence of side dominance on MPP are scarce.

Aging is another factor analysed in previous studies with potential to influence MTM parameters. Significantly higher stiffness was found in plantar flexors in older persons[25] and in rectus femoris and biceps brachialis[10]. Due to the narrow age range of our sample (18-50 years), the life-span effect of aging on MTM parameters could not be assessed.

Interestingly, we observed a numerical (but not statistically relevant) increase in stiffness between measurements (from ANp_MTM_M1 to ANp_MTM_M2). This is consistent with previous work from Nair *et al*11 that reported significantly higher values at 10 min *vs* 0 min (baseline) in both genders and on both sides, which may be explained by the thixotropy phenomenon, a physiological property of muscles in which resistance to movement increases with time at rest and is reduced by movement[23].

In this study, the influence of the method to identify the point of interest to perform measurements, by ultrasound or by anatomical reference, on MTM results was to our knowledge the first time analysed. Ultrasound does not seem to add value for the identification of the point of interest for MTM assessment, suggesting that MPP of lower lumbar myofascia can be reliably assessed by MTM without the need for previous imaging guidance. However, this result should be confirmed in future studies as the sample size is small, making difficult to capture statistically significant differences. Additionally, only the intra-rate reliability (IRR) was assessed.

MTM and SWE measurements of axial MPP showed good IRR in healthy subjects, supporting previous evidence of a very good/excellent IRR for MTM measurements of stiffness (ICC≥0.90) and tone (ICC≥0.96)[14,26,27], and with SWE for shear modulus (ICC≥0.91)[14,17,18], but less so for elasticity (ICC≥0.59). One previous study showed that, the MTM device also has a high within-day and between day intra-reader reliability, although lower for elasticity[15]. This low ICC value for elasticity in one determination (ANp_ MTM_M2) may be related with common variation associated to small sample size. Overall, this analysis of IRR (a score of the consistency in ratings given by the same person across multiple instances) should be complemented in future studies, by inter-rate reliability (measures the consistency in ratings given by various judges) to allow test validity completion, for these devices.

An intriguing result in our study of lower lumbar myofascia arises from the non-significant correlation between resting stiffness measured by MTM and shear modulus measured by SWE. Contrary to a previous study which found a significant correlation between measurements obtained with both techniques, in the homogeneous resting gastrocnemius (r=0.46-0.54, p <0.05)[22], the lack of correlation in this study may have several explanations. First, MTM and SWE estimate a measure of stiffness through different mechanisms, therefore, differences in values may result from the respective methods. Also, MTM can only measure stiffness to a depth of about 2 cm, which may have influenced our non-significant correlational results. The MTM is measuring more superficial and heterogeneous myofascial (and overlying adipose tissues), whereas SWE measures more deeper and homogeneous mid-bulk multifidus muscle. It is important to stress, that MTM technique does not specifically exclude the localized superficial tissue overlaying the muscle of interest. Another explanation might be related with time to perform the assessments. To correlate results obtained by MTM and SWE, we considered measurements obtained in the same USp. SWE (Usp_SWE) results were obtained in M1 (10 minutes relax time) and MTM (USp_MTM_M2), which means 10 minutes later. For future studies, comparative studies of both devices with standardized imaging phantoms and/or comparing measurements at similar depths of homogeneous tissue (like gastrocnemius) might be useful to clarify and improve understanding of our results.

Considering all these concerns, it seems that MTM provides a reliable, accurate and sensitive method to objectively and non-invasively evaluate physical properties of superficial skeletal muscles. This type of MTM device (handheld, portable and economical) represents a user-friendly methodology to be applied to research and eventually to clinical practice.

This is the first study of lower lumbar myofascia where MPPs were evaluated using two different localization techniques (anatomical reference and ultrasound guided) to identify the point of interest for performing measurements, stratified by gender and side dominance. Since this study was performed with a convenience sample (small, involving young people under 50 years, without level of physical activity stratification), and inter-reader reliability was not assessed, our results are preliminary and should be further confirmed in future studies.

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CONFLICTS OF INTEREST

None of the authors declared conflicts of interest.

