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ORIGINAL ARTICLE

Hamstring stiffness pattern during contraction in healthy individuals: analysis by ultrasound-based shear wave elastography

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Abstract

Purpose To assess the stiffness of hamstring muscles during isometric contractions in healthy individuals, using ultrasound-based shear wave elastography to (1) determine the intra- and inter-day assessment repeatability, (2) characterize the stiffness of semitendinosus (ST) and biceps femoris long head (BFlh) along the contraction intensity, and (3) characterize stiffness distribution among the hamstring muscles and inter-limb symmetry.

Methods Two experiments were conducted. In experiment I ($n = 12$), the intra-day repeatability in assessing the BFlh and ST stiffness were determined at intensities between 10–60% of maximal voluntary isometric contraction (MVIC) in a single session. In experiment II ($n = 11$), the stiffness of the hamstring muscles of both thighs was assessed at 20% of MVIC in the first session; and retested (for one randomly chosen thigh) in a second session. Isometric contraction of knee flexors was performed with the knee flexed at 30° and with the hip in a neutral position.

Results Moderate-to-very-high intra- and inter-day repeatability was found (ICC = 0.69–0.93). The BFlh/ST stiffness ratio increased with contraction intensity. At 20% of MVIC, the ST showed the highest stiffness among the hamstring muscles ($p < 0.02$), with no differences between the remaining hamstring muscles ($p > 0.474$). No differences were found between limbs ($p = 0.12$).

Conclusions The stiffness distribution among the hamstring muscles during submaximal isometric contractions is heterogeneous, but symmetric between limbs, and changes depending on the contraction intensity. Shear wave elastography is a reliable tool to assess the stiffness of hamstring muscles during contraction.

Keywords Surface EMG · Shear modulus · Skeletal muscle · Biceps femoris short head · Semitendinosus

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Abbreviations

BFlh	Biceps femoris long head
BFlh/ST	Biceps femoris long head to semitendinosus ratio
BFsh	Biceps femoris short head
d	Cohen effect size
ICC	Intraclass correlation coefficient
MVIC	Maximum voluntary isometric contractions
r	Pearson correlation coefficient

sEMG	Surface electromyography
SEM	Standard error of measurement
SM	Semimembranosus
ST	Semitendinosus
SWE	Ultrasound-based shear wave elastography

Introduction

The use of ultrasound-based shear wave elastography (SWE) to assess in vivo the stiffness of different soft tissues has grown in recent years (Bercoff et al. 2004; Sigrist et al. 2017). Since it enables the quantification of shear wave velocity, which allows the muscle shear modulus (i.e., index of stiffness) to be estimated, SWE has been proposed as a means to assess the local muscle stiffness during contraction (Hug et al. 2015), in a wide range of intensities (Ateş et al. 2015), and at different muscle lengths (Sasaki et al. 2014). The muscle shear modulus response during contraction along with the contraction intensity has been reported to be associated with myoelectric activity (Nordez and Hug 2010), and consequently, interpreted as a valid way to represent the active forces derived from contractile machinery (Hug et al. 2015). Therefore, the SWE technique has been suggested as an effective way to investigate stiffness differences between muscles along different contraction intensities, which can be helpful to understand several human motor control processes (e.g., intermuscular coordination) and musculoskeletal injury mechanisms (e.g., muscle strain) in humans.

The hamstring muscle group is an important anatomical structure for human performance (Morin et al. 2015), and is often of great interest for sports science and health professionals due to the high occurrence of strain injury (Feeley et al. 2008). For instance, hamstring strain injury is the most common musculoskeletal injury subtype in footballers (Ekstrand et al. 2011), and has increased ~ 4% annually between 2001 and 2014 in elite European football athletes (Ekstrand et al. 2016). Based on T2 relaxation quantification using functional magnetic resonance imaging, a previous research group has reported that the mechanism of hamstring strain injury may relate to an alteration of the load within the hamstring muscles during contraction (Schuermans et al. 2014, 2016); in particular the biceps femoris long head (BFlh) and the semitendinosus (ST). However, the T2 relaxation is not an appropriate parameter to reflect muscle load. Instead, SWE may be more adequate to infer about the load sharing strategy (Bouillard et al. 2012, 2014). Moreover, previous biomechanical studies have suggested that BFlh is the hamstring muscle with the highest stiffness during contraction, and hence more prone to injury (Schache et al. 2012; Dolman et al. 2014). This has also recently been observed in vivo using SWE during a passive condition (i.e., no muscle contraction) (Umegaki et al. 2015a, b; Le Sant et al. 2015; Miyamoto et al. 2017); while it has been shown that ST stiffness was the lowest among the hamstring muscles. However, measurements under passive conditions are unlikely to reflect the stiffness response during contraction (Shinohara et al. 2010; Lacourpaille et al. 2017). Therefore, the stiffness of each hamstring muscle during contraction remains to be examined in vivo.

Interestingly, previous studies examining the muscle shear modulus during contraction have reported a nonlinear response with contraction intensity (Bouillard et al. 2012), and a heterogeneous shear modulus between synergistic muscles (Bouillard et al. 2012; Lacourpaille et al. 2017). The nonlinear response opposes what is expected with the myoelectric response (i.e., linear response) during an isometric contraction at a given joint angle (Kellis and Katis 2008; Campy et al. 2009). However, it is unknown if a similar response would also occur in the hamstring muscles. Important to note that conflicting conclusions have been reported regarding the activation of hamstring muscles during contraction based on surface electromyographic (sEMG) assessments (Kumazaki et al. 2012; Zebis et al. 2013; Tsaklis et al. 2015). Moreover, from a clinical perspective, it is usual to compare the muscular properties between limbs. To date, few studies have used SWE to examine if the stiffness of bilateral muscles would be similar (Alfuraih et al. 2018); however, to our knowledge, the inter-limb muscle shear modulus symmetry has not been investigated in the hamstring muscles, and in particular, during contraction. Therefore, it remains to be determined if healthy individuals present inter-limb hamstring muscle stiffness symmetry. This may provide new insights into several research, and clinical, topics. Nonetheless, to use SWE to investigate the stiffness of hamstring muscles during contraction, the first step is to confirm the muscle shear assessment repeatability and quantify its typical measurement error.

