



Original research

Hamstrings passive and active shear modulus: Implications of conventional static stretching and warmup

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ABSTRACT

Purpose: This study compares the acute effects of a static stretching and a warmup protocol on the active and passive shear modulus of the hamstring muscles.

Methods: Muscle shear modulus was assessed at rest and during isometric contractions at 20 % of maximal voluntary isometric contraction (MVIC).

Results: After stretching, the passive shear modulus pattern was not altered, while at 20 % MVIC the biceps femoris short head (BFsh) and semimembranosus showed a shear modulus increase and decrease, respectively, which resulted on BFsh-SM pair differences (pre: 3.8 ± 16.8 vs. post: 39.3 ± 25.1 kPa; $p < 0.001$; $d = 1.66$) which was accompanied by a decrease of 18.3 % on MVIC. Following the warmup protocol, passive shear modulus remained unchanged, while active shear modulus was decreased for the semitendinosus (pre: 65.3 ± 13.5 vs. post: 60.3 ± 12.3 kPa; $p = 0.035$; $d = 0.4$). However, this difference was within the standard error of measurement (10.54 kPa), and did not impact the force production, since it increased only 1.4 % after the warmup.

Conclusions: The results of this study suggest that the passive and active shear modulus responses of the individual hamstring muscles to static stretching are muscle-specific and that passive and active hamstring shear modulus are not changed by a standard warmup intervention.

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Practical implications

- The present hamstring stretching protocol decreases the shear modulus of the semimembranosus muscle, which may be useful when the goal is to inhibit this muscle.
- The stretching protocol led to a shear modulus increase of the biceps femoris short head, which may be beneficial when the goal is to enhance the contribution of this muscle.
- A warmup protocol designed for running seems not to influence the mechanical properties of the hamstrings and knee flexion force production.

1. Introduction

The hamstrings continue to draw attention in the sports medicine/science field due to the continuously increasing number of injuries.¹

Therefore, a further understanding of the mechanical properties of the hamstring muscles, such as stiffness and shear modulus, measured following two different conditions, such as a warmup and stretching often performed prior to exercise or competition, may provide functionally relevant information. Indeed, warmup and stretching have been shown to affect the mechanical and physiological properties of muscles^{2–5} through increases in the maximum range of motion^{6–8} (ROM) and a decrease in the muscle shear modulus.³ Stretching is thought to affect the viscoelastic and thixotropic properties of muscles,^{9,10} while warmup exercises are thought to affect the viscoelastic and thixotropic properties,^{10,11} and in addition, have been shown to increase muscle temperature.¹² Increased ROM¹³ and decreased muscle stiffness¹⁴ of the hamstring muscles have been associated with a decreased risk of hamstring strain injury.

It has been suggested that changes in the shear modulus of an active muscle (i.e., measured during muscle contraction) are linearly correlated with changes in torque production during submaximal isometric contractions.¹⁵ Previous studies have reported between-muscle differences in the shear modulus for different tasks,^{16,17} suggesting that the neuromuscular coordination between synergistic muscles, that is, the

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load sharing strategy, could be task-dependent.^{16,18} If indeed the force sharing between muscles is different for different tasks in humans, it would confirm the extensive results obtained in animal studies where direct and multiple simultaneous muscle force measurements were performed for different tasks.^{18,20–22} Therefore, as reported by Boulliard et al.,¹⁶ measuring changes in the shear modulus of muscles for different tasks may provide an indirect, and as of yet non-validated way to determine load sharing strategies in human movement. Needless to say, if indeed shear wave elastography is being used to make inferences about the force sharing among muscles, direct validation, which has not been done to date, would be of the essence.

Previous studies have measured the passive hamstring shear modulus and reported a decrease after static stretching^{4,5,23} and after conventional warmup routines.³ However, the muscle-specific responses to any type of warmup exercise have not been described. The individual hamstring muscles have different inter- and intra-muscular passive and active shear modulus properties,^{24–26} but their passive shear modulus appears to respond in the same direction (i.e., decrease) to static stretching^{4,5,23} and warmup.³ The effects of static stretching and warmup regimes on active shear modulus have also not been examined. In this regard, it should be noted that while passive stiffness is primarily related to the mechanical properties of the elastic components (i.e. both parallel and series elastic components) but also the ever-present weakly bound cross-bridges, active stiffness essentially relates to parallel muscle contraction force, derived from cross-bridges.^{27,28}

Although warmup and stretching studies have been shown to decrease the passive shear modulus, these two interventions could produce a different response on force production since the warmup increases muscle temperature, which has a positive effect on force production,²⁹ while static stretching reduces the maximal voluntary force.³⁰ The purpose of this study was to determine the acute effects of static stretching and conventional warmup protocols on the active and passive shear modulus of the hamstring muscles. We hypothesized that passive and active shear modulus of the hamstring muscles decreases following the static stretching and the warmup protocols.