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SUPPLEMENTARY TABLES

SUPPLEMENTARY TABLE S1. SHEAR MODULUS OF AXIAL LUMBAR MUSCLES ASSESSED BY ELASTROGRAPHY (SWE)

	Total (N=17)	Male (N=8)	Female (N=9)
USp_SWE median both sides – Shear modulus (kPa)	12.9 (11.9;16.5)	13.3 (12.5;14.7)	15.6 (13.7;19.4)
USp_SWE Left – Shear modulus (kPa)	13.7 (12.0;17.4)	12.8 (11.4;15.6)	15.0 (12.1;19.3)
USp_SWE Right – Shear modulus (kPa)	14.2 (13.0;18.0)	13.6 (13.0;14.7)	17.4 (12.6;18.1)
p-value – Shear modulus right vs left	0.33	0.33	0.59

Kilopascal (KPa); results in median (interquartile ranges). Mann-Whitney U test for continuous independent non-normal samples. Wilcoxon signed-rank test for continuous dependent non-normal samples. No statistical difference between genders was found for any parameter ($p>0.05$). USp_SWE: Shear modulus assessed by elastography over a point defined by ultrasonography.

SUPPLEMENTARY TABLE S2. STIFFNESS (N/M) OF LOWER LUMBAR MUSCLES ASSESSED BY MTM, COMPARING GENDERS AND SIDES

Measurement point	Total (n=17)	Male (n=8)	Female (n=9)	p-value
ANp_MTM_M1 mean both sides	215 (204;377)	291 (215;377)	204 (197;265)	0.02
ANp_MTM_M1 left	216 (199;343)	285 (201;345)	210 (187;280)	0.08
ANp_MTM_M1 right	230 (202;303)	298 (230;348)	211 (195;252)	0.01
p-value left vs right	0.69	0.80	0.73	-
ANp_MTM_M2 mean both sides	298 (215;356)	355 (315;364)	215 (214;218)	0.05
ANp_MTM_M2 left	275 (210;355)	315 (291;355)	210 (200;229)	0.14
ANp_MTM_M2 right	321 (221;373)	373 (338;395)	221 (207;230)	0.02
p-value left vs right	0.70	0.60	0.89	-
USp_MTM_M2 mean both sides	261 (233;314)	305 (271;366)	233 (219;251)	0.02
USp_MTM_M2 left	267 (232;319)	305 (271;366)	244 (217;263)	0.05
USp_MTM_M2 right	270 (234;309)	300 (270;325)	224 (207;259)	0.01
p-value left vs right	0.87	1.00	0.52	-

Mann-Whitney U test for continuous independent non-normal samples (between gender differences). Wilcoxon signed rank test (for comparing two groups of dependent non-normally distributed values). Units expressed as Median (interquantile range).
MTM: Myotonometry; USp: ultrasound-guided point; ANp: anatomical point; M1: timepoint one; M2: timepoint 2

SUPPLEMENTARY TABLE S3. TONE (HZ) OF LOWER LUMBAR MUSCLES ASSESSED BY MTM, COMPARING GENDERS AND SIDES

Measurement point	Total (n=17)	Male (n=8)	Female (n=9)	p-value
ANp_MTM_M1 mean both sides	13.6 (13.1;15.3)	14.6(13.6;17.0)	13.2(12.7;14.5)	0.01
ANp_MTM_M1 left	13.9 (12.8;16.4)	14.5 (13.6;15.2)	12.9(12.6;15.2)	0.04
ANp_MTM_M1 right	13.8 (13.2;15.3)	15.1 (13.7;16.9)	13.2(12.8;14.0)	0.01
p-value left vs right	0.89	0.69	1.00	-
ANp_MTM_M2 mean both sides	14.8 (13.3;16.5)	16.4 (15.0;16.5)	13.3(12.3;13.5)	0.04
ANp_MTM_M2 left	14.2 (12.9;16.2)	16.0 (14.2;16.2)	12.9(12.0;13.7)	0.07
ANp_MTM_M2 right	15.4 (13.4;16.7)	16.6 (15.7;17.1)	13.4(12.6;13.8)	0.02
p-value left vs right	1.00	0.66	0.96	-
USp_MTM_M2 mean both sides	14.9 (13.6;15.5)	15.4 (15.0;17.1)	13.6(13.0;14.2)	0.01
USp_MTM_M2 left	14.8 (13.6;15.7)	15.1 (14.8;15.8)	13.6(12.7;14.4)	0.02
USp_MTM_M2 right	14.9 (13.7;15.8)	15.4 (15.0;18.3)	13.7(12.6;14.7)	0.01
p-value left vs right	0.74	0.56	0.80	-

Mann-Whitney U test for continuous independent non-normal samples (between gender differences). Wilcoxon signed rank test (for comparing two groups of dependent non-normally distributed values). Units expressed as Median (interquartile range). MTM: Myotonometry; USp: ultrasound-guided point; ANp: anatomical point; M1: timepoint one; M2: timepoint 2

