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Non-uniform distribution of passive muscle stiffness within hamstring

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Limited information is available on whether stiffness is different within and between the constituents of the hamstring, that is, the biceps femoris long head (BFlh), semitendinosus (ST), and semimembranosus (SM). Therefore, understanding of hamstring injuries and stretching effect on hamstring stiffness is difficult. The present study primarily aimed to identify whether passive muscle stiffness differs between the BFlh, ST, and SM and between the proximal, middle, and distal sites within each muscle. Secondly, the effect of stretching exercise on the heterogeneity in passive muscle stiffness was examined. In the lengthened hamstring positions by extending the knee joint or flexing the hip joint, passive muscle shear modulus (a measure of stiffness) at the proximal, middle, and distal sites of the BFlh, ST, and SM was measured by using ultrasound shear wave elastography. Furthermore, before and after five repetitions of 90-seconds static stretching for the hamstring, passive muscle shear modulus at the proximal and distal sites of the SM was measured. The shear modulus was significantly higher in the SM than in the BFlh and ST and higher at the distal site than the proximal site in all muscles. After the stretching, the higher shear modulus at the distal site of the SM compared to the proximal site was still observed (pre-stretching: +80%, post-stretching: +81%). These findings indicate that passive muscle stiffness varies within the hamstring regardless of performing stretching exercise and that passive muscle stiffness is not highest at the proximal site of the SM where a stretching-type hamstring strain typically occurs.

KEYWORDS
elastography, hamstring strain injury, semimembranosus, shear modulus, stretching, ultrasound

1 | INTRODUCTION

Mechanical properties of passive muscles are related to sport discipline,[1] athletic performance,[2] and muscle injury risk.[3,4] The hamstring is one of the primary muscle groups, being involved in many movements, and is commonly injured in sports.[5,6] For example, a stretching type of hamstring strain occurs during stretching movements performed to an extreme range of motion (ROM) as seen in gymnastics and dancing and most frequently occurs at the proximal site of the semimembranosus (SM).[6] Thus, evidence on mechanical properties distribution between and within the constituents of the hamstring, that is, the biceps femoris long head (BFlh), semitendinosus (ST), and SM, is important to advance our understanding of hamstring injuries (eg, why the stretching-type hamstring strain occurs at the specific site of the

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SM) and to allow the clinicians to perform optimal treatment. Nevertheless, limited information is available on the mechanical properties of the hamstring.

Ultrasound shear wave elastography (SWE) has been used to quantify localized shear modulus (a measure of stiffness, expressed in the unit of Pascal) of individual muscles,[1,7-12] based on the propagation speed of remotely induced shear waves.[13,14] Contradictory findings have been reported regarding the shear modulus of the hamstring in passively lengthened positions. Hirata et al[7] and Miyamoto et al[9] showed that, among the hamstring muscles, SM had the highest shear modulus, whereas Le Sant et al[8] reported that the BFlh had the highest shear modulus although the testing posture was similar (ie supine) in the Hirata et al's and Le Sant et al's studies. These contradictions may be due to different measurement sites for muscle shear modulus. Specifically, the muscle shear modulus was measured at 50% of thigh-length in the studies of Hirata et al[7] and Miyamoto et al,[9] whereas the measurement was performed over the muscle belly but not strictly controlled in Le Sant et al's study.[8] Heterogenous distribution of shear modulus within a muscle was previously reported for biarticular muscles (such as the medial and lateral gastrocnemii[15] and rectus femoris[16]) where muscle injuries including muscle strain more frequently occur compared to mono-articular muscles.[17] Based on these findings, it is likely that passive muscle stiffness varies not only between but also within the BFlh, ST, and SM. However, this has not been thoroughly examined.

Stretching exercise is widely and commonly performed in sports practice with the aim to reduce the risk of muscle injuries.[18] It is also widely believed that most hamstring strains occur due to the hamstring being stiff.[19,20] Although there is some evidence suggesting an effect of stretching exercise on muscle injuries (eg, McHugh & Cosgrave[21]), a scientific consensus has not yet been reached. Therefore, we need to pursue an understanding of how stretching exercise modifies the stiffness, with special emphasis on the focal stiffness distribution.

As a first step toward the above goal, the present study primarily aimed to identify whether passive muscle stiffness differs between the BFlh, ST, and SM and between the proximal, middle, and distal sites within each muscle. Based on the previous finding that the stretching-type hamstring strain most frequently occurs at the proximal site of the SM in the hamstring,[6] we hypothesized that passive muscle stiffness is highest thereat. Secondly, we aimed to examine the effect of stretching exercise on the within-muscle heterogeneity in passive muscle stiffness, if any. Based on the previous finding that, along the length within a muscle, a large stretching effect is observed at a relatively stiff region,[22] we hypothesized that the greatest stretching effect would be observed at the site which exhibits the highest stiffness, leading to diminished, or at least decreased, heterogeneity of stiffness distribution after stretching exercise.