This study, composed by two experiments, was conducted to achieve the following objectives: (1) determine the intra- (for ST and BFlh) and inter-day (for all hamstring muscles) repeatability of shear modulus assessment; (2) examine the association between shear modulus and myoelectric activation of ST and BFlh with contraction intensity; and (3) examine the stiffness distribution of the hamstring muscles and (4) their inter-limb symmetry, during a submaximal isometric contraction in healthy individuals. The hamstring contraction was performed isometrically in a knee flexion exercise. We hypothesized that: (1) an acceptable reliability would be obtained in assessing the hamstring muscles shear modulus during contraction; (2) both BFlh and ST shear modulus would increase with knee joint torque and sEMG; (3) the BFlh of healthy individuals would present a greater shear modulus compared to the remaining hamstring muscles, and (4) there is inter-limb symmetry in the hamstring muscles stiffness.

Materials and methods

Participants

Twelve healthy and physically active adults participated in the experiment I (nine males and three females; age: 24.5 ± 8.63 years; height: 1.71 ± 0.09 m; body mass: 69 ± 6.27 kg;); while 11 male individuals (age: 29 ± 9.4 years; height: 1.73 ± 0.07 m; body mass: 70.0 ± 8.9 kg) volunteered to the experiment II. The minimal sample size was estimated for a p value of 0.05, 80% statistical power, effect size of 0.2, and 0.8 Pearson correlation (G*Power software, v3.1.9.2). People from both sexes were included (in experiment I), since it has been reported no statistical differences in muscle shear modulus during contraction between sexes (Souron et al. 2016). Participants reported no history of lower limb injuries in the past 2 years (including history of hamstring strain injury), and performed recreational sports at the time of the study. Consent was obtained from all the participants. Participants were instructed to not perform strength or flexibility training 72 h prior to study testing session. The Ethics Committee of the Faculty of Human Kinetics (University of Lisbon) approved this study (approval number: 21/2016). The procedures were conducted according to the principles expressed in the Declaration of Helsinki.

Equipment and variables

Dynamometry

The knee torque was measured at a sample rate of 1 kHz through an isokinetic dynamometer (Biodex System 3 research, Shirley, NY, USA). The participants were positioned prone, with the assessed knee flexed at 30°, the hip in neutral position (i.e., 0°), and the lateral femoral condyle aligned with the dynamometer axis. The ankle of the tested limb was immobilized at approximately 90° using an orthosis and tape. This procedure was always conducted by the same examiner. The contralateral lower limb rested in a neutral position during the tests (Fig. 1).

Surface electromyography

For experiment I (see description below), the activation of BFlh and ST was assessed at a sample rate of 1 kHz using sEMG through a telemetric system (Plux, Lisbon, Portugal). Before the electrode placement [bipolar Al/AgCl electrodes with an inter-electrode (center-to-center) 20-mm distance; AMBU, Ballerup, Denmark], the skin was shaved, slightly roughened using abrasive sandpaper, and cleaned with alcohol. Electrodes were placed over the ST and BFlh muscles bellies, at ~40% proximal-to-distal femur length, next to the ultrasound probe cast (Fig. 1). Ultrasound was used to guide the electrodes placement according to the fascicles direction. Tape was used to cover the electrodes to avoid contact to the gel used for ultrasound probe. The ground electrode was fixed over the medial malleolus of the contralateral limb. The acquisition and processing signal was carried out under International Society of Electrophysiology and Kinesiology (ISEK) standards.

Ultrasound-based shear wave elastography

The muscle shear modulus was assessed using an ultrasound scanner (Aixplorer, v11; Supersonic Imagine, Aix-en-Provence, France) in SWE mode (musculoskeletal preset, penetrate mode, smoothing level 5, persistence off; scale: 0–800 kPa), coupled with a linear transducer array (SL10-2, 2–10 MHz. Super Linear, Vermon, Tours, France). The SWE technique has been previously described in detail (Bercoff et al. 2004). Briefly, a pushing beam is created to generate the propagation of a shear wave within the muscle. Then, ultrafast ultrasound sequences are used to measure the shear wave velocity (Vs) using a time-of-flight algorithm in each pixel of the map. Assuming a linear elastic behavior, a shear modulus (μ) is calculated using vs as follows $\mu = \rho \times Vs^2$, where ρ is the muscle mass density (1000 kg/m^3). The push frequency (that generated the elastogram window) was set automatically by the ultrasound equipment to approximately 1 Hz (range 0.8–1.4 Hz), depending of the elastogram window size and position (i.e., frequency decreases for larger windows and at higher depths).

To ensure a stable measure of the muscle shear modulus during the contractions, a plastic cast was fixed (using bi-adhesive tape) to the skin on the cutaneous projection of each muscle mid-cross-sectional area, at at ~55%, ~20%, ~55%, and ~43% of the distal-to-proximal femur length for the BFlh, biceps femoris short head (BFsh, ST, and SM, respectively). To find these regions of interest, both longitudinal and transverse scans were performed. In experiment I, only the BFlh and ST was assessed, due to the difficulties in performing feasible measurements on SM and BFsh in higher contraction intensities, and also due the higher number of contractions needed to cover all the muscles which would increase the assessment duration and produce higher index of knee flexors fatigue. The cast orientation was determined using ultrasound (B-mode), according to the fascicle orientation during submaximal contraction in each muscle (Fig. 1). This cast allowed the examiners to always place the probe in the same site for each measurement (Fig. 1). These sites were chosen since we previously observed that these positions corresponded to the largest cross-sectional muscle areas were fascicles could be well visualized during