2. Methods

2.1. Study type: observational cross-sectional study

Forty physically active, male adults (Experiment I: N = 20, age: 23.8 ± 4.6 years; height: 174.6 ± 7.5 cm; body mass: 70.4 ± 9.8 kg; Experiment II: N = 20, age: 25.8 ± 3.7 years; height: 176.5 ± 8.7 cm; body mass: 76.8 ± 9.4 kg) without a history of hamstring strain injury participated in this study. Sample size was calculated with a 0.67 effect size of a previous study³¹ and 90 % of power. Participants were instructed to avoid strenuous activities 24 h before the test to minimize any confounding effect of fatigue. All participants read and signed an informed consent form prior to participating in the study. The Ethical Committee at the Faculty of Human Kinetics at the University of Lisbon approved the study (#5/2021).

The muscle shear modulus was assessed using two ultrasound scanners (Aixplorer, v11; Supersonic Imagine, Aix-en-Provence, France; Aixplorer, v12; Supersonic Imagine, Aix-en-Provence, France) in SWE mode (musculoskeletal preset, penetrate mode, smoothing level 5, opacity 100 %), coupled with a linear transducer array (SL10-2, 2–10 MHz, Super Linear, Vermon, Tours, France). For passive measures (i.e., rest condition) a scale of 0–100 kPa was used, and for active measures (i.e., contraction condition) a scale of 0–800 kPa was used. 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).³² It should be noted that the machine output is Young's modulus, which corresponds to a linear displacement, whereas the shear modulus corresponds to an angular displacement. Therefore, to estimate the shear modulus it is necessary to have some considerations. Moreover, the Young's modulus has been related to the shear modulus

for muscle tissue through the experimentally measured wave speed, using the following equation³³:

$$E = 2(1 + \nu)3G = 3G = \rho V^2$$

where E the Young's modulus, ν , Poisson's ratio, G , shear modulus, ρ is the muscle mass density (1 g/cm³) and V is the shear wave speed. Given the high-water content of soft tissue, the Poisson's ratio, ν , of muscle is near 0.5, approximating that of an incompressible medium.

However, this shear modulus calculation assumes isotropy of the tissue. However, it is known that muscle is anisotropic. The anisotropy of skeletal muscle fibers therefore requires a longitudinal transducer orientation for SWE, aligned with the fascicle orientation (since the shear waves preferably propagate in the fiber direction), to obtain accurate shear modulus values.³⁴

The shear modulus of the target muscles was assessed in areas where the hyperechoic lines delineating the muscle fascicles (i.e., perimysial membranes) were well identified. This region of interest corresponded to ~43 % (semimembranosus, SM), ~55 % (biceps femoris long head, BFlh), ~55 % (semitendinosus, ST), and ~20 % (biceps femoris short head, BFsh) of the distal-to-proximal femur length measured from the lateral femoral condyle to the greater trochanter.²⁴ To ensure a stable measure of the muscle shear modulus during the contractions, a plastic cast was fixed to the skin using bi-adhesive tape on the cutaneous projection of each muscle's mid-cross-sectional area (Fig. 1-A). To maximize the window of opportunity of the effects in both tasks, both examiners collected data simultaneously in the pairs BFlh + SM and ST + BFsh. The utilization of casts and the measurement with two examiners was previously validated.³¹ To guarantee the quality of the data, the area and percentage of shear wave filling of the elastogram window were measured.

Each group of participants visited the laboratory once. In both experiments, individuals were tested for shear wave measurements in a prone position with the hip in the neutral anatomical position, the knee at 30° of flexion,³⁵ and the ankle was restrained with an orthosis to prevent dorsiflexion during knee flexion contractions on an isokinetic dynamometer (System 3; Biodex Medical Systems, Shirley, NY) (Fig. 1-B). The right limb was tested. After positioning the casts for shear wave measurements, the passive shear modulus was assessed for each muscle. For this purpose, two video clips of 20 s were recorded using minimal transducer pressure. Participants performed 20 submaximal isometric contractions just to familiarize themselves with the dynamometer. Then, two maximal voluntary isometric contraction (MVIC) trials lasting 3 s each separated by a 1-min rest. Participants were instructed to flex the knee as hard as possible. The contraction level corresponding to 20 % of their MVIC was determined and presented to participants using visual feedback on a computer screen. At this intensity, two video clips of ~30 s were obtained for each muscle for the active shear modulus assessment. Immediately following the static stretching (Fig. 2-A) and the warmup (Fig. 2-B) protocols (within ~30 s for all individuals), participants were positioned in the same way as for the pre-task measurements. Conditions and the pre-testing measurements were repeated. The order of the muscles tested and the ultrasound devices used was randomized for both tasks.

The static stretching protocol was similar to that published by Umegaki et al.⁴ Briefly, participants laid in a supine position, with one hip flexed at 90°. The knee was extended to the maximum tolerable range of motion without pain (Fig. 1-B). Five repetitions of 60 s were performed with an interval of 30 s between repetitions. A trained strength and conditioning professional administered the passive stretching intervention. During stretching, participants were instructed to maintain the tibia in a neutral position.

