

Research article

## Acute and Prolonged Effects of Stretching on Shear Modulus of the Pectoralis Minor Muscle

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### Abstract

Increased muscle stiffness of the pectoralis minor (PMi) could deteriorate shoulder function. Stretching is useful for maintaining and improving muscle stiffness in rehabilitation and sport practice. However, the acute and prolonged effect of stretching on the PMi muscle stiffness is unclear due to limited methodology for assessing individual muscle stiffness. Using shear wave elastography, we explored the responses of shear modulus to stretching in the PMi over time. The first experiment ( $n = 20$ ) aimed to clarify the acute change in the shear modulus during stretching. The shear modulus was measured at intervals of  $30 \text{ s} \times 10$  sets. The second experiment ( $n = 16$ ) aimed to observe and compare the prolonged effect of different durations of stretching on the shear modulus. Short and long stretching duration groups underwent  $30 \text{ s} \times 1$  set and  $30 \text{ s} \times 10$  sets, respectively. The assessments of shear modulus were conducted before, immediately after, and at 5, 10, and 15 min post-stretching. In experiment I, the shear modulus decreased immediately after a bout (30 s) of stretching ( $p < 0.001$ , change:  $-2.3 \text{ kPa}$ , effect size:  $r = 0.72$ ) and further decreased after 3 repetitions (i.e., 90 s) of stretching ( $p = 0.03$ , change:  $-1.0 \text{ kPa}$ , effect size:  $r = 0.53$ ). In experiment II, the change in the shear modulus after stretching was greater in the long duration group than in the short duration group ( $p = 0.013$ , group mean difference:  $-2.5 \text{ kPa}$ , partial  $\eta^2 = 0.36$ ). The shear modulus of PMi decreased immediately after stretching, and stretching for a long duration was promising to maintain the decreased shear modulus. The acute and prolonged effects on the PMi shear modulus provide information relevant to minimum and persistent stretching time in rehabilitation and sport practice.

**Key words:** Elastography; time-course; shoulder; stiffness; ultrasonography.

### Introduction

The pectoralis minor (PMi) is a triangular muscle in the upper part of the chest and beneath the pectoralis major muscle. The PMi generally originates from the third, fourth, and fifth ribs and inserts into the medial and superior margins of the anterior portion of the scapula coracoid process (Lee et al., 2018). The PMi function affects scapular movement (Borstad, 2006; Borstad and Ludewig, 2005) and the increase in PMi stiffness might cause abnormal

movement and position of the scapula (i.e., scapular dyskinesis) (Kibler et al., 2013). Many previous studies have examined the effect of interventions lengthening the PMi on the rounded shoulder posture, scapular movement, and patient self-reported outcome (Gutierrez-Espinoza et al., 2019; Lee et al., 2015; Rosa et al., 2017; Wang et al., 1999), indicating that the stretching of PMi is significant in rehabilitation and sports settings. However, few studies have directly determined the muscle mechanical response of the PMi in resistance to stretching interventions.

One muscle mechanical response to stretching is an acute change in the muscle mechanical properties during lengthening, which is an attractive topic in muscle biomechanics research and rehabilitation settings. The acute effect of stretching on the ankle passive torque and displacement of the muscle-tendon unit of the triceps surae (Hirata et al., 2016; Ikeda et al., 2020; Kay et al., 2015; Konrad and Tilp, 2020), and the passive torque and stiffness of the hamstring muscles (Herda et al., 2011; Nakao et al., 2019; Nordez et al., 2006) have shown in the literature. Moreover, some studies have provided information on the minimum time necessary for the stretching of the triceps surae (Nakamura et al., 2013; Toft et al., 1989). Another muscle mechanical response is muscle stiffness after stretching, which is referred to as the prolonged effect of stretching. Previous studies that examined the prolonged effect of stretching on the muscle-tendon unit reported a change in the ankle passive torque and muscle-tendon unit over time after stretching (Konrad et al., 2019; Konrad and Tilp, 2020; Mizuno et al., 2013) and compared the stiffness calculated from the ankle passive torque between the different stretching durations (Ryan et al., 2008). These studies advocated the appropriate timing for performing stretching and for when therapists and coaches should apply stretching before sports practice and motor control exercise. However, the most of previous studies considering the acute and prolonged effects of stretching on the muscle mechanical response are limited to lower limb muscles and not extended to the PMi muscle. Because the range of motion and passive torque are not available to assess the mechanical behavior of an individual muscle due to a large number of muscles around the shoulder joint.

