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Changes in passive and active hamstrings shear modulus are not related after a warmup protocol

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ABSTRACT

This study aimed to determine whether changes in hamstrings passive and active shear modulus after a warmup protocol are correlated. Twenty males without a history of hamstring strain injury participated. Muscle shear modulus was assessed using ultrasound-based shear wave elastography at rest and during isometric contractions at 20% of maximal voluntary isometric effort before and immediately after a warmup protocol. Changes in passive shear modulus did not seem to be associated with changes in active shear modulus. The results of this study suggest that changes in passive and active hamstring shear modulus are not associated after a standardized warmup intervention.

1. Introduction

Warmup is commonly performed prior to sport activities (Behm et al., 2016; McGowan et al., 2015) and has been shown to affect the mechanical and physiological properties of muscles, particularly muscle stiffness (Cummings et al., 2022; Morales-Artacho et al., 2017). Stiffness can be divided into passive and active (Miyamoto and Hirata, 2019; Vaz et al., 2021). Stiffness *in vivo* is a continuously varying property of skeletal muscle, depending especially on the muscle activation/force (Ateş et al., 2015), as well as on factors such as previous activity (Sadeghi et al., 2018) and muscle temperature (Souron et al., 2019). While passive stiffness is primarily related to the mechanical properties of elastic components (i.e. both parallel and in series elastic components) and to the ever-present weakly bound cross-bridges, active stiffness essentially relates to muscle force, derived from cross-bridges (Ford et al., 1981; Huxley and Simmons, 1973). Several methods have been used to assess muscle stiffness (Kubo, 2014; Marshall et al., 2016; Morgan, 1977). Previous studies have used ultrasound-based shear wave elastography (SWE) for stiffness determination of *in vivo* human muscle (Morales-Artacho et al., 2017; Zhi et al., 2022). It should be noted that SWE provides an estimate of the local muscle shear modulus through the quantification of the shear wave velocity (Bercoff et al., 2004) which does not correspond directly to muscle stiffness. Indeed, stiffness is a functional property that is influenced by the shape of the specimen, while SWE provides a material property that is not affected by the geometry of the system.

An increase in the biceps brachialis was seen in passive shear modulus from an elbow angle of 60° to 180°. However, at different contraction intensities (25 %, 50 % and 75 % MVIC) the active shear modulus did not increase in some angles and intensities, indicating that the contribution of the passive and active mechanical properties are affected differently (Zimmer et al., 2023). On other hand, a similar passive and active shear modulus pattern was seen on five neck muscles with a significant shear modulus increase observed at each level of effort, with the magnitude of the increase in shear modulus with force being muscle-dependent (Dieterich et al., 2017).

To the best of our knowledge, no study has analyzed the relationship between the possible change in passive and active shear modulus after a given task. Nevertheless, a previous study reported a moderate relationship between active and passive knee flexor stiffness in humans (Blackburn et al., 2004). If a relationship between passive and active shear modulus existed, it would be possible to perform one shear modulus measurement for a given contractile condition and derive the others through the known relationship, thereby minimizing data collection and processing. Furthermore, shear moduli in most previous studies were measured for the passive state (Avrillon et al., 2020;

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Miyamoto et al., 2020; Morales-Artacho et al., 2017; Umegaki et al., 2015; Zhi et al., 2022). If changes in passive and active shear modulus are not associated, important information about the effect of exercise on the muscle might be missing.

The hamstring is a biarticular muscle group consisting of four muscles (biceps femoris short head (BFsh), biceps femoris long head (BFlh), semitendinosus (ST), and semimembranosus (SM)), with the bi-articular muscles (BFlh, SM and ST) acting simultaneously at two joints by participating in knee flexion and hip extension. The hamstrings continue to draw attention in the sports medicine/science field due to the number of injuries (Ekstrand et al., 2022). It has been reported that the hamstring's passive shear modulus decreases after a warmup (Morales-Artacho et al., 2017). However, the possible association of passive and active changes in the shear modulus after a warmup have not been examined. Since passive forces are transmitted by the connective tissue matrix, including the epi-, peri-, and endomysium and the tendon (Hill, 1968; Street, 1983), while active forces are related to structural intracellular proteins, such as actin and myosin (interaction changes between them), a minute influence of titin, tiny contribution of changes in the mechanical properties of desmin, nebulin and dystrophin (Freundt and Linke, 2019; Herzog, 2018; Li et al., 2020); it is unlikely that changes in passive and active muscle shear modulus are correlated.