The warmup consisted of a low intensity aerobic exercise, followed by dynamic mobilization and calisthenics of the lower limb. The aerobic exercise consisted of 5 min of running at 7.2 km/h on a treadmill. Dynamic mobilization consisted of ~8 min of calisthenic exercises

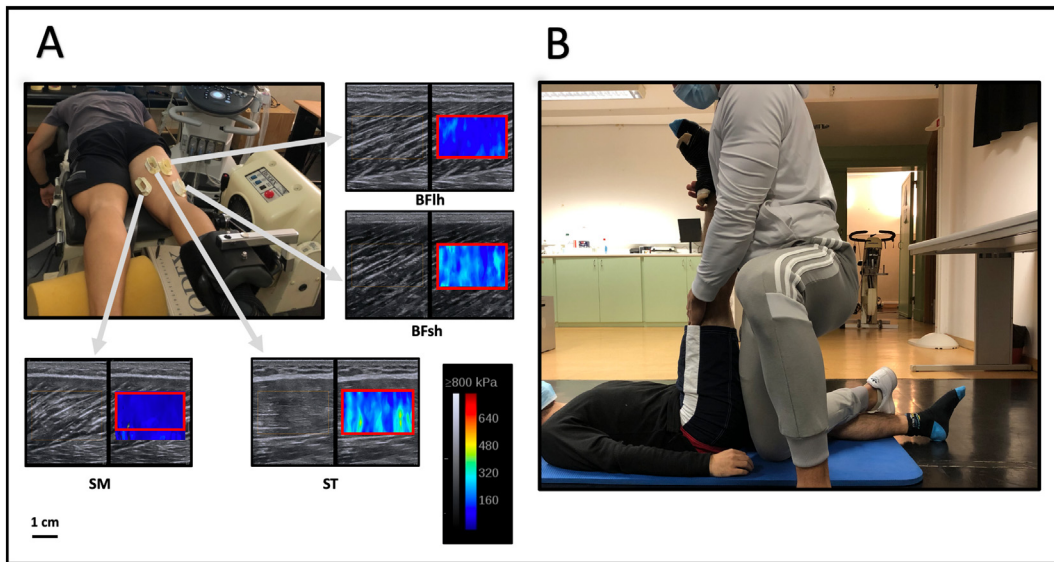


Fig. 1. (A) Shear modulus assessment at 20% of maximal voluntary isometric contraction pre-stretching for biceps femoris long head (BFh), semimembranosus (SM), semitendinosus (ST) and biceps femoris short head (BFsh) during the knee flexion exercise. The red box corresponds to the window for calculating the average shear modulus. (B) Position of the static stretching protocol: started at 90° knee flexion and 90° hip flexion and ended at the maximum range of motion without pain.

comprising 3 sets of a circuit composed of 15 repetitions of bodyweight squats, 6 repetitions of squat jumps, skipping in low, medium, and high range of motion with a duration of 10 s for each skipping range of motion. The time of rest between sets was 30 s. Most warmup protocols consist of general, running, and stretching components.³⁶ However, in this study, the stretching component was excluded to prevent interference with the interpretation of the results. Therefore, this warmup protocol was designed to include both general and running-specific components.

Dynamometer and elastography data were synchronized using an external switch that started data collection for all devices at the same time and was acquired using a Biopac acquisition system (MP100, Santa Barbara, CA). Elastography data were processed using automated Matlab routines (The Mathworks Inc., Natick, MA). 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 in the elastogram window was selected by avoiding