2 | METHODS

Our study comprised two experiments. First, we investigated the inter- and intra-muscle variabilities of passive muscle stiffness within the hamstring (Experiment 1). Second, we examined the acute effect of static stretching on the within-muscle variability of passive muscle stiffness (Experiment 2). In both Experiment 1 and Experiment 2, all subjects were healthy young adults and had no apparent neurological, orthopedic, or neuromuscular problems. Subjects could participate in sports activities, but not in any strength or flexibility training at the time of the study. They were asked to refrain from strenuous exercise 24 hours before the experiments. None of them felt fatigue or pain in their lower legs on the experimental days. Before participating in the study, all subjects were fully informed of the purpose and experimental procedures and provided their written consent. This study was approved by the institutional ethics committee and performed in accordance with the Declaration of Helsinki.

2.1 | Experiment 1

2.1.1 | Subjects

With reference to our preliminary results (n = 5; proximal shear modulus = 28.4 ± 8.4 kPa, middle shear modulus = 45.6 ± 9.2 kPa, distal shear modulus = 58.4 ± 11.0 kPa), a priori power analysis with an assumed type 1 error of 0.05 and a statistical power of 0.80 was conducted to find a statistically significant difference in passive muscle shear modulus between the three sites (proximal, middle, distal). The minimum sample size was estimated to be 12. Thus, 13 men were recruited (171.3 ± 4.0 cm, 65.2 ± 3.5 kg, 22.5 ± 3.0 years; mean ± SD). Six subjects were involved in regular training and competitions (sepak takraw [2], endurance running [1], judo [1], kendo [1], dance [1]), four were recreationally active, and the others were sedentary.

2.1.2 | Design

The hamstring (ie the BFlh, ST, and SM) is a biarticular muscle group that crosses both the knee and hip joints. Thus, the muscles can be lengthened by either passive knee extension or hip flexion. To fulfill the aim of Experiment 1, two tasks were performed: knee extension and hip flexion tasks.

In the knee extension task, the passive lengthening of the hamstring was conducted by passive knee extension in the hip-flexed position, whereas in the hip flexion task, the hamstring lengthening was conducted by passive hip flexion in the knee-extended position. Except for the difference in knee and hip joint angles at which muscle shear modulus measurements were conducted, the protocols and variables for analyses were the same between the two tasks (see following sections). The two tasks were conducted in a randomized order on separate days across subjects.

2.1.3 | Experimental setup and procedure

In the knee extension task, subjects lay supine on an examining bed. The left hip was flexed at 90° from the lying position (0°) with the knee flexed at 90° (full extension = 0°) (Figure 1A). This posture was defined as the shortened position in the knee extension task. The rotation axis of the left knee joint was aligned with that of the dynamometer. The lever arm of the dynamometer was attached to the left lower leg. In accordance with a previous study,[9] a foam pad was placed in front of the thigh so that the hip joint angle could not change during passive lengthening. The pelvis was firmly secured to the bed with non-elastic straps to avoid posterior pelvic tilt. In the hip flexion task, subjects lay supine on a bed with the hip at 0° and the knee fully extended (Figure 1C). This posture was defined as the shortened position in the hip flexion task. The rotation axis of the left hip joint was aligned with that of the dynamometer. The lever arm of the dynamometer was attached to the left leg at both the anterior thigh and posterior lower leg so that the knee joint could not flex during passive lengthening. The ankle joint was fixed at approximately 10° of plantar flexion using footplate and non-elastic straps. The pelvis was firmly secured to the bed with non-elastic straps. Then, the knee and hip joints were passively rotated from the shortened position in the knee extension and hip flexion tasks, respectively, to the angle at which the subjects started experiencing pain, at an angular velocity of 2°/s. This angular velocity was used to avoid or minimize the stretch reflex. In each task, the difference in joint angle between the shortened position and the angle at which the subjects started experiencing pain was the maximal ROM. Also, in each task, the angle which corresponds to 80% of ROM from the shortened position was defined as the lengthened position (Figure 1B,D).

In order to determine the measurement sites for shear moduli and electromyographic (EMG) activity (see below) before data acquisition, the proximal and distal muscle-tendon junctions (ie the proximal and distal ends of the muscle belly) of each of the BFlh, ST, and SM were identified first in the shortened position and then in the lengthened position, using B-mode ultrasonography (Aixplorer Ver. 8, Supersonic Imagine) with a linear array probe (SL15-4, Supersonic Imagine). The measurement sites of the shear moduli and EMG activity of each muscle in the shortened and lengthened positions were the mediolateral centers at the levels of 25% (proximal), 50% (middle), and 75% (distal) of the muscle belly length in the corresponding positions (Figure 2). For the shear modulus measurement in both the shortened and lengthened positions, the probe position and