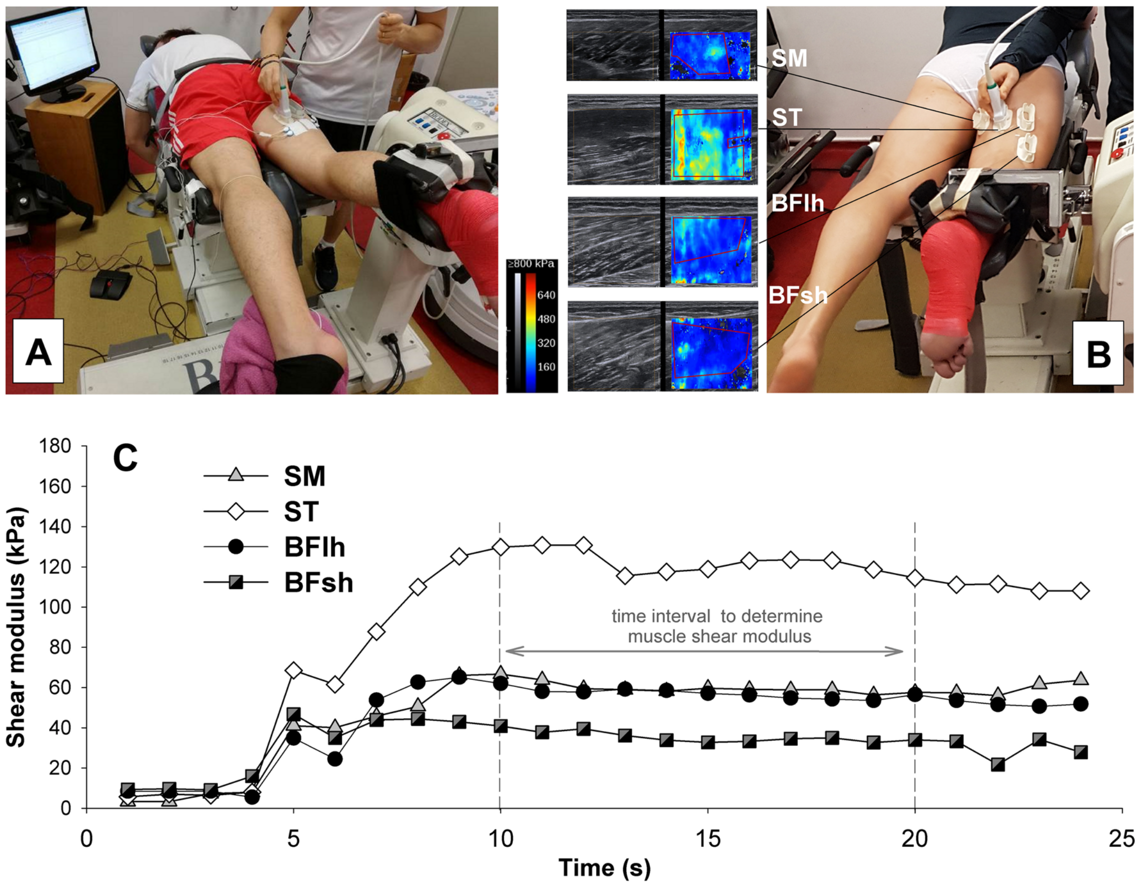

Fig. 1 Experimental setting used to perform knee flexion isometric contractions in (**a**) experiment I, where and both semitendinosus (ST) and biceps femoris long head (BFlh) shear modulus, and activation (i.e., by means of sEMG), were assessed along the contraction intensity; and used in (**b**) experiment II, where the shear modulus of all hamstring muscle heads were assessed at 20% of maximal isometric voluntary contraction. **c** Raw shear modulus response of the hamstring muscles from one participant (#3) of experiment II, from rest (0–5 s) to the target contraction intensity (5–20 s). The shear modulus calculation was performed in the area circumscribed in red on each elastogram showed in **b**. The gray dots in **c** indicate the timeline interval (10–20 s) considered for data processing, where the shear modulus of each hamstring muscle was calculated. *BFsh* biceps femoris short head, *SM* semimembranosus. Sonograms shown in **b** in both brightness (left) and shear wave elastography (right) correspond to the data shown in **c**

contraction. Examiners were trained to perform the measurements with the minimal pressure on the skin. Clip videos with both B-mode and elastogram windows included were recorded during the tests.

Protocol

Experiment I

Participants performed one testing session. Prior to the testing, participants performed several submaximal contractions for ~5 min to familiarize with the experimental setup and to warm-up. The testing consisted of measuring the knee flexion torque, and the ST and BFlh activation and shear modulus during a 15-s knee flexion isometric contraction test, at different submaximal intensities (i.e., 10, 20, 30, 40, 50 and 60% of maximum voluntary isometric contraction, MVIC). Visual feedback was given to participants during the testing, so the target knee torque intensity could be performed. Only the dominant lower limb (i.e., preferred limb to kick a ball) was tested.

After participants positioning, two 5-s knee flexor MVIC repetitions were initially performed to determine the submaximal contraction intensities, and for sEMG amplitude normalization purposes. Then, the participants were asked to perform isometric knee flexor contractions with different intensities in a random order, by following a line set on the screen that displayed the torque produced. Each contraction lasted 15 s. For each intensity, the contraction was performed twice. The examiner held the probe to the cast during the contraction and ensured that the shear modulus was properly measured and displayed on the ultrasound screen. When the shear modulus elastogram was not fully filled over the muscle region of interest, an additional repetition was performed. At the end of the submaximal contractions, two additional 5-s knee flexor MVIC repetitions were performed to determine the fatigue index (i.e., % of MVIC decrease). 1 min rest was given between all the knee flexors contraction repetitions (i.e., a total of 28 repetitions).

Experiment II

This experiment was conducted after experiment I. Participants were tested in two sessions, separated by 7 days, at approximately the same time of the day. In the first session, both lower limbs were tested, while in the second session only one limb (randomly chosen) was retested. At the start of the first session, two 5 s knee flexors MVIC were performed (1 min rest between trials) in each lower limb. The highest MVIC trial, among lower limbs, was used to determine the target knee flexion intensity. Thus, the same torque was performed during the knee flexion in both lower limbs during the experiment. After 5 min rest, the shear modulus of each hamstring muscle was measured during a knee flexion 15 s isometric contraction at 20% of MVIC, using visual feedback (1 min rest between trials). This submaximal contraction intensity was chosen as we noted an accepted repeatability in experiment I (please see the "Results" section). Two trials were performed for each muscle assessment, representing a total of eight trials per limb. In the end of the first session, two MVIC trials (1 min rest between trials) were performed in the lower limb with the highest performance to determine the fatigue index. For the second session, only one limb (randomly chosen) was tested to assess inter-day repeatability by performing the same knee flexion torque as in the first session. The location for the ultrasound cast placement was marked on the skin in first session, to ensure the same ultrasound probe location on the second session. Before the placement of the casts, ultrasound scanning was performed to ensure the proper probe alignment in respect to muscle fascicles during the contraction.

Data processing

In both experiments, the physiological data were synchronized using an external switch that simultaneously started the data collection of all equipment, and acquired using a Biopac acquisition system (MP100, Santa Barbara, USA). Data were processed using automatized Matlab routines (The Mathworks Inc., Natick, Massachusetts, USA).