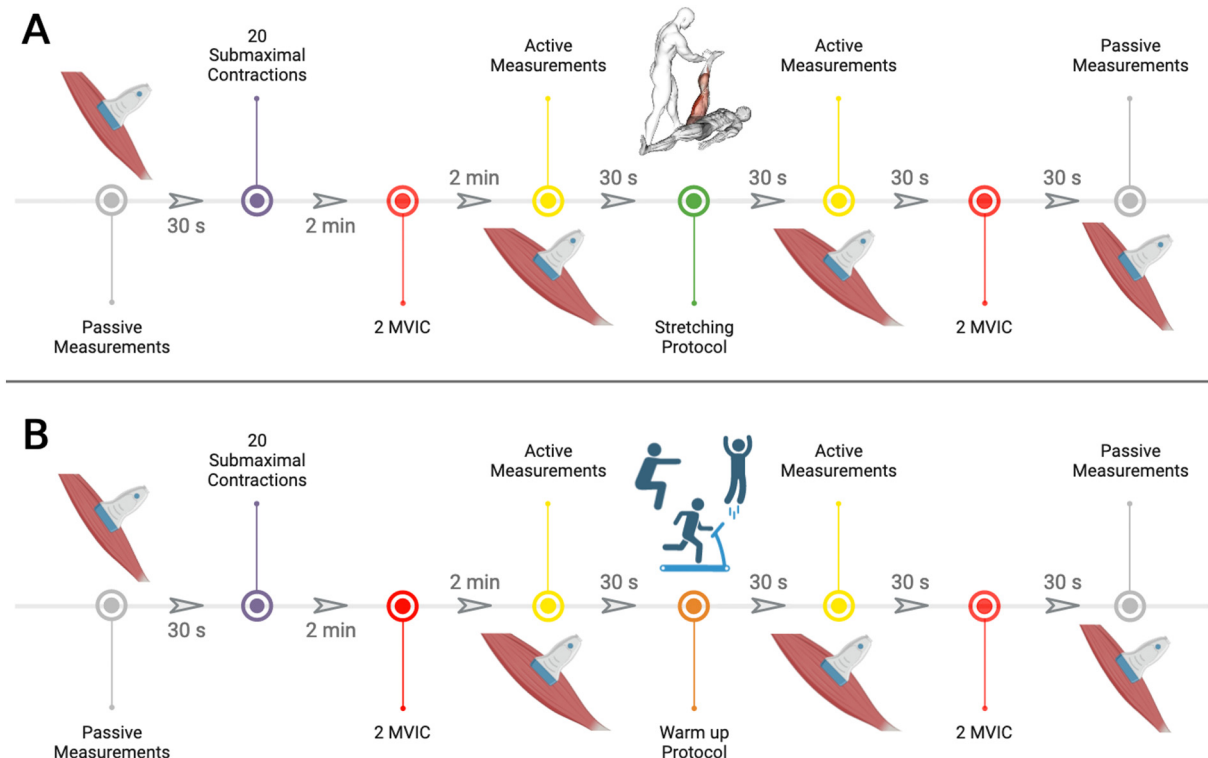


Fig. 2. Timeline of the (A) stretching and (B) warmup protocol. MVC, maximal voluntary isometric contraction.

aponeuroses, tissue artifacts (e.g., vessels) and saturation points, and the elastic values were averaged to obtain a representative muscle value. Values were then divided by 3 to better represent the muscle shear elastic modulus.³² Shear modulus data were plotted on a graph and the most stable 20 s were visually identified, averaged, and considered for analysis.

Data analysis was performed using IBM SPSS Statistics 25.0 (IBM Corporation, Armonk, NY). The Shapiro–Wilk test was used to determine if the data distribution was normal. Using data from the pre-testing stretching experiment, the standard error of measurement (SEM) of passive and active shear modulus for each muscle was determined, and used to interpret whether the effects of the protocols are covered or not by the error of measurement. The stretching and warmup tasks and muscle (BFsh, BFlh, SM and ST) effects, as well as their interaction, were examined by conducting a two-way repeated measures ANOVA [pre vs. post \times muscle (BFsh, BFlh, SM, and ST)] for the passive and active shear modulus values. Post-hoc analysis was conducted using Bonferroni correction to determine the differences within each factor. A paired sample *t*-test was used for the pre-post comparison of knee flexion MVIC. The partial eta squared (η_p^2) values were reported as a measure of the effect size of the ANOVA's findings, classified as small ($\eta_p^2 = 0.01$ – 0.05), medium ($\eta_p^2 = 0.06$ – 0.013), and large ($\eta_p^2 > 0.14$) effects.³⁷ Cohen *d* effect sizes were determined and classified as small ($d \leq 0.2$ – <0.5), medium ($d \geq 0.5$ – <0.8), and large ($d \geq 0.8$) for normally distributed data.³⁷

3. Results

3.1. Methodological findings

Large elastography map areas were obtained for both the stretching (>4.4 cm²) and warmup (4.5 cm²) conditions. Additionally, the

elastogram window filling was high for both the stretching (>99.2 %) and warmup (>99 %) conditions. Considerations on image quality can be seen in the Supplementary Table 1. The passive shear modulus SEM for each muscle was: BFlh = 0.56 kPa, BFsh = 1.27 kPa, SM = 1.02 kPa, and ST = 1.17 kPa; while the active shear modulus SEM of each muscle was: BFlh = 8.54 kPa, BFsh = 12.72 kPa, SM = 8.65 kPa, and ST = 10.54 kPa.

3.2. Static stretching

The knee flexor MVIC decreased about 18.3 ± 2.3 % after the static stretching (pre: 98.9 ± 18.8 Nm; post: 80.8 ± 18.4 Nm; $p < 0.001$; $d = 0.97$).

Static stretching induced no differences in passive shear modulus (Fig. 3-A) for the moment factor ($p = 0.996$; $\eta_p^2 < 0.001$) or for the moment \times muscle interaction ($p = 0.070$; $\eta_p^2 = 0.116$). However, a significant difference was seen for the muscle factor ($p < 0.001$; $\eta_p^2 = 0.759$). The post hoc analysis can be seen in Supplementary Table 2. Post-hoc analysis revealed significant differences between BFlh-BFsh (4.8 ± 1.6 vs. 9.4 ± 2.2 kPa; $p < 0.001$; $d = 2.39$), BFlh-SM (4.8 ± 1.6 vs. 5.8 ± 1.3 kPa; $p = 0.026$; $d = 0.69$), BFlh-ST (4.8 ± 1.6 vs. 5.2 ± 1.3 kPa; $p = 0.086$; $d = 0.27$), BFsh-SM (9.4 ± 2.2 vs. 5.8 ± 1.3 kPa; $p < 0.001$; $d = 2.00$) and BFsh-ST (9.4 ± 2.2 vs. 5.2 ± 1.3 kPa; $p < 0.001$; $d = 2.32$).