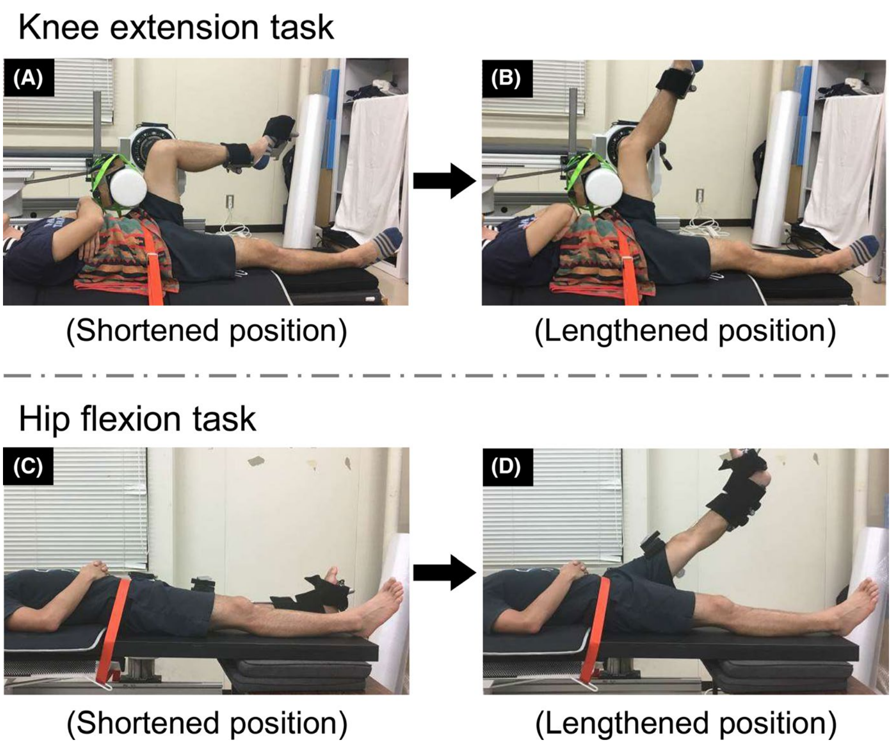

FIGURE 1 Experimental setup in the knee extension (A, B) and hip flexion (C, D) tasks. A and B show the shortened position. The lengthened (B, D) position was defined as the angle which corresponds to 80% of maximal range of motion in both tasks

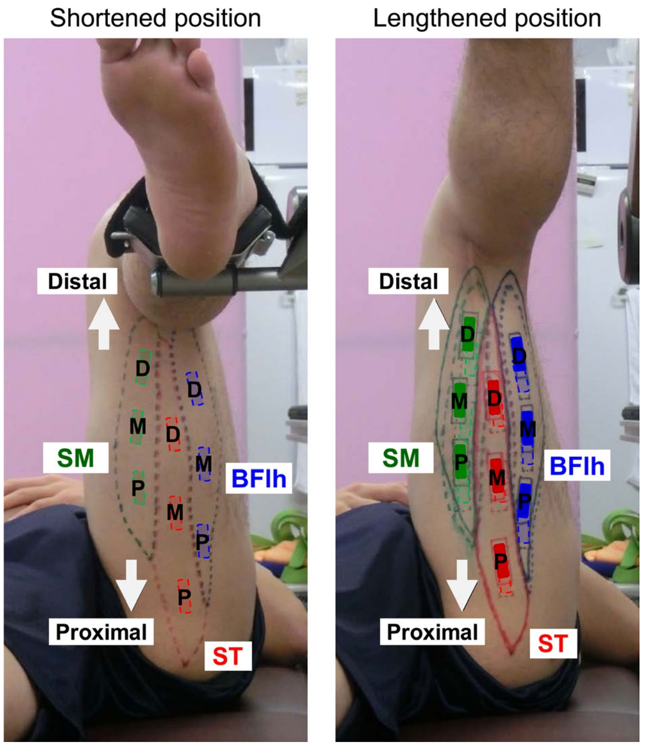

FIGURE 2 Schematic representing the muscle belly of the biceps femoris long head (BFlh: blue), semitendinosus (ST: red), and semimembranosus (SM: green) and the measurement locations (ie, positions of ultrasound probe and electromyographic electrode) at the proximal (P), middle (M), and distal (D) sites in the shortened position (dashed line) and lengthened position (solid line) of the knee extension task [Correction added on 22 July 2020, after online publication: Figure 2 has been corrected in this version]

orientation were determined before data acquisition so that fascicles could be identified within the B-mode image and that a stable color distribution could be acquired for a few seconds. These locations were marked on the skin with a waterproof marker. Also, skin preparation that included shaving, rubbing with sandpaper, and cleaning with alcohol for EMG measurements was performed.

After these preparation steps, the testing session was initiated. It is very difficult (virtually impossible) to simultaneously measure the muscle shear modulus and EMG signal at all sites of interest mentioned above, because of an insufficient area of the muscles on the skin surface, resulting in EMG cross talk from adjacent muscles. Therefore, the measurements of shear modulus and EMG were performed separately. In both tasks, first, the joint angles were set corresponding to the shortened position, and then, the shear moduli were measured (the required time: 3-4 minutes). The shear modulus was measured using the aforementioned ultrasound scanner (Aixplorer) and probe, in SWE mode (musculo-skeletal preset, persistence = med, smoothing = 5). The order in which the nine SWE measurement sites were tested in the shortened position was randomized across subjects. Throughout the scanning, care was taken not to press and deform the muscles. Next, the EMG activity in this position was measured (the required time including attachment of the electrodes: ~3 minutes). After active surface electrodes (DE-2.1, DELSYS) were placed at 9 sites of interest, EMG signals were recorded using two identical EMG systems (maximum number of inputs: 8 channels per system; Bagnoli-8 EMG System, DELSYS). Then, the joint angles were set corresponding to the lengthened position. Based on our preliminary and previous studies,[23] we considered that after 2 minutes at 80% of ROM, there is no longer any considerable change in stress relaxation. Thus, to account for the effect of stress relaxation, the position was held for 2 minutes before the measurements of the shear moduli were taken. The order in which the nine SWE measurement sites were tested in the lengthened position was randomized across subjects. Finally, the EMG activity in the lengthened position was measured after the second placement of the electrodes. During measurements of SWE and EMG, the subject stayed in the lengthened position for about 8-9 minutes continuously (ie wait for 2 minutes, SWE measurement for 3-4 minutes, and EMG measurement including electrodes placement for ~3 minutes). Throughout the testing session, the subjects were requested to relax completely. Additionally, to normalize the EMG data (see below), in both tasks, measurements of two knee flexion maximal voluntary isometric contractions (MVCs) and two hip extension MVCs were performed in each of the shortened and lengthened positions.