The sEMG signals were band-passed filtered (10–500 Hz), full-wave rectified and smoothed with a low-pass filter (12 Hz, 4th order Butterworth). Then, the signals were amplitude normalized to the maximal values determined from the MVIC. For the shear modulus calculation, each clip exported from Aixplorer's software was sequenced in .jpeg images. Image processing converted each pixel of the color map into a value of the shear modulus based on the recorded color scale. The largest region of interest (ROI) in the elastogram window was determined by avoiding aponeurosis and tissues artifacts (e.g., vessels), and the shear modulus values were averaged for a representative muscle value. The average shear modulus was divided by 3, to better represent the muscle shear elastic modulus (Bercoff et al. 2004). The average from the 5th to the 15th second of the isometric contraction was calculated for knee torque, shear modulus, and sEMG, and used for statistical analysis as representative values for the target contraction intensity. As the change of muscle shear modulus along the contraction intensity reflects the variation of muscle localized tension, the inter-muscular loading strategy between two synergistic muscles can be inferred from relative shear modulus changes between muscles. Thus, the BFlh/ST ratio for both shear modulus and activation (from experiment I) were determined for each contraction intensity, and used for analysis to infer about the load sharing strategy along the contraction intensity.

Statistical analysis

Data analysis was performed using the IBM SPSS Statistics 22.0 (IBM Corporation, New York, USA). Normality was confirmed using Shapiro–Wilk test. Both the intra- and inter-day repeatability for the muscle shear modulus assessment was determined by calculating the intraclass coefficient correlation ($ICC_{2,1}$ for experiment I, and $ICC_{2,k}$ for experiment II), the standard error of measurement, and the Pearson correlation coefficient (r). The standard error of measurement was calculated as standard deviation of the difference scores divided by $\sqrt{2}$.

For the experiment I, paired t tests were performed to determine whether differences existed between the shear modulus assessments in each contraction intensity, and to determine if MVIC changed after the protocol; in addition, Cohen d effect size was calculated. A 2 × 6 repeated measures ANOVA [muscles (BFlh, ST) × intensity (10, 20, 30, 40, 50, and 60% of MVIC)] was performed for the shear modulus and sEMG activation variables. These ANOVAs were followed by a post hoc analysis with Bonferroni test when appropriate. Paired t tests were performed to compare the shear modulus or sEMG in each percentile of MVIC between muscles. One-way ANOVA (1 × 6) was performed for each the BFlh/ST shear modulus and sEMG ratios. The relation between shear modulus and sEMG assessment was also determined by calculating the Pearson correlation coefficient (r) for each participant.

For experiment II, a repeated measures ANOVA approach was performed [two limbs (dominant, non-dominant) × four muscles (BFlh, BFsh, ST, SM)] was performed to examine shear modulus differences between limbs and hamstring muscles; and a paired t test was performed to examine differences in the BFh/ST shear modulus ratio. Post hoc analysis was performed with the Bonferroni test. In addition, Cohen's effect size (*d*) was calculated to provide clinical meaningfulness of the differences. Significance was set at $p < 0.05$.

Both ICCs and *r* were classified as: "little" (0.00–0.25), "low" (0.26–0.49), "moderate" (0.50–0.69), "high" (0.70–0.89), and "very high" (0.90–1.00). Significance was set at 0.05.

Results

In both experiments, a trivial to small decrease of MVIC was observed after the experimental testing (experiment I: −5%; $p = 0.04$; $d = 0.17$ first session of experiment II: −5%%; $p = 0.03$; $d = -0.28$). A high-to-very-high intra-day repeatability was found for BFlh and ST shear modulus assessment (Table 1); while for the hamstring muscles shear modulus inter-day assessment a moderate-to-very-high repeatability was found (Table 2).

Table 1 Intra-day repeatability outcomes for the shear modulus assessment in both biceps femoris long head and semitendinosus muscles, during isometric contractions at different intensities

	Shear modulus (kPa)		Repeatability outcomes			
% MVIC	Measure 1	Measure 2	$ICC_{2,1}$ [95% CI]	SEM	*p* value of *t* test	*d*
Biceps femoris long head						
10	11.3 ± 8.3	14.1 ± 14.1	0.81 [0.48–0.94]	4.6	0.20	0.25
20	31.9 ± 25.3	37.5 ± 24.3	0.86 [0.59–0.96]	7.1	0.16	0.22
30	60.0 ± 31.5	59.4 ± 31.1	0.86 [0.58–0.96]	8.2	0.91	0.02
40	76.1 ± 28.6	78.2 ± 33.9	0.93 [0.76–0.98]	5.2	0.56	0.07
50	79.2 ± 31.1	81.8 ± 34.2	0.89 [0.67–0.97]	6.3	0.62	0.07
60	83.0 ± 29.0	81.0 ± 39.5	0.72 [0.27–0.91]	11.4	0.80	0.06
Semitendinosus						
10	48.2 ± 21.2	43.8 ± 20.2	0.93 [0.72–0.98]	3.7	0.06	0.21
20	79.5 ± 29.8	81.5 ± 34.1	0.89 [0.67–0.97]	7.3	0.66	0.06
30	88.3 ± 38.6	97.0 ± 40.0	0.87 [0.61–0.96]	9.0	0.15	0.22
40	86.8 ± 41.3	92.0 ± 40.3	0.91 [0.73–0.97]	6.5	0.31	0.13
50	85.6 ± 31.3	98.3 ± 31.5	0.84 [0.29–0.96]	7.6	0.01	0.41
60	74.8 ± 32.7	80.6 ± 30.0	0.85 [0.58–0.95]	7.8	0.26	0.19
BFlh/ST						
10	0.28 ± 0.20	0.33 ± 0.25	0.79 [0.43–0.93]	0.10	0.60	0.22
20	0.42 ± 0.41	0.50 ± 0.30	0.72 [0.29–0.91]	0.18	0.63	0.20
30	0.70 ± 0.34	0.67 ± 0.43	0.92 [0.75–0.98]	0.10	0.84	0.08
40	1.03 ± 0.61	0.96 ± 0.54	0.93 [0.79–0.98]	0.15	0.78	0.12
50	1.05 ± 0.59	0.92 ± 0.43	0.62 [0.13–0.87]	0.30	0.53	0.26
60	1.29 ± 0.60	1.20 ± 0.76	0.84 [0.55–0.95]	0.26	0.75	0.13