Regarding the active shear modulus (Fig. 3-B) there was no difference for the moment factor ($p = 0.756$; $\eta_p^2 = 0.005$). However, a significant interaction was seen moment \times muscle ($p < 0.001$; $\eta_p^2 = 0.785$) was also significant. Post hoc testing (Supplementary Table 3) revealed that the ST active shear modulus (71.66 ± 16.67 kPa) was significantly different from all other muscles' pre-task [BFlh (47.0 ± 10.0 kPa; $p < 0.001$; $d = 1.8$), BFsh (46.5 ± 14.8 kPa; $p < 0.001$; $d =$

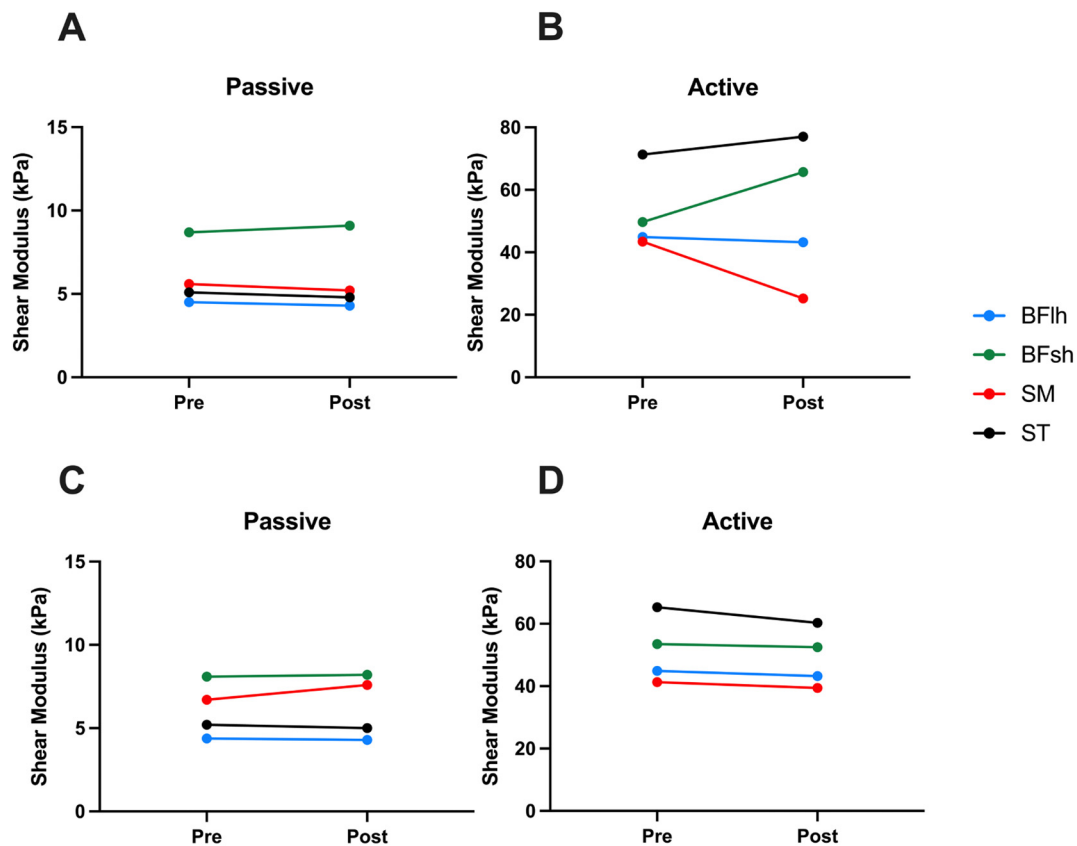


Fig. 3. Acute effects of a stretching protocol on (A) passive and (B) active shear modulus and acute effects of warmup protocol on (C) passive and (D) active shear modulus of the biceps femoris long head (BFlh), biceps femoris short head (BFsh), semimembranosus (SM), and semitendinosus (ST).

*Significant pre-post differences ($p < 0.05$).