2.1.4 | Data analysis

For SWE data, processing was performed using the software of the ultrasound apparatus. A circular area as large as possible while excluding aponeurosis and subcutaneous adipose tissue was selected as the region of interest for the shear modulus calculation (≥13 mm diameter). The values of two measurements at each site in each position were averaged, and the average was used for further analyses. The coefficients of variation of two measurements were <2.0%, with intra-class correlation coefficients of >.988. For EMG data, the root-mean-square values of EMG signals (EMG-RMS) over 1 second period were calculated for all sites in each position in each task. Then, the EMG-RMS value was normalized to that obtained during MVC. The EMG-RMS under passive conditions in the shortened and lengthened positions was normalized to that during MVC in the corresponding positions, respectively. In each position, the highest value among two knee flexion MVCs and two hip extension MVCs was used to normalize the EMG data.

2.1.5 | Statistics

For shear modulus and EMG-RMS data in each task, three-way analyses of variance (ANOVAs) [position (shortened, lengthened) × muscle (BFlh, ST, SM) × site (proximal, middle, distal)] with repeated measures were performed. To analyze the change in shear modulus between shortened and lengthened positions, a two-way ANOVA (muscle × site) with repeated measures was performed. When appropriate, post-hoc Bonferroni analyses were performed. The significance level for all comparisons was set at $\alpha = .05$. Data are presented as means ± SDs. The statistical analyses were performed using statistical software (SPSS Statistics Ver. 26, IBM).

2.2 | Experiment 2

2.2.1 | Subjects

With reference to our preliminary results (n = 5; proximal shear modulus before stretching = 67.4 ± 14.0 kPa, distal shear modulus before stretching = 126.0 ± 32.4 kPa, proximal shear modulus after stretching = 51.2 ± 16.0 kPa, distal shear modulus after stretching = 102.7 ± 39.7 kPa), a priori power analysis with an assumed type 1 error of 0.05 and a statistical power of 0.80 was conducted to find a statistically significant interaction. The minimum sample size was estimated to be 14. Eighteen men (172.2 ± 4.0 cm, 65.2 ± 3.5 kg, 22.5 ± 3.0 years; mean ± SD) participated in Experiment 2. Eleven subjects were involved in regular training and competitions (sepak takraw [3], dance [2], track and field [1], endurance running [1], baseball [1], soccer [1], rugby [1], kendo [1]), five were recreationally active, and the others were sedentary.

2.2.2 | Experimental setup and procedure

The experimental setup and procedure of measuring muscle shear modulus were almost the same as those used in Experiment 1. Because the effect of stretching gradually disappeared within minutes after the stretching,[24] only the proximal and distal sites of the SM were studied. Additionally, the effect of acute static stretching on the SM shear modulus is reported to be similar between knee extension and hip flexion stretching exercises.[9] Thus, the knee extension task (not hip flexion task) and hamstring stretching exercise by passive knee extension were adopted.

First, in order to determine the lengthened position, the knee joint was passively rotated to the angle at which the subjects started experiencing pain, at an angular velocity of 2°/s. Next, the SM shear moduli and EMG activity at the proximal and distal sites were measured in the lengthened position (PRE). Then, using the same dynamometer, five repetitions of 90-seconds static stretching (inter-repetition rest period = 30 seconds) were administered by passively extending the knee joint to the angle at which each subject started experiencing pain and holding it for 90 seconds. The stretching knee joint angle was re-determined for each repetition. Throughout the stretching exercise, the subjects were instructed to relax completely. Immediately after the completion of the 5 repetitions of stretching, the knee joint angle was set at the same as that of the lengthened position in PRE measurement. This position was held for 2 minutes before the measurements of the shear moduli were taken (POST). The order in which the two SWE measurement sites were tested in both the shortened and lengthened positions was randomized across subjects. Then, the EMG activity in this lengthened position was measured. Finally, the knee joint was again passively rotated to the angle at which the subjects started experiencing pain in order to examine the effectiveness of stretching exercise (ie the difference in maximal knee joint angle between PRE and POST). Throughout the measurements and stretching exercise, the subjects were requested to relax completely. Additionally, to normalize the EMG data, two knee flexion and two hip flexion MVCs were measured in the lengthened position.

2.2.3 | Statistics

For shear modulus and EMG-RMS data, two-way ANOVAs [time (PRE, POST) × site (proximal, distal)] with repeated measures were performed. When appropriate, post-hoc paired t tests were performed. Further, regarding the change in shear modulus between PRE and POST measurements, a paired t test was performed. The significance level for all comparisons was set at $\alpha = .05$.