BFlh/ST biceps femoris long head to semitendinosus ration, *d* Cohen effect size, *ICC* intraclass correlation coefficient, *MVIC* maximal voluntary isometric contraction, *SEM* standard error of measurement

Table 2 Inter-day reliability outcomes for the shear modulus assessment in biceps femoris long head, biceps femoris short head, semitendinosus and semimembranosus muscles, during isometric contractions at 20% of maximal voluntary isometric contraction

	Mean ± SD					
	Day 1	Day 2	$ICC_{2,k}$ (95% CI)	SEM	*p* value	*d*
BFlh	48.52 ± 15.48	45.34 ± 19.67	0.84 (0.52–0.95)	8.03	0.68	0.18
BFsh	63.16 ± 21.62	64.79 ± 28.09	0.69 (0.22–0.91)	16.40	0.88	0.06
SM	54.58 ± 16.71	53.76 ± 18.08	0.82 (0.45–0.95)	6.71	0.91	0.05
ST	80.55 ± 21.11	82.22 ± 19.87	0.72 (0.27–0.91)	12.99	0.85	0.08
BFlh/ST	0.65 ± 0.35	0.62 ± 0.45	0.91 (0.71–0.98)	0.10	0.87	0.03

BFlh biceps femoris long head, *BFsh* biceps femoris short head, *BFlh/ST* biceps femoris long head to semitendinosus ratio, *d* Cohen effect size, *ICC* intraclass correlation coefficient, *r* Pearson correlation coefficient, *SM* semimembranosus, *ST* semitendinosus, *SEM* standard error of measurement

Relation between muscle shear modulus and EMG activation

Most of the individuals sEMG-shear modulus relationship for the BFlh was found to be moderate-to-very-high (very low relation: $n=1$; moderate relation: $n=1$; high relation: $n=4$; very high relation: $n=6$; mean = 0.82; median $r=0.90$; overall sample $r=0.73$), while for the ST the sEMG-shear modulus relation was seen very low to low (very low relation: $n=3$; low relation: $n=2$; moderate relation: $n=5$; high relation: $n=1$; very high relation: $n=1$; mean = 0.51; median $r=0.55$; overall sample $r=0.22$) (Fig. 2).

Effects of contraction intensity on muscle shear modulus and EMG activation

Figure 3 shows the muscle shear modulus and activation at the different isometric contraction intensities. For shear modulus, a significant effect was observed for the muscle ($p=0.032$), intensity ($p<0.001$), and for the muscle × intensity interaction ($p=0.036$). For muscle activation, a significant effect was observed for the muscle ($p=0.009$), intensity ($p<0.001$), and for the muscle × intensity interaction ($p=0.019$). Regarding the BFlh/ST ratio, an effect was observed for intensity in shear modulus ($p<0.001$), but not for activation ($p=0.085$).

Stiffness distribution among hamstring muscles and inter-limb symmetry

The mean shear modulus of the hamstring muscles in both lower limbs, as well the BFlh/ST shear modulus ratio, is shown in Fig. 4. No effect was found for limb ($p=0.317$) nor for muscle × limb interaction ($p=0.778$), but an effect was observed for muscle ($p<0.001$). Post hoc analysis revealed that the shear modulus of ST (80.6 ± 21.1 kPa) was higher than BFsh (63.2 ± 21.6 kPa, $d=0.81$, p = 0.022), SM (54.6 ± 16.7 kPa, $d=1.36$, $p=0.005$) and BFlh (48.5 ± 15.5 kPa, $d=1.73$, $p=0.001$). No differences were observed between BFlh, BFsh, and SM ($p>0.474$). No differences were seen in the BFlh/ST shear modulus ratio between limbs.

Discussion

To our knowledge, this is the first study that examined the shear modulus of the hamstring muscles in healthy individuals using SWE throughout a range of submaximal isometric contraction intensities. We found: (1) high-to-very-high intra- and inter-day repeatability in the hamstring shear modulus; (2) both the BFlh and ST showed a nonlinear shear modulus increase with contraction intensity, while only BFlh showed a moderate-to-very-high shear modulus-sEMG activity relationship; (3) ST showed the highest shear modulus among the hamstring muscles, although the BFlh/ST shear modulus ratio increased with the contraction intensity; and (4) inter-limb symmetry in hamstring muscles shear modulus was observed when the measurements were performed at the same proximal-to-distal muscle length.

An accurate quantification of the force produced by individual muscles still remains a major challenge. Previous studies have elucidated the validity of muscle shear modulus during contraction as an index of muscle force by correlating shear modulus against sEMG measurements (Ateş et al. 2015; Nordez and Hug 2010); and showing that measurements can be performed with moderate to high repeatability (Sasaki et al. 2014; Ateş et al. 2015). However, to our knowledge, no studies have been performed on the hamstring muscles. In regard to validity, most of participants showed a moderate-to-very-high shear modulus-sEMG relationship for BFlh (i.e., 11 in 12 participants), and a low-to-moderate relationship for ST (i.e., 10 in 12 participants). We speculate that the low shear modulus-EMG relationship found for the ST in most of the participants could be due to factors underlying the shear wave speed quantification (e.g., changes in fascicle orientation during contraction), and the undesired electric signals derived from other muscles (i.e., crosstalk). A previous study obtained a similar conclusion for another muscle, i.e., tibialis anterior (Raiteri et al. 2016). For instance, the area of ST projection to the skin where the electrodes were placed may have decreased (or moved out) during contraction causing other muscles to become closer to the location of the surface electrodes. This potentially favored crosstalk from other muscles (e.g., semimembranosus). However, the present results do not allow us to conclude if there was a crosstalk affecting the recording of ST activation; thus this should be investigated in the future, as well as examining the validity of shear modulus measurement when inferring about muscle stiffness during contraction. Nevertheless, a moderate-to-very-high correlation was found for BFlh in most of the participants, which reinforces the validity of shear modulus assessment.