The purpose of this study was to investigate the association between changes in the hamstrings' passive and active shear modulus after a conventional warmup. We hypothesized that changes in passive and active shear modulus are not correlated.

2. Methods

2.1. Sample

Twenty physically active males (age: 24.4 ± 4.9 years; height: 175.7 ± 8.2 cm; body mass: 73.3 ± 10.5 kg) without a history of hamstring strain injury participated in this study. Participants were instructed to avoid strenuous activities 24 h before the test to minimize any confounding effect of fatigue. All participants provided written 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).

2.2. Equipment and variables

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). The SWE method has been previously detailed (Ricardo Pimenta et al., 2023d). Briefly, the muscle shear modulus was assessed on the region of interest (ROI) for each hamstring muscle, corresponding to ~ 43 %, 55 %, 55 %, and 20 % of distal-to proximal femur length for the SM, BFlh, ST, and BFsh, respectively. To ensure a stable measure of the muscle shear modulus during muscle contraction and to guarantee that the same ROI was used for measurement before and after the warmup, a plastic cast was fixed to the skin using bi-adhesive tape on the cutaneous projection of each muscle's mid-cross-sectional area (Ricardo Pimenta et al., 2023b).

2.3. Protocol

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 on an isokinetic dynamometer (System 3; Biodex Medical Systems, Shirley, NY) Pimenta et al. (2023a). Testing started by assessing the passive shear modulus for each muscle. For this purpose, one vid***eoclip of 20 s was recorded by an experienced examiner using minimal transducer pressure. Then, two MVIC trials lasting 3 s each separated by a 2 min rest. Participants were instructed to flex their 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 (Ricardo Pimenta et al., 2023c). At this intensity, two vid***eoclips of ~ 30 s were obtained for each muscle (a total of 4 contractions, since only two muscles were measured in each contraction) for the active shear modulus assessment. Immediately following the warmup protocols (within 20 s for all individuals), participants were placed in the same position and the pre-testing measurements were repeated, measuring this time first the active and then the passive shear modulus. The order of the muscles tested, and the ultrasound device used were randomized. The warmup consisted of low-intensity aerobic exercise, followed by lower limb dynamic mobilization and calisthenics (Ricardo Pimenta et al., 2023c).

2.4. Data processing

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) (Mendes et al., 2018). The shear modulus calculation has been previously described (R. Pimenta et al., 2023). Briefly, shear modulus data were plotted on a graph and the most stable 20 s (for active measurements) were visually identified, averaged, and considered for analysis. Additionally, in the active state the average of the two repetitions was used for the statistical analysis.

2.5. Statistical 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. The percentage of change between passive and active shear modulus was calculated using the following equation:

$$\text{Percentage change} = (\text{POST} - \text{PRE}) / \text{PRE} \times 100$$

Moreover, the percentage of change in passive and active shear modulus was determined using Pearson's and Spearman's coefficient, for normally and not normally distributed data, respectively. The correlation coefficients were classified as weak (<0.3), moderate (0.3–0.7), and strong (>0.7) (Sheskin, 2000). Statistical significance was set at $p < 0.05$. The intraclass coefficient correlation (ICC) was used to determine the intra-examiner repeatability (ICC2,1) of active shear modulus assessment. The ICCs were classified as poor (<0.5), moderate (≥0.5–<0.75), good (≥0.75– 0.9) and excellent (>0.9) (Koo and Li, 2016). Since only one passive shear modulus measurement was made (thus not allowing for calculating the ICC), and for a better understanding of the variability of the passive shear modulus, the average, range (minimum and maximum), standard deviation, and coefficient of variation (CV) of the mean shear modulus during the 20 s of data collection were calculated at each time point (pre- and post-task). For active shear modulus, the average, range (minimum and maximum) and standard deviation of the percentage of difference between the two measurements was calculated for each time point (pre- and post-task).