3 | RESULTS

3.1 | Experiment 1

The maximal ROM from the shortened position was 59.9 ± 13.6° in the hip flexion task and 71.1 ± 12.8° in the knee extension task. Accordingly, the lengthened position (ie 80% of ROM) was 47.9 ± 10.9° in the hip flexion task and 56.9 ± 10.2° in the knee extension task.

In the hip flexion task, for shear modulus, a three-way ANOVA showed a significant interaction of position × muscle ×site ($P < .05$). Follow-up post-hoc tests revealed that, in the shortened position, the ST shear modulus was significantly greater in the following order: distal > middle>proximal ($P < .05$) (Figure 3A). In the lengthened position, there were significant main effects of both muscle ($P < .01$) and site ($P < .001$) factors, with no significant interaction of muscle × site. Post-hoc tests revealed that shear modulus was significantly greater in the SM than in the BFlh ($P < .001$)

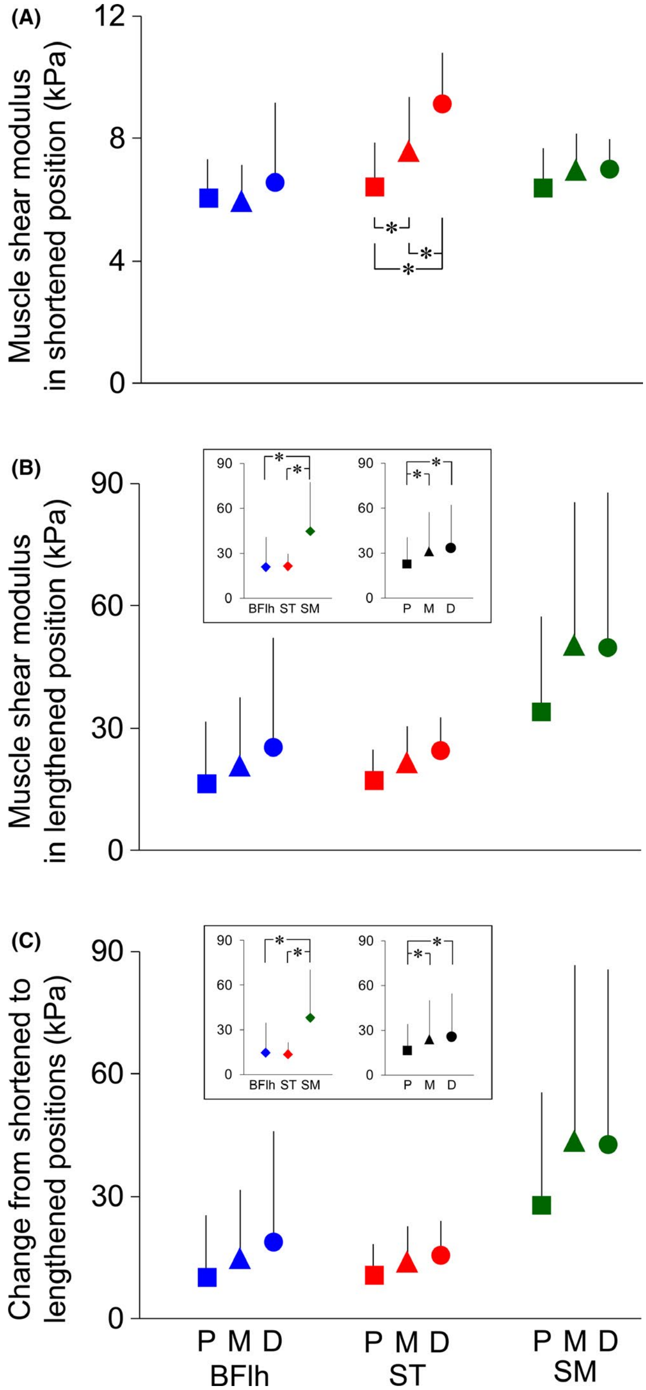

FIGURE 3 Shear modulus of the biceps femoris long head (BFlh), semitendinosus (ST), and semimembranosus (SM) at the proximal (P), middle (M), and distal (D) sites in the hip flexion task of Experiment 1. A, muscle shear modulus in the shortened position, B, muscle shear modulus in the lengthened position, C, the change in shear modulus between shortened and lengthened positions. *Significant difference at $P < .05$