With respect to repeatability, previous studies assessing the muscle shear modulus during contraction have reported high-to-very-high reliability outcomes (Yoshitake et al. 2014; Sasaki et al. 2014; Lapole et al. 2015; Hug et al. 2015). This study is the first to examine both the intra- and inter-day repeatability of the hamstring muscles shear modulus assessment during contraction. This is an important methodological issue, since the shear modulus assessment reliability is muscle-specific due to variations in structural properties and muscle architecture (Hug et al. 2015). Although the hamstring muscles present a complex muscle architecture (i.e., which can affect the measurements accuracy and reliability), we noted high-to-very high repeatability when

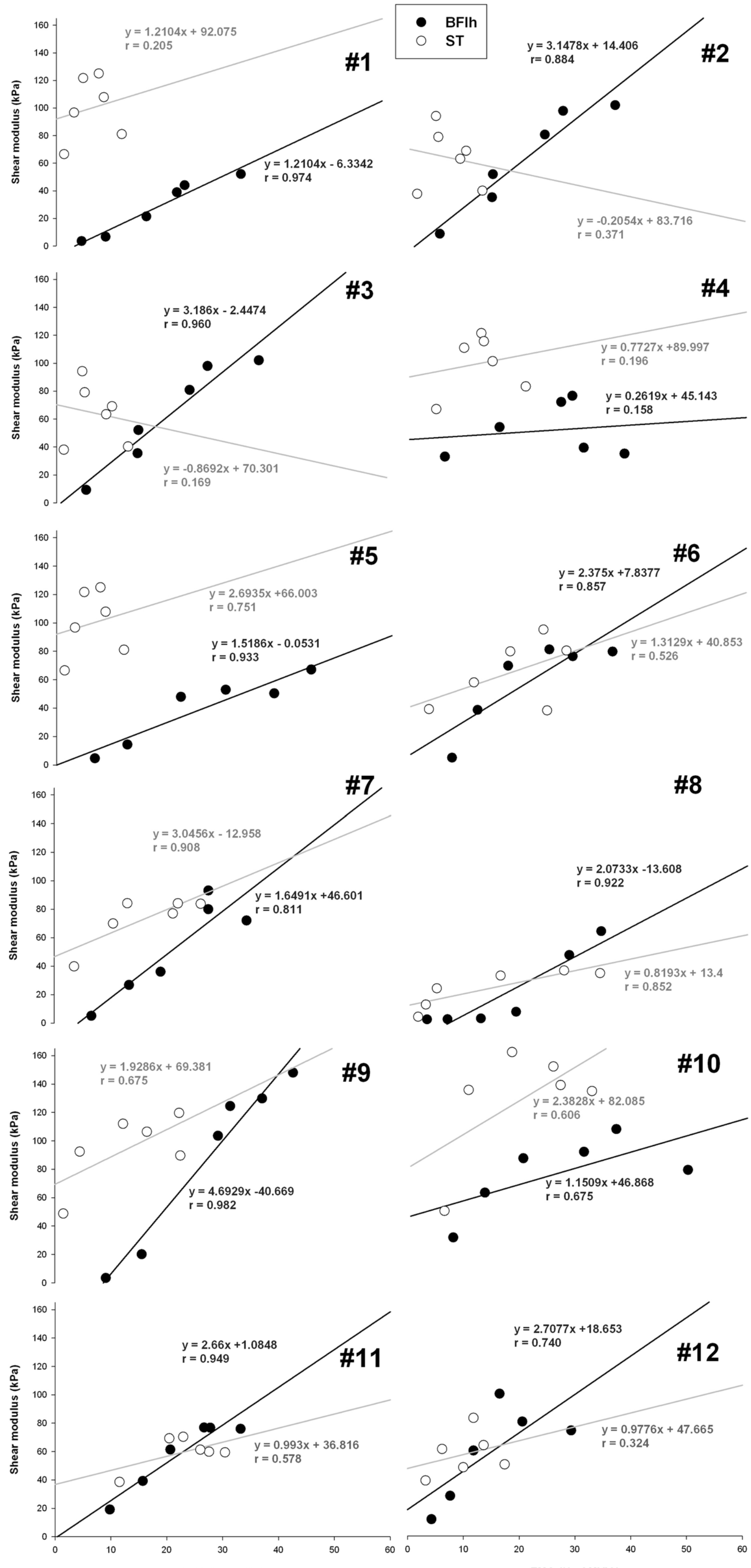

Fig. 2 Relation between the muscle activation (by means of surface EMG) and shear modulus during the isometric knee flexors contractions at different intensities of the participants biceps femoris long head (BFlh) and semitendinosus (ST)