3. Results

The pre-task passive shear modulus variability measures were: BFlh = 4.2 ± 1.0 kPa (0.65–5.65 kPa; CV = 25 %); BFsh = 8.1 ± 2.6 kPa (range, 1.1–12.4; CV = 33 %); SM = 6.2 ± 2.3 kPa (range, 0.77–13.1 kPa; CV = 38 %); ST = 4.9 ± 1.4 kPa (range, 0.49–6.9 kPa; CV = 28 %). Moreover, the post-task values were: BFlh = 4.1 ± 1.2 kPa (0.45–6.42

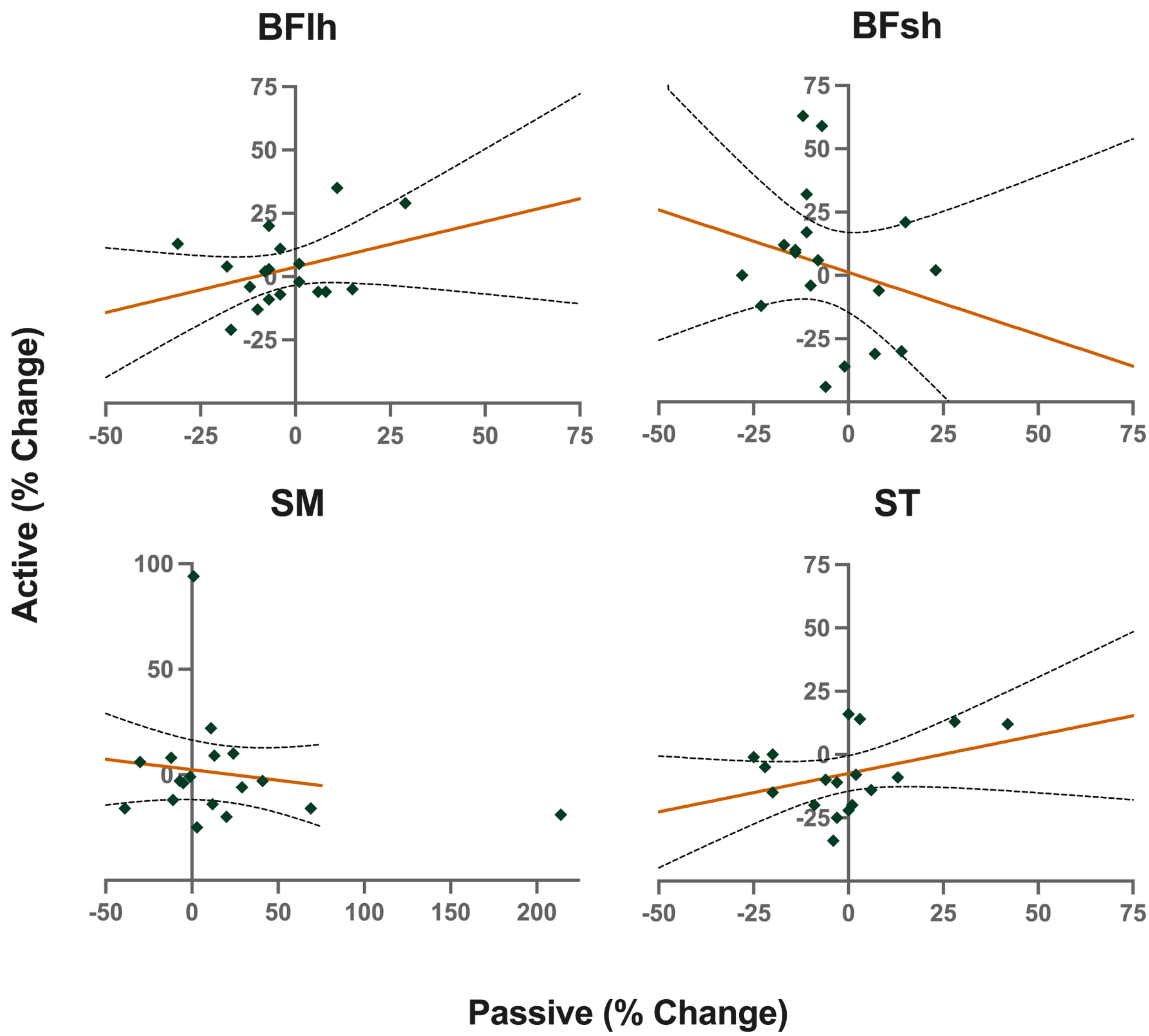

Fig. 1.