SUPPLEMENTARY TABLE S4. DECREMENT (INVERSE OF ELASTICITY) OF LOWER LUMBAR MUSCLES ASSESSED BY MTM COMPARING GENDERS AND SIDES

Measurement point	Total (n=17)	Male (n=8)	Female (n=9)	p-value
ANp_MTM_M1 median both sides	1.12 (0.95;1.19)	1.14 (0.96;1.19)	1.10 (0.90;1.21)	0.73
ANp_MTM_M1 left	1.05 (0.94;1.20)	1.15 (0.94;1.20)	1.03 (0.92;1.19)	0.80
ANp_MTM_M1 right	1.15 (0.97;1.23)	1.15 (1.04;1.23)	13.2 (12.8;14.0)	0.89
p-value left vs right	0.70	0.69	1.00	-
ANp_MTM_M2 median both sides	1.21 (1.02;1.32)	1.29 (1.21;1.32)	1.02 (1.00;1.22)	0.49
ANp_MTM_M2 left	1.20 (1.07;1.33)	1.25 (1.22;1.33)	1.07 (0.95;1.19)	0.44
ANp_MTM_M2 right	1.21 (1.07;1.31)	1.26 (1.18;1.31)	1.07 (1.04;1.25)	0.30
p-value left vs right	0.91	0.89	1.00	-
USp_MTM_M2 mean both sides	1.08 (1.01;1.38)	1.08 (1.03;1.39)	1.03 (1.00;1.35)	0.81
USp_MTM_M2 left	1.10 (0.98;1.38)	1.07 (0.97;1.44)	1.11 (0.98;1.38)	0.96
USp_MTM_M2 right	1.11 (1.00;1.32)	1.14 (1.10;1.36)	1.00 (0.93;1.32)	0.28
p-value left vs right	1.00	0.57	0.65	-

Mann-Whitney U test for continuous independent non-normal samples (between gender differences). Wilcoxon signed rank test (for comparing two groups of dependent non-normally distributed values). Units expressed as Median (IQR). MTM: Myotonometry; USp: ultrasound-guided point; ANp: anatomical point; M1: timepoint one; M2: timepoint 2.

SUPPLEMENTARY TABLE S5. INTRA-OBSERVER RELIABILITY OF REPEATED MEASUREMENTS OF LOWER LUMBAR MUSCLE PROPERTIES ASSESSED BY MTM AND SWE, IN BOTH SIDES

	MTM measurements, ICC (95% CI)	
Stiffness	ANp_MTM_M1: left side | right side	0.98 (0.95;0.99) | 0.98 (0.97;1.00)
	ANp_MTM_M2: left side | right side	0.98 (0.92;1.00) | 0.90 (0.64;0.97)
	USp_MTM_M2: left side | right side	0.99 (0.96;1.00) | 0.99 (0.98;1.00)
Decrement	ANp_MTM_M1: left side | right side	0.96 (0.89;0.99) | 0.98 (0.94;0.99)
	ANp_MTM_M2: left side | right side	0.95 (0.82;0.99) | 0.59 (-0.13;0.88)
	USp_MTM_M2: left side |right side	0.88 (0.66;0.96) | 0.98 (0.95;0.99)
Tone	ANp_MTM_M1: left side | right side	0.99 (0.96;1.00) | 0.97 (0.93;0.99)
	ANp_MTM_M2: left side | right side	0.98 (0.94;1.00) | 0.96 (0.84;0.99)
	USp_MTM_M2: left side | right side	0.96 (0.89;0.99) | 0.99 (0.98;1.00)
	SWE measurements, ICC (95% CI)	
Shear modulus	USp_SWE: left side | right side	0.85 (0.69;0.94) | 0.94 (0.87;0.98)

MTM: myotonometry; SWE: shear wave elastography; ICC: intraclass correlation coefficient; 95% CI: 95% confidence interval; USp_SWE: mean of three SWE measurements on the multifidus; ANp_MTM_M1: mean value of three consecutive measurements performed by MTM at baseline on a region of the multifidus muscle marked without the help of any imaging technique; ANp_MTM_M2: mean value of three consecutive measurements performed by MTM after 10min on a region of the multifidus muscle marked without the help of any imaging technique; USp_MTM_M2: mean value of three consecutive measurements performed by MTM after 10min on a region of the multifidus muscle marked by US-SWE