1.6), SM (42.7 ± 8.6 kPa; $p < 0.001$; $d = 2.2$). At post-task, significant differences were seen between BFlh-BFsh (45.9 ± 13.3 vs. 63.7 ± 19.9 kPa; $p = 0.037$; $d = 1.1$), BFlh-SM (45.9 ± 13.3 vs. 24.4 ± 10.6 kPa; $p < 0.001$; $d = 1.8$), BFlh-ST (45.9 ± 13.3 vs. 72.4 ± 20.4 kPa; $p < 0.001$; $d = 1.5$), BFsh-SM (63.7 ± 19.9 vs. 24.4 ± 10.6 kPa; $p < 0.001$; $d = 2.5$) and SM-ST (24.4 ± 10.6 vs. 72.4 ± 20.3 kPa; $p < 0.001$; $d = 3.0$).

Moreover, the muscle factor was also significant ($p < 0.001$; $\eta_p^2 = 0.806$). Post-hoc analysis (Supplementary Table 2 (active values)) revealed significant differences between BFlh-SM (46.5 ± 10.5 vs. 33.5 ± 8.9 kPa; $p < 0.001$; $d = 1.3$), BFlh-ST (46.5 ± 10.5 vs. 72.0 ± 17.0 kPa; $p < 0.001$; $d = 1.8$), BFsh-SM (55.1 ± 15.2 vs. 33.5 ± 8.9 kPa; $p < 0.001$; $d = 1.7$), BFsh-ST (55.1 ± 15.2 vs. 72.0 ± 17.0 kPa; $p = 0.006$; $d = 1.05$) and SM-ST (33.5 ± 8.9 vs. 72.0 ± 17.0 kPa; $p < 0.001$; $d = 2.8$).

3.3. Warmup

The knee flexion MVIC increased 1.4 ± 4.1 % after the warmup (pre: 99.6 ± 21.9 Nm; post: 100.9 ± 22.8 Nm; $p = 0.03$; $d = 0.06$).

The warmup did not lead to significant differences in passive shear modulus (Fig. 3-C) for the moment factor ($p = 0.607$; $\eta_p^2 = 0.016$) or for the moment \times muscle interaction ($p = 0.059$; $\eta_p^2 = 0.135$). However, a significant difference was seen for the muscle factor ($p < 0.001$; $\eta_p^2 = 0.552$). The post hoc analysis can be seen in Supplementary Table 4. Post-hoc analysis revealed significant differences between BFlh-BFsh (4.14 ± 1.16 vs. 7.8 ± 2.5 kPa; $p < 0.001$; $d = 1.9$), BFlh-SM (4.1 ± 1.2 vs. 6.9 ± 3.5 kPa; $p = 0.006$; $d = 1.1$), BFlh-ST (4.14 ± 1.16 vs. 4.8 ± 1.4 kPa; $p = 0.019$; $d = 0.5$), BFsh-SM (7.8 ± 2.5 vs. 6.9 ± 3.5 kPa; $p < 0.001$; $d = 0.3$) and BFsh-ST (7.8 ± 2.5 vs. post: 4.8 ± 1.4 kPa; $p = 0.039$; $d = 1.5$).

Regarding the active shear modulus (Fig. 3-D) there was no significant moment \times muscle interaction ($p = 0.484$; $\eta_p^2 = 0.042$). However, significant differences were seen for the muscle ($p < 0.001$; $\eta_p^2 = 0.432$) and moment ($p = 0.033$; $\eta_p^2 = 0.218$) factors. Post-hoc analysis (Supplementary Table 4) revealed significant differences between muscles, BFlh-ST (40.6 ± 13.7 vs. 62.8 ± 11.9 kPa; $p < 0.001$; $d = 1.7$), BFsh-SM (53.0 ± 15.6 vs. 40.4 ± 8.4 kPa; $p = 0.042$; $d = 1.0$) and SM-ST (40.4 ± 8.4 vs. 62.8 ± 11.9 kPa; $p < 0.001$; $d = 2.2$).

Moreover, post hoc testing (Supplementary Table 5) revealed only significant differences between moments (pre-post task) for the ST muscle (pre: 65.3 ± 13.5 kPa; post: 60.3 ± 12.3 kPa; $p = 0.035$; $d = 0.4$).

4. Discussion

In this study we examined hamstrings' passive (i.e., at rest) and active (i.e., at 20 % of MVIC) shear modulus before and after static stretching and warmup protocols. To the best of our knowledge, this is the first study to examine the acute effects of stretching and warmup protocols on passive and active shear modulus of all hamstring muscles, as previous studies have only assessed passive properties and have not differentiated between the short and long heads of the biceps femoris. The main findings were (i) stretching induces changes on active shear modulus of the BFsh and SM, which showed opposing responses to stretching (increase and decrease), respectively; (ii) the warmup protocol did not alter the passive shear modulus, as there was only a physiologically irrelevant decrease in the ST active shear modulus.

Since the hamstring muscles have different intra- and inter-muscular properties in the passive and active state,^{24,26} we considered the pre-post task differences physiologically relevant between muscles when they exceed the pre-task differences. Moreover, for the moment factor, we considered that a physiologically meaningful effect was present when the difference was beyond the SEM.