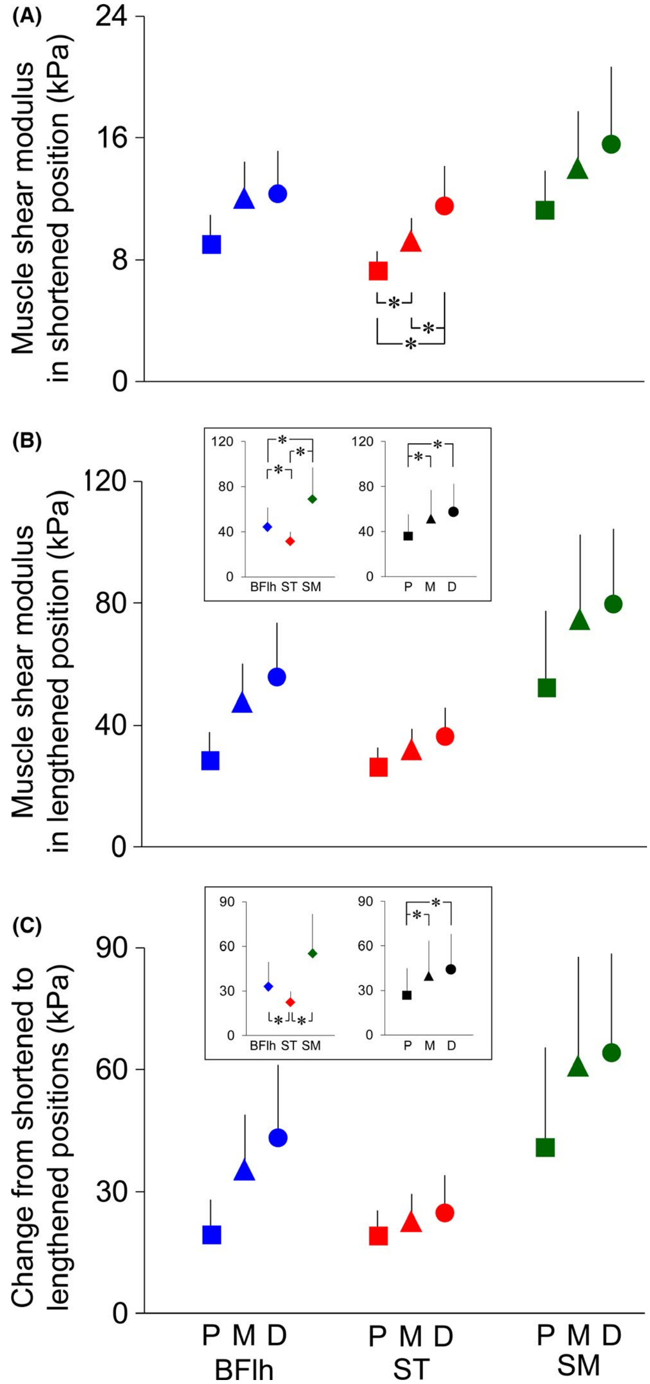

FIGURE 4 Shear modulus of the biceps femoris long head (BFlh), semitendinosus (ST), and semimembranosus (SM) at the proximal (P), middle (M), and distal (D) sites in the knee extension task of Experiment 1. A, muscle shear modulus in the shortened position, B, muscle shear modulus in the lengthened position, C, the change in shear modulus between shortened and lengthened positions. *Significant difference at $P < .05$

and ST ($P < .05$) and that shear modulus was significantly smaller at the proximal site than at the middle ($P < .01$) and distal sites ($P < .01$) (Figure 3B). Regarding the change in shear modulus between shortened and lengthened positions, there were significant main effects of both muscle ($P < .01$) and site ($P < .01$) factors, with no significant interaction of muscle × site. Post-hoc tests revealed that the change in shear modulus was significantly greater in the SM than in the BFlh ($P < .001$) and ST ($P < .01$) and that the change was significantly smaller at the proximal site than at the middle

($P < .01$) and distal sites ($P < .05$) (Figure 3C). For EMG-RMS, there was a significant interaction of position × muscle × site ($P < .05$). Follow-up post-hoc tests revealed that in the shortened position EMG-RMS was significantly smaller at the distal site (0.7 ± 0.5% MVC) than at the proximal (1.1 ± 0.7% MVC, $P < .05$) and middle sites (1.3 ± 0.6% MVC, $P < .001$) whereas in the lengthened position there were no significant main effects or interaction of muscle and site factors.

In the knee extension task, three-way ANOVA showed a significant interaction of position × muscle × site ($P < .05$). Follow-up post-hoc tests revealed that, in the shortened position, the ST shear modulus was significantly greater in the following order: distal > middle > proximal ($P < .05$) (Figure 4A). In the lengthened position, there were significant main effects of both muscle ($P < .001$) and site ($P < .001$) factors, with no significant interaction of muscle × site. Post-hoc tests revealed that shear modulus was significantly smaller at the proximal site than at the middle and distal sites ($P < .001$) and that shear modulus was significantly greater in the following order: SM > ST > BFlh ($P < .001$) (Figure 4B). Regarding the change in shear modulus between shortened and lengthened positions, there were significant main effects of both muscle ($P < .01$) and site ($P < .001$) factors, with no significant interaction of muscle × site. Post-hoc tests revealed that the change in shear modulus was significantly smaller in the ST than in the BFlh ($P < .01$) and SM ($P < .01$) and that the change was significantly greater in the following order: distal > middle > proximal ($P < .001$) (Figure 4C). For EMG-RMS, three-way ANOVA revealed that there was a significant muscle × site interaction ($P < .01$), with no significant interaction of position × muscle × site. Follow-up post-hoc tests revealed that EMG-RMS of the ST (2.1 ± 1.1% MVC) was significantly greater than that of the SM (1.5 ± 0.8% MVC, $P < .05$) and that EMG-RMS at the proximal site (2.5 ± 1.1% MVC) was significantly greater than that at the middle (1.6 ± 1.2% MVC, $P < .01$) and distal sites (1.4 ± 1.0% MVC, $P < .01$).