Table 1
Individual percentage differences in passive and active shear modulus (kPa) for a warmup task across all hamstring muscles. Red cells correspond to a missing value.

Participant	BFlh		BFsh		SM		ST	
	Passive	Active	Passive	Active	Passive	Active	Passive	Active
1	−31 %	13 %	−6%	−44 %	1 %	94 %	0 %	16 %
2		−24 %		−18 %		−8%		3 %
3	6 %	−6%	−12 %	63 %	12 %	−14 %	28 %	13 %
4	−7%	−9%	8 %	−6%	−12 %	8 %	−4%	−34 %
5		−39 %		12 %		−13 %		5 %
6	29 %	29 %	−14 %	10 %	20 %	−20 %	13 %	−9%
7	−7%	20 %	−11 %	17 %	−11 %	−12 %	−25 %	−1%
8	8 %	−6%	14 %	−30 %	69 %	−16 %	−3%	−11 %
9	−10 %	−13 %	−10 %	−4%	29 %	−6%	−20 %	−15 %
10	11 %	35 %	15 %	21 %	−7%	−3%	0 %	-22 %
11	−4%	−7%	−8%	6 %	214 %	−19 %	1 %	−20 %
12	15 %	−5%	7 %	−31 %	24 %	10 %	42 %	12 %
13	−8%	2 %	−14 %	9 %	41 %	−3%	3 %	14 %
14	−17 %	−21 %	−7%	59 %	3 %	−25 %	−9%	−20 %
15	1 %	−2%	−11 %	32 %	−30 %	6 %	6 %	−14 %
16	1 %	5 %	−28 %	0 %	−5%	−4%	−3%	−25 %
17	−7%	3 %	−1%	−36 %	11 %	22 %	−20 %	0 %
18	−18 %	4 %	−17 %	12 %	−1%	−1%	−22 %	−5%
19	−12 %	−4%	23 %	2 %	13 %	9 %	−6%	−10 %
20	−4%	11 %	−23 %	−12 %	−39 %	−16 %	2 %	−8%

Abbreviations: BFlh, Biceps Femoris long head; BFsh, Biceps Femoris short head; SM, semimembranosus; ST, semitendinosus.

kPa; CV = 30 %); BFsh = 7.5 ± 2.3 kPa (range, 1.0–10.73; CV = 30 %); SM = 7.5 ± 4.8 kPa (range, 0.78–22.7 kPa; CV = 65 %); ST = 4.8 ± 1.4 kPa (range, 0.49–6.74 kPa; CV = 30 %).

Regarding the active state the variability values pre-task between repetitions were: BFlh = -1.4 ± 42.7 % (-55–165 %); BFsh = 1.3 ± 45.4 % (-53–176 %); SM = -2.3 ± 16.8 % (-28–42 %); ST = 1.1 %±14.3 % (-25–28 %). Furthermore, the post-task values were: BFlh = 20.6 ± 50.7 % (–22–186 %); BFsh = 1.4 ± 27.2 % (-43–60 %); SM = 2.9 ± 11.7 % (-16–38 %); ST = -5.1 %±15.6 % (-26–31 %). The ICC values of active shear modulus indicated good repeatability at pre-task [BFlh = 0.76 (0.5–0.90); BFsh = 0.71(0.40–0.88); SM = 0.86(0.69–0.94); ST = 0.82 (0.59–0.92)], as well as at post-task [BFlh = 0.78(0.52–0.91); BFsh = 0.63(0.27–0.84); SM = 0.86(0.68–0.94); ST = 0.73(0.44–0.88)].

Correlations between changes in passive and active shear modulus were not significant and ranged from weak to moderate (Fig. 1). Individual responses can be seen in Table 1.

4. Discussion

In this study, we compared the pre-post changes on hamstrings' passive (i.e., at rest) and active (i.e., at 20 % of MVIC) shear modulus after a warmup task. To the best of our knowledge, this is the first study to determine the correlation between passive and active shear modulus changes in all hamstring muscles. The main finding was that pre-post changes in hamstrings' passive and active shear modulus were not associated.