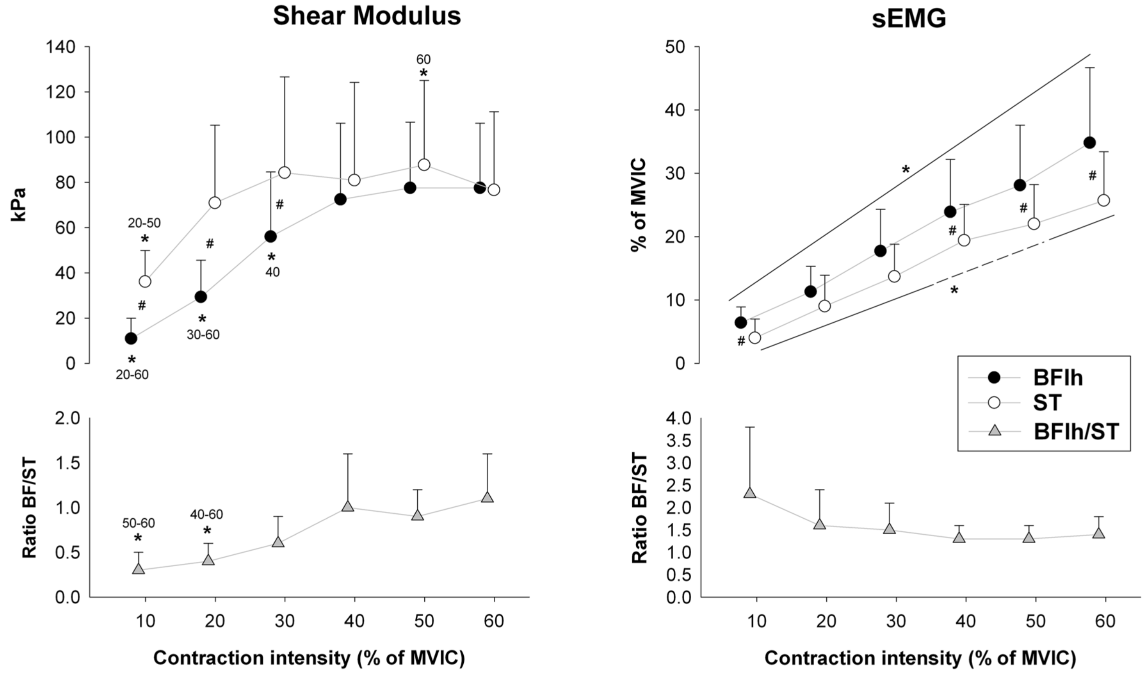

Fig. 3 Shear modulus (left) and muscle activation (right) values for the biceps femoris long head (BFlh) and semitendinosus (ST) during the isometric knee flexors contractions, presented as mean (standard deviation); and, at the bottom, the BFlh/ST ratio presented for both variables. *MVIC* maximal voluntary isometric contraction, *sEMG* surface electromyography. #Significant different between muscles ($p<0.05$). *Significant different between intensities ($p<0.05$). (1) the contraction intensity which has difference is indicated near the asterisk symbol; (2) in sEMG, differences between ST intensities were seen for all except repetition four to five (indicated with a dashed line)

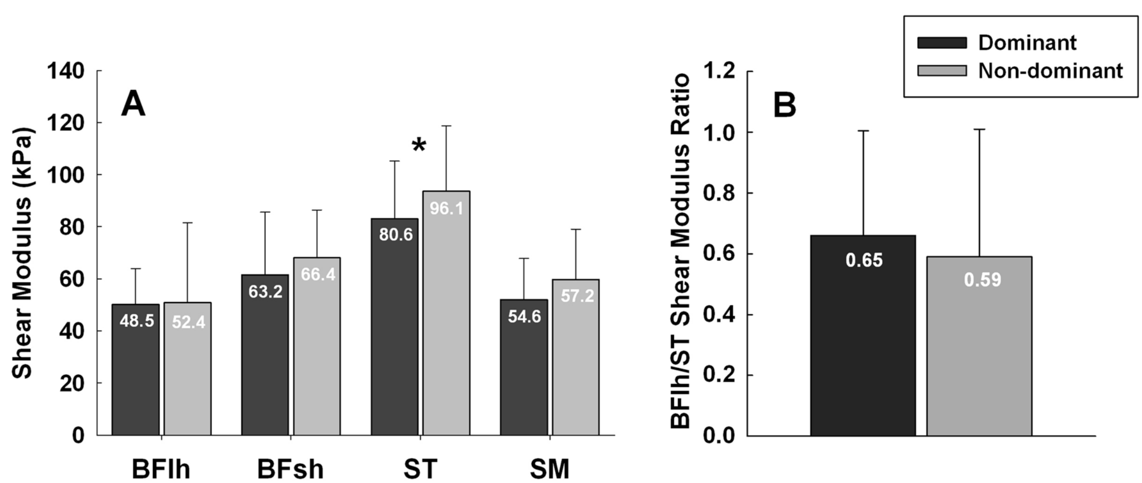

Fig. 4 **a** Shear modulus of the individual hamstring muscles, and **b** biceps femoris long head to semitendinosus (BFlh/ST) shear modulus ratio of both dominant and non-dominant limbs. *BFlh* biceps femoris long head, *BFsh* biceps femoris short head, *ST* semitendinosus, *SM* semimembranosus. *Significant differences between the ST and the other hamstring muscles, in both dominant and non-dominant limbs ($p<0.05$). Average values are shown inside the bars

assessing the hamstring shear modulus during contraction. We propose that two factors contributed to the repeatable measurements. First, isometric contractions were performed rather than slow dynamic contractions, as used in previous studies (Ateş et al. 2015; Nordez and Hug 2010). We decided on this procedure after observing a low-to-very-low repeatability when hamstring shear modulus was assessed in a previous pilot testing (not published) during dynamic slow contractions, i.e., 10% of MVIC per second. In addition, the shear modulus assessment is currently limited to the reading frequency of ~ 1 Hz, which makes it difficult to study in dynamic situations. We think that measurement during isometric contractions over a given interval time favored shear wave speed quantification and processing due to lower fascicle motion. Second, measurements were always taken at the same proximal-to-distal muscle length, by orienting the ultrasound probe according to the fascicles orientation with minimal pressure, and with the help of a plastic cast that was fixed to the skin to ensure a stable and constant measurement in a fixed site during the contraction intensity. These aspects have been reported as essential to minimize the methodological error (Kot et al. 2012; Alfuraih et al. 2018), and we believe that these procedures have improved the shear modulus repeatability for a given contraction intensity.The BFlh and ST shear moduli were seen to increase nonlinearly with contraction intensity, with ST showing the highest stiffness index for most of the contraction intensities. We noted that the ST shear modulus only increased to up to 30% of MVIC, while the BFlh increased up to 50% of MVIC. Given that the shear modulus of BFlh and ST showed little change between 40–60% of MVIC, it might be possible that other important knee flexors (e.g., gastrocnemius, semimembranosus, biceps femoris short head, gracilis, and sartorius) have contributed to the torque increase. Unfortunately, these muscles were not assessed due to the aforementioned methodological constraints. Therefore, this issue remains to be examined. In addition, the different shear modulus increase rate with contraction intensity between ST and BFlh might suggest a preferential loading request on certain muscles compared to other synergists. Although previous studies have reported a linear muscle shear modulus increase with contraction intensity up to 60% of MVIC (Lapole et al. 2015; Ateş et al. 2015), this may not be the case for all muscles. For instance, it was recently demonstrated that the muscle shear modulus in some neck extensors muscles did not increase with contraction intensity, despite the increase in other synergistic muscles (Dieterich et al. 2017). A similar finding was seen for brachialis during elbow flexion (Bouillard et al. 2012). Thus, we propose that the load sharing strategy across synergistic muscles might evoke a preferential load (i.e., shear modulus) increase in some muscles at certain contraction intensities, but not for others. Consequently, an increase in the contraction intensity (i.e., greater joint torque) might not reflect a greater muscle shear modulus. It is interesting to note that the BFlh/ST shear modulus ratio significantly increased with the contraction intensity due to both ST shear modulus decrease (i.e., from 50 to 60% of MVIC) and BFlh shear modulus increase with contraction intensity. This suggests that the load sharing strategy within the hamstring may evoke a greater BFlh tension when contraction intensity increases, and consequently might explain why BFlh (but not ST) strain injury occurs during the end of the swing phase where an intense hamstring contraction is thought to occur (Heiderscheit et al. 2010; Schmitt et al. 2012).