Previous studies reported that passive knee extension static stretching is an effective method to reduce the passive shear modulus of all hamstring muscles.^{4,5,23} However, the magnitude of stretching

effects was reported to be greater in BFlh in two of the aforementioned studies.^{4,5} For instance, Nakao et al.⁵ reported a decrease in all hamstring muscles after stretching (5×30 s repetitions), with BFlh revealing the greatest decrease (-27.8 %), followed by SM (i.e. -24.1 %) and ST (i.e. -13.8 %). Umegaki et al.⁴ also reported a decrease in all hamstring muscles (5×60 s repetitions), with the BFlh showing the greatest decrease (-23 %), followed by the ST (-20.3 %), and SM (-18.9 %). Moreover, despite not presenting absolute or relative values for the passive shear modulus, Miyamoto et al.²³ also reported a decrease in passive shear modulus at a given joint angle for BFlh, ST, and SM. In the present study, a significant effect was only seen for the muscle factor. This result may be explained with the different intra- and inter-muscular properties of muscles in the passive and active states.^{24,26} However, a significant effect was seen for the pre-post task moment. One possible explanation for our results and those reported in the literature could be the fact that stretching affects the extracellular matrix (ECM). Since the ECM is viscoelastic, a significant decrease in passive properties (due to the influence of titin in passive force)³⁸ may occur if the ECM is under tension, thus contributing to muscle shear modulus. However, with the ECM slack (our study), the contribution to passive muscle shear modulus would be expected to be negligible, therefore not resulting in a difference in passive properties. Finally, it is possible that testing at long muscle lengths with great passive force,³⁹ the effects of stretching might be more pronounced than at short muscle length. Nevertheless, the reason behind the different findings between studies remains unknown, but joint angle and muscle length may play an important role.^{39,40}

Indeed, it should also be noted that shear wave measurements were performed using different methods between studies. The passive measurements in the present study were performed with the hip in the neutral anatomical position and the knee flexed at 30° (i.e., with the hamstring muscles slack).⁴¹ Nakao et al.⁵ measured the hamstring shear modulus with the hip flexed at 90° and the knee extended at 135° (full knee extension = 180°), while Miyamoto et al.²³ measured hamstring shear modulus during passive hamstring stretching. Umegaki et al.⁴ compared the stretching effects with the hamstrings at (presumably) slack length (i.e. hip at 90° and knee at 90°) and lengthened (i.e. hip at 90° and knee at 135°) positions. Therefore, one possible explanation for the different results may be due to the different testing positions. It should be noted that the passive shear modulus of the BFlh is the most sensitive among the hamstring muscles to changes in hip flexion,⁴⁰ and that testing in the Umegaki et al.⁴ study was performed with the hip flexed at 90° . Thus, whether the different positions (and muscle lengths) used for testing can explain the different results requires further investigation.

Regarding the active shear modulus, the results demonstrated a significant effect between muscles. However, as previously mentioned, the effect is attributed to the different properties between the individual hamstring muscles. Nevertheless, a significant moment \times muscle interaction was seen after 5 min of static stretching, characterized by opposite responses in the active shear modulus of the SM and BFsh. Stretching resulted in a 42 % decrease in the active shear modulus in SM and a 32 % increase in the active shear modulus in BFsh (Fig. 3-B). These effects were not only statistically significant but also greater than the corresponding SEM, and impacted the force production by decreasing 18 %, which suggests that they may be physiologically relevant. Indeed, the effect size was large on the BFsh-SM pair differences since they started with a similar value but changed in different directions after the task, which led to an increase >900 % (pre: 3.8 ± 16.8 kPa; post: 39.3 ± 25.1 kPa; $p < 0.001$; $d = 1.66$). Changes in the active shear modulus may be associated with changes in the load sharing among the hamstring muscles.^{15,35,42} Previous studies suggest that the SM has greater passive force during passive knee extension stretching compared to the remaining hamstring muscles.^{4,5,23,40} Thus, it is perceivable that the SM may respond with lower active force for a given knee flexion torque due to static stretching-induced inhibition of

muscle activation.⁴³ This inhibition related to stretching and the putative between-muscle compensation is related to possible differences in the force-length relationship between hamstring muscles. Furthermore, a 4-week stretching program that mobilizes the hip reported that the greatest decrease in the shear modulus was seen for the SM.⁴⁴ To compensate for the decrease in contribution to the knee flexor torque, the BFsh may increase its force, reflected by an increase in the active shear modulus. Maximal BFsh stretching can only be achieved when the knee is fully extended. Therefore, the BFsh may not have been passively loaded in the stretching maneuver used in the present study, and thus, stretch-induced inhibition would not have been present. Consequently, BFsh may have produced the observed load sharing compensation. One possible explanation, as previously mentioned, is that stretching might elongate the tendon. Thus, for a given muscle tendon unit length might shorten the average sarcomere length. If the hamstrings are working on the ascending limb of the force-length relationship, shorter sarcomere lengths would be associated with less force capacity which explains the results.