There were no significant associations between passive and active shear modulus for any of the hamstring muscles after the warmup protocol performed here. One possible explanation is the different sources of passive and active mechanical properties in skeletal muscles. Previous studies (Freundt and Linke, 2019; Herzog, 2018; Li et al., 2020) reported a lack of association between passive and active stiffness with a minute contribution of titin. The role of titin is evident when analyzing children with spastic cerebral palsy, since their passive muscle stiffness is typically greater than in typically developing children (Brandenburg et al., 2016). This increased stiffness is normally associated with a vast increase in the collagen matrix, and occurs despite an approximate 50 % decrease in titin content in muscles of children with spastic cerebral palsy compared to typically developed children (Leonard et al., 2019). Thus, single sarcomeres and isolated myofibrils, in which titin produces virtually all of the passive force, are about half as stiff in children with cerebral palsy compared to typically developing children (Leonard et al., 2019).

It should be noted that our results are in accordance with findings of previous studies of *ex vivo* animal (Prado et al., 2005) and *in vivo* human plantar flexor muscles after a seated calf raise (Hunter et al., 2001), but oppose the moderate relationship found between active and passive knee flexor stiffness in humans analyzed with knee extension stretching task for passive and knee flexion task for active measurements (Blackburn et al., 2004). However, Hunter et al. (2001) and Blackburn et al. (2004) used joint torque–angle measurements to determine muscle stiffness, while we measured the shear modulus (a localized mechanical tissue property) and the true relationship between longitudinal stiffness and shear modulus is more complicated than for an isotropic material which could be different between muscles and will depend crucially on muscle lengths and activation. Moreover, despite several studies reporting stiffness when using SWE methodology, it should be taken into consideration that stiffness is a property of a structure or component of a structure, which depends on various descriptive physical dimensions. On the other hand, the shear modulus is an intrinsic property of the constituent material and is not influenced by specimen geometry. Therefore, while two different objects composed of the same material will have the same shear modulus, they may display differing stiffness. While passive stiffness is mainly influenced by the extracellular matrix (Meyer and Lieber, 2018; Wheatley, 2020), active stiffness is attributed to the parallel attached cross-bridges (Kubo, 2014; Marshall et al., 2016; Morgan, 1977); other factors would have a small effect and could probably only be measured with a highly sensitive method. Nevertheless, the changes seen in the active shear modulus post-warm-up may also have been caused by changes in muscle recruitment pattern, as it has been reported to change after a warm-up (Babault et al., 2022). In any case, muscle activity has been highly related to shear modulus (Nordez and Hug, 2010). Considering that SWE measurements provide a proxy of a localized tissue mechanical property (Bercoff et al., 2004) rather than a global measure of joint stiffness, we consider that our findings support the notion that an association between the muscle passive and active mechanical properties is unlikely.

This study has some limitations. Firstly, the sample size was small, and we measured only one muscle group at one intensity. However, the present study was primarily designed as a pilot study which could inform future full-sized studies that encompass broader scope (multiple muscles, activation levels and different warmup protocols). Moreover, shear modulus measurements were performed only at a single site. The present findings are based on the assumption that the effects were not site-dependent. Furthermore, it is possible that the changes in active shear modulus were caused by a different activation strategy between synergists rather than by the mechanical effects of the warm-up on the muscle tissue. However, due to the effects of fatigue and the technical limitations of SWE, it would not be feasible to perform the measurements at a higher %MVIC. Finally, the reliability of shear modulus measurements was moderate to good, which is a sign of the limitations of using shear modulus as a muscle property assessment method.

5. Conclusion

The present study results indicated that the percentage of change in passive and active shear modulus are not associated after a warmup protocol. Therefore, future studies should be careful when assuming that either the passive or active measurements provide a full picture of the task effect on a given muscle when using SWE methodology.

CRediT authorship contribution statement

Ricardo Pimenta: Conceptualization, Data Curation, Writing-original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis and Methodology. **José P. Correia:** Writing – review & editing, Formal analysis, Methodology. **João R. Vaz:** Writing – review & editing, Formal analysis. **António P. Veloso:** Writting – review & editing, Formal analysis, Supervision. **Walter Herzog:** Writing – review & editing, Validation, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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