3.2 | Experiment 2

After five repetitions of a 90-s static stretching exercise, maximal knee joint angle was significantly increased (PRE: 68.7 ± 13.2°, POST: 75.8 ± 17.3°; $P < .05$). For shear modulus, two-way ANOVA showed a significant interaction of time × site ($P < .05$). Post-hoc tests revealed that at both PRE and POST measurements shear modulus was significantly greater at the distal site than at the proximal site ($P < .001$) and that at both sites shear modulus was significantly reduced at POST compared to PRE ($P < .001$) (Figure 5B). The absolute change in shear modulus between PRE and POST was significantly greater at the distal site (−21.8 ± 13.5 kPa) than at the proximal site (−13.8 ± 10.7 kPa, $P < .001$). In contrast, when the shear modulus change was expressed as the percent change from the PRE measurement, no significant difference was found between the proximal and distal sites (−20.3 ± 14.9% and −19.4 ± 16.4%, respectively) (Figure 5B). For EMG-RMS, there was a significant interaction of time × site ($P < .05$). Post-hoc t tests revealed that at both PRE and POST measurements, EMG-RMS was significantly smaller at the distal site (0.8 ± 0.4% MVC at PRE and 0.7 ± 0.4% MVC at POST) than at the proximal (1.6 ± 1.2% MVC at PRE and 1.4 ± 0.9% MVC at POST). Further, no significant difference between PRE and POST measurements was found at the proximal or distal site.

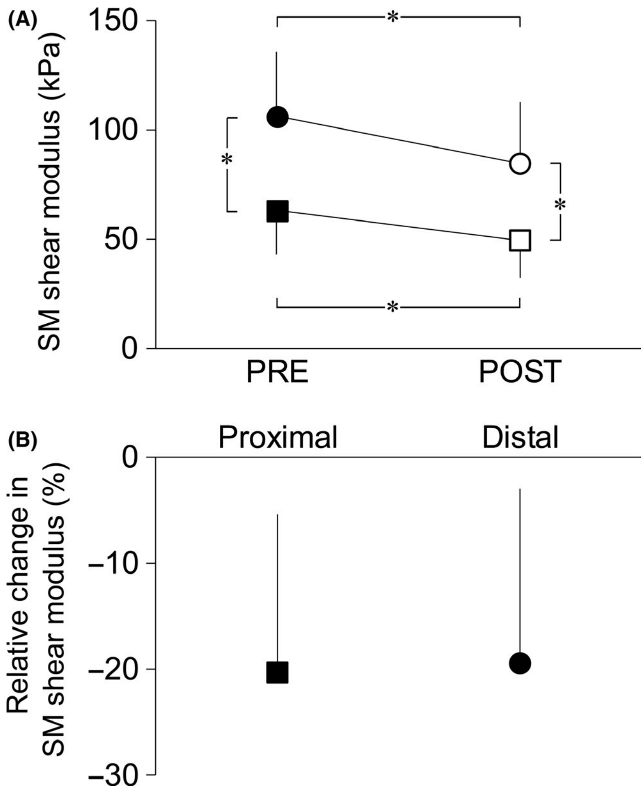

FIGURE 5 Shear modulus of the semimembranosus (SM) at the proximal and distal sites in Experiment 2. A, muscle shear modulus before (PRE) and after (POST) stretching, B, relative change in muscle shear modulus between PRE and POST measurements. *Significant difference at $P < .05$

4 | DISCUSSION

The present study focused on the inter- and intra-muscular distribution of passive muscle stiffness, with multiple proximo-distal shear modulus measurements using ultrasound SWE for the hamstring. Our hypotheses were as follows: (a) passive muscle stiffness would be highest at the proximal site of the SM in the hamstring and (b) the greatest stretching effect would be observed at the site which exhibits the highest stiffness, leading to diminished, or at least decreased, heterogeneity of stiffness distribution after stretching exercise. The present finding that the shear modulus was highest in the SM among the hamstring and lower at the proximal site than the middle and distal sites in the lengthened position is not in line with our first hypothesis. We also found that, although the absolute change in shear modulus by stretching exercise was greater at the distal than at the proximal site, the relative change was similar between the proximal and distal sites. This did not completely support our second hypothesis. Although all subjects in the present study have not suffered from hamstring strains, the present findings allow us to speculate that passive muscle stiffness is not highest at the site where hamstring strains typically occur, and that stretching exercise does not necessarily reduce such heterogenous distribution of passive muscle stiffness.

Before stretching, significant differences in muscle shear modulus were observed among the three muscles and among the three intra-muscular sites. Specifically, the SM displayed higher passive muscle stiffness than the BFlh and ST. This finding seems to be linked with the observation that the stretching-type hamstring strain frequently occurs in the SM.[6] However, the stretching-type hamstring strain most frequently occurs at the proximal site of the SM,[6] whereas in the present study, the highest shear modulus was observed at the distal site of the SM. The present study shows for the first time in vivo that different sites within the hamstring can behave in mechanically different ways, but that the stiffest site within the hamstring does not correspond to the site where hamstring strains frequently occur. On the other hand, it is likely that high stiffness in one area of a muscle forces other areas of the muscle to produce most of the strain during lengthening, hence the latter area being more prone to injury. This is speculative but may be relevant for future studies.