The functional implications of the potential changes in hamstring load sharing after stretching remain unclear. However, it could translate into a change in the knee external/internal rotator torque, thus affecting knee joint stability during functional tasks, such as jumping and running, which may increase injury risk.⁴⁵ It should be noted that SM is the muscle most frequently affected in hamstring stretch-type injuries.⁴⁶ Additionally, as the tendon is arranged in series with the muscle component⁴⁷ and the tendon shear modulus would expectedly decrease with stretching, which will result in a greater elongation for a given muscle force. Therefore, the increase in BFsh shear modulus (which is related to force) could potentially increase the strain of the common BF tendon, for the same amount of muscle force, and probably it could be injured more readily.⁴⁸ In this regard, it is worth noting that the BFsh muscle/tendon is the most frequently injured hamstring muscle.⁴⁹ However, this study did not measure tendon shear modulus. This possible contribution to injury risk warrants further investigation. Moreover, whether an asymmetric loading between the lateral and medial hamstring heads is associated with changes in hamstring active shear modulus should be a matter of further research.

The knee flexor MVIC decreased 18 % after the static stretching protocol, which is in agreement with previous studies that reported a decrease in maximal force production with static stretching of 3–5 min duration.^{50,51} This change in MVIC was accompanied by an altered hamstring active shear modulus pattern; while the SM active shear modulus decreased, the BFsh active shear modulus increased. Since SM is reported to have a larger physiological cross-sectional area than the BFsh,⁴⁸ it is possible that the BFsh loading compensation (i.e. increased active shear modulus) is insufficient to avoid knee flexor maximal strength loss with stretching. This result requires further investigation.

Regarding the warmup protocol and contrary to our hypotheses and previous literature,^{3,52} there were no significant effects of warmup on passive shear modulus of the hamstring muscles, with only a significant effect for muscle which has been previously explained. Regarding the active shear modulus only a significant difference was seen for ST. However, this decrease in ST shear modulus was small and likely not physiologically relevant, as the SEM value (i.e., 10.54 kPa) was greater than the change in shear modulus (i.e., 5 kPa). Moreover, even though there was a significant post-warmup increase in knee flexion MVIC, the trivial effect size and the magnitude of the change (1.4 Nm) suggest that it should not be interpreted as a physiologically meaningful increase after the warmup intervention. Thus, it appears that the decrease in active ST shear modulus had a small influence on knee flexor force. Therefore, the present findings suggest that a practical conventional warmup protocol as used here does not seem to alter passive and active shear modulus in the hamstring muscles.

This study has limitations. Firstly, shear modulus measurements were performed only at a single site. Only one site was chosen, as measuring in more regions would increase the time required for data

collection and thus prevent examination of the acute effects of stretching and warmup protocols on the shear modulus. The present findings are based on the assumption that the effects were not site-dependent. Secondly, although a considerable number of knee flexor muscles were assessed, we cannot rule out the participation of other synergistic muscles crossing the knee (e.g. gastrocnemius and gracilis) which might affect the results and some of the conclusions. Thirdly, the time between the end of the interventions (stretching and warmup) and the beginning of the shear wave measurements must be considered, which was controlled to be the same (approximately 20 s). Fourthly, passive muscle conditions were not checked using EMG. However, individuals were instructed to fully relax during the measurements. Fifthly, the muscle active shear modulus was measured only in one knee flexor contraction intensity (i.e. 20 % of MVIC); we are unaware whether the magnitude of the effects is the same at other contraction intensities. Moreover, muscle temperature was not determined after the warmup protocol, consequently, we cannot eliminate the possibility that the effects of the warmup protocol were caused by changes in muscle temperature. Finally, it is possible that the warmup protocol designed for running could not sufficiently impact the hamstrings muscles.

5. Conclusion

From the results of this study, it is possible to indicate that static hamstring stretching alters the shear modulus of hamstrings muscles, which showed a decrease of SM and an increase of BFsh in the active but not the passive state. However, a conventional warmup protocol has no effect on the shear modulus in either passive or active conditions. Future studies should examine the functional and clinical implications of altered (passive and active) hamstring shear modulus.

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Confirmation of ethical compliance

All participants read and signed an informed consent prior to participating in the study. The Ethical Committee at the Faculty of Human Kinetics at the University of Lisbon approved the study (#5/2021). All methods were performed in accordance with the Declaration of Helsinki.

CRediT authorship contribution statement

Ricardo Pimenta contributed to the conception and design of the study, collected and processed all the data, performed the statistical analysis and interpretation of the data, produced the first draft of the manuscript, reviewed the article critically for important intellectual content and edited subsequent versions of the manuscript. José Pedro Correia contributed to the data collection and analysis, and manuscript editing. João Rocha Vaz participated in the conception and design of the study, and interpretation of the data. António Prieto Veloso participated in the interpretation of the data and editing of the manuscript. Walter Herzog contributed to the analysis and interpretation of the data, editing, and reviewing of the manuscript critically for important intellectual content.

Declaration of interest statement

The authors declare that they have no competing interests.

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