Among the hamstring muscles, the ST exhibited significantly highest stiffness at 20% of MVIC, but also at most of the submaximal isometric contractions when compared to BFlh. Interestingly, the shear modulus distribution across the hamstring muscles found in experiment II is similar to the T2 relaxation response of hamstring muscles after knee-related exercises observed in previous studies (Schuermans et al. 2016; Mendez-Villanueva et al. 2016; Bourne et al. 2016). This might explain the greater ST volume gain (compared to other hamstring muscles) after resistance training using knee flexion exercise (Bourne et al. 2016). However, it is unknown whether the muscle shear modulus associates with T2 relaxation, or other outcomes reflecting muscle activity (e.g., sEMG activity). It should be noted that T2 relaxation reflects the muscle metabolic activity (Cagnie et al. 2011), while shear modulus reflects the tissue stiffness (Hug et al. 2015); and functional magnetic resonance imaging can only be used after the muscle contraction in a resting condition, while the SWE can be used in real-time during contraction. Also, the shear modulus results are different from sEMG studies, in which similar (Tsaklis et al. 2015) or greater (Kumazaki et al. 2012; Zebis et al. 2013) BFlh activation was found compared to ST. This contradictory result suggests that the EMG may skew the analysis regarding the estimation of the muscle localized tension, i.e., probably due to sEMG limitations; please see (Martinez Valdes et al. 2018), which makes it impossible to perform comparisons between muscles.

In a passive condition, the ST is reported to have the lowest stiffness (i.e., shear modulus) among the hamstring muscles regardless of the hip and knee positions (Umegaki et al. 2015a; Le Sant et al. 2015; Ichihashi et al. 2016). However, we noted a higher stiffness in ST compared to the remaining hamstring muscles, for most of submaximal contraction intensities. As the stiffness observed during contraction is thought to be derived from the active component of the contractile element (Hug et al. 2015), this indicates that a preferential load is given to ST in low contraction intensities. Moreover, several studies have suggested that hamstring muscle stiffness may increase the strain injury risk (McHugh et al. 1999; Watsford et al. 2010; Marshall et al. 2016). On one hand, the results of the present study do not support this suggestion; since ST is reported to be the muscle with the lowest strain injury among the hamstring muscles (Hallén and Ekstrand 2014), we noted that the highest stiffness among the hamstring muscles for most of the contraction intensities. Thus, it is difficult to hypothesize if the risk of strain injury relates to the individual hamstring muscle (absolute) stiffness generated during contraction. It may be possible that this is not the case, but rather, strain injury is related to the intrinsic tissue ability to resist force and deform during (eccentric) contraction.

We also verified that healthy individuals have identical hamstring muscle stiffness between limbs during contraction at 20% of MVIC. It is important to note that inter-limb comparisons were performed for the same percentage of muscle length. Previous research has reported heterogeneous shear modulus within a muscle during passive stretching (Le Sant et al. 2017); which indicates that a stiffness heterogeneity may exist within the muscle during contraction. Based on this possibility, care was taken to perform the shear modulus measurements in an analogous site (i.e., for same muscle length, where the highest muscle thickness without visible intramuscular aponeurosis could be seen). Since it is possible that small regional differences exist within a muscle, future studies may compare regional differences in hamstring muscles during contraction. Nonetheless, the present findings suggest that health and sports science professionals may consider analyzing inter-limb (a)symmetry in populations where differences are expected (or hypothesized) to exist between limbs (e.g., after a strain injury occurrence).

The findings of the present study should be interpreted with caution regarding some methodological considerations. Only two hamstring muscles (ST and BFlh) were tested for the shear modulus and sEMG activation along the contraction intensity in experiment I for two main reasons. First, they are able to simultaneously measure shear modulus and activation during contraction (i.e., biceps femoris short head and semimembranosus have low representation at the skin level for sEMG assessment). Second, because increasing the number of muscles to be tested would imply a much higher number of knee flexion contractions which would lead to higher levels of fatigue and increase the duration of the testing session. A similar reason underlies why the hamstring muscles were tested only at 20% of MVIC in experiment II. Therefore, the hamstring load sharing pattern in other contraction intensities remains to examined. A small MVIC decrease (− 5%) was also seen at the end of the assessments. However, we assume that this did not affect the study conclusions, since the effect was trivial to small ($d = 0.17$–0.28) and the repetitions were randomized. The present results should also be carefully interpreted when comparing with other muscle contraction types and intensities. Shear modulus measurements were performed only up to 60% of MVIC because in higher intensities the elastograms are less stable, and in most situations the shear modulus was saturated and prone to artifacts. Therefore, we cannot be certain if, for instance, the BFlh/ST shear modulus ratio follows the tendency observed in contraction intensities below the 60% of MVIC. Finally, the muscle shear modulus was assessed in only one joint configuration, where we felt confidence in performing stable (and reliable) measurements. It is possible that the results may change depending on the muscle–tendon length; however, this remains to be examined in the future.

Conclusion

A moderate-to-very-high intra- and inter-day repeatability was noted in assessing the hamstring muscles' shear modulus using ultrasound-based shear wave elastography, during submaximal isometric contractions, when the probe was placed on the same site. A high correlation was found between the shear modulus and the myoelectric activation of biceps femoris long head along the contraction intensity. This was not the case for the semitendinosus, possibly due to methodological limitations. We also noted a nonlinear muscle shear modulus response, where the semitendinosus to biceps femoris long head shear modulus ratio increased with the contraction intensity, suggesting that the load sharing strategy among the hamstring muscle depends on the contraction intensity. This may explain why the biceps femoris long head has a higher incidence of strain injury in situations when a higher intensity hamstring contraction is required (e.g., sprint). Nonetheless, this response was contrary to the muscle myoelectric activation, and consequently, puts into question the validity of surface electromyography to infer about muscle tension. A heterogeneous stiffness distribution between the hamstring muscles at 20% of knee flexors maximal voluntary isometric contraction capacity was also observed, although inter-limb symmetry was noted. Here, the semitendinosus presented the highest stiffness index. The present findings open up new possibilities to investigate hamstring intermuscular coordination processes during contraction using shear wave elastography.

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Author contributions All authors conceived and designed the research. TF and BM conducted experiments. TN processed the data. TF and BM analyzed data. TF and BM wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest None of the authors has any conflict of interest to disclose. JV is funded by P20GM109090.

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