In both Experiment 1 and Experiment 2, there was an intra-muscular variability of EMG activity although the subjects were requested to completely relax throughout the measurements. The present study showed that the EMG activity at the distal site was comparable to or smaller than that at proximal and/or middle sites, suggesting that the spatial variabilities in EMG activity cannot explain the heterogenous stiffness distribution within a muscle, that is, highest stiffness at the distal site. A plausible explanation for the intra-muscular variability of stiffness is that the hamstring muscle cross-sectional area (CSA) is smaller at the proximal and distal sites than at the middle site. Such differences in the muscle CSA among the proximal, middle, and distal sites of the hamstring were confirmed in previous studies using MRI[25] as well as in the present study using transverse extended field-of-view ultrasonography (data not shown). Theoretically, the smaller CSA at the proximal and distal sites should result in higher stress compared to the middle (larger CSA) site. In the present study, however, the muscle shear modulus at the distal site was similar to that at the middle site but substantially different from that at the proximal site. Therefore, factors other than muscle CSA and EMG activity would be responsible for the intra-muscular variability in muscle shear modulus.

As an alternative possible explanation for the heterogenous distribution of passive muscle stiffness, we propose the distribution of intra-muscular connective tissue. There is a growing body of evidence indicating that intra-muscular connective tissues (consisting mainly of collagen) such as perimysium and epimysium strongly affect passive muscle stiffness.[26-28] Additionally, sarcomere length varies between proximal and distal sites within an in vivo human muscle.[29] Besides, ex vivo[30] and in vivo[31] studies reported that passive tension generated at the site close to the extremity being lengthened is higher than the opposite site. This consideration strengthens the hypothesis of higher stress applied to the distal site of the hamstring during passive knee extension (eg, knee extension task in the present study), but does not hold for our finding observed in the hip flexion task. Further studies are warranted to examine the precise mechanisms underlying the spatial variations in muscle shear modulus within the hamstring.

In Experiment 2, the maximal ROM and the SM shear modulus (measured in the same lengthened position) were significantly increased and decreased, respectively, by stretching exercise. This indicates the substantial effectiveness of static stretching exercise. With regard to SM shear modulus, the large reduction in absolute shear modulus (in kPa) was observed at the distal site compared to the proximal site whereas the relative reduction was similar between the proximal and distal sites. This does not support our second hypothesis and indicates that stretching exercise cannot substantially reduce the heterogenous distribution of passive muscle stiffness. Although the reasons for the discrepancy between the hypothesis and the result are unknown, the aforementioned mechanism responsible for the spatial variations in muscle shear modulus, which are unaffected by an acute bout of static stretching (eg, intra-muscular connective tissue content) might be involved. Future works should explore this possibility and gain insight into the clinical relevance of spatial variations in passive muscle stiffness.

The present study has some limitations. First, in order to avoid the potential effect of thixotropic property of a muscle on passive muscle stiffness, passive loading/unloading cycles were performed before the measurement in previous studies[1,8,32] but not in the present study. It is well known that the effect of thixotropic property of a muscle on passive muscle stiffness can be largely reduced by relatively small amounts of movement.[33,34] In the present study, before the measurement, passive stretching maneuvers were performed several times to determine the maximal angle and the measurement sites of shear modulus and EMG activity. Thus, the effect of the absence of cyclic conditioning on the present results should be minor. Second, although the lengthened position was defined as the angle which corresponds to 80% of ROM, this is not an extremely stretched position where the stretching-type hamstring strain most often occurs.[6] Thus, it remains unknown from the present study whether the distribution of passive muscle stiffness in an extremely stretched position is similar to that observed in the present study. On the other hand, the change in shear modulus between shortened and lengthened positions was significantly greater in the SM and at the middle and distal sites. Therefore, it is reasonable to assume that, in an extremely stretched position, the SM shear modulus is highest among the hamstring and higher at the distal site than proximal site, as observed in the present study. Third, the SWE system used in the present study does not directly quantify the tissues shear modulus but quantifies the shear wave speed. Although some assumptions are needed for obtaining the tissue shear modulus from the shear wave speed, the tissue density is a matter of concern in the case of the present study, as intra-muscular composition such as fat and connective tissue could differ between the proximo-distal sites. Thus, we performed additional analyses to test the robustness of our claims by re-analyzing the SWE data in terms of shear wave speed instead of shear modulus. The additional analyses demonstrated the same tendency for inter- and intra-muscular variations and for the stretching effect, confirming our claims.

In conclusion, we provided evidence that passive muscle stiffness varies not only between the hamstring constituents (ie, BFlh, ST, and SM) but also between sites within the muscles. Specifically, passive muscle stiffness is not highest at the site where hamstring strains typically occur. We also found that the intra-muscular heterogeneity of passive muscle stiffness remained after an acute bout of static stretching, suggesting that stretching exercise does not necessarily reduce such heterogenous distribution of passive muscle stiffness.

5 | PERSPECTIVE

Several MRI studies have indicated that hamstring strain can be of two types[5,6]: a sprinting type most frequently occurring in BFlh[5,35,36] and a stretching type most frequently occurring at the proximal site of the SM.[6] Thus, the present finding that passive muscle stiffness is not highest thereat is surprising. On the other hand, although the precise risk factors and/or mechanisms for hamstring strain are unclear, the present study focused only on the passive muscle stiffness. Thus, it would be worth focusing on heterogeneity of stress and/or strain (ie, elongation) within the hamstring. Such findings may give a hint for the high rate of muscle- and site-specific occurrence of hamstring strain and help clinicians perform optimal treatment for injury prevention and rehabilitation.

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CONFLICT OF INTEREST

There is no conflict of interest, financial or otherwise, to declare.

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