Ultrasound shear wave elastography (SWE) is used as a reliable and valid method to assess the shear modulus of an individual muscle (i.e., the surrogate of muscle stiffness, the higher the shear modulus, the stiffer the muscle) (Hug et al., 2015; Koo et al., 2013; Lacourpaille et al., 2012). Previous studies found a decrease in the shear modulus of the medial gastrocnemius muscle immediately after stretching (Nakamura et al., 2014) and the change in each medial and lateral head of the gastrocnemius muscle along with time after stretching (Taniguchi et al., 2015). SWE is a promising technology for assessing the acute and prolonged effects of stretching on an individual muscle. However, no studies have determined the mechanical response of the PMi muscle to stretching.

Taking advantage of SWE, we explored the responses of shear modulus to stretching over time in PMi, which is considered in shoulder rehabilitation and sport practice. The first experiment aimed to clarify the acute change in shear modulus during stretching. The second experiment aimed to observe the prolonged effect of stretching on the shear modulus and to compare the effects of different stretching durations. We hypothesized that the shear modulus declines steeply after several tens of seconds of stretching and then gradually in experiment I. The prolonged effect on the shear modulus would be greater in long-duration stretching than in short-duration stretching in experiment II.

## Methods

### Participants

Twenty healthy men (age:  $25.0 \pm 3.2$ yr; weight:  $65.4 \pm 3.1$  kg; height:  $1.7 \pm 0.05$  m) were enrolled in experiment I. Of these, 16 healthy men (age:  $25.3 \pm 3.2$ yr; weight:  $65.2 \pm 7.7$  kg; height:  $1.71 \pm 0.05$  m) participated in experiment II and were randomly assigned to a short duration group ( $n = 8$ ) and a long duration group ( $n = 8$ ) using a computerized random number function in Microsoft Excel (Microsoft Corp., Redmond, Washington). The exclusion criteria were: (i) recent ( $< 3$  years) orthopedic injury/disability in upper limbs or spine, (ii) severe cardiovascular or neurological disease, (iii) engagement in daily flexibility training ( $> 1$  hour·day<sup>-1</sup>) and sports/activities where the use of an arm is predominant. All the participants were sedentary or university students who recreationally performed activities. The experimental procedures were approved by the ethics committee of our institute (R0233) and the subjects were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed and consent document to participate in this study. All subjects provided informed consent.

### Experimental set-up

Subjects sat on a stool with 90° shoulder abduction and 90° elbow flexion. The interventional upper limb was placed on a platform and then relaxed as much as possible. The joint angles for this posture were measured using a goniometer. The posture was defined as the initial posture in experiments I and II (Figure 1a).

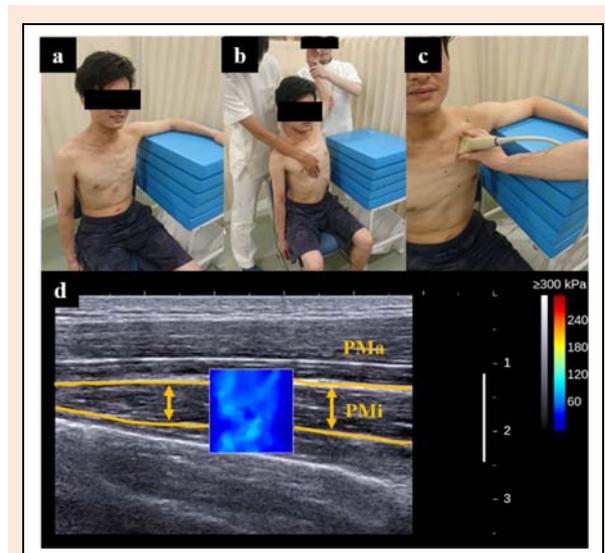
### Protocol

The study design was a laboratory-based cross-sectional

study. The subjects refrained from excessive training 24 hours before the experiments. We asked the subjects to be in the initial posture 15 min prior to the experiments. The dominant and non-dominant upper limbs were defined as the control and interventional limbs, respectively.

In experiment I, the shear modulus of the PMi was acquired after each stretching. The shear modulus at first (Baseline) was measured before stretching for the interventional limb. Shear moduli were measured during the 1 min intervals of 30 s  $\times$  10 sets (= 5 min) stretching. The data were acquired at 11 timepoints in the interventional limb. For the control limb, shear moduli were measured before and after the rest period, which had the same duration as the interventional limb (Figure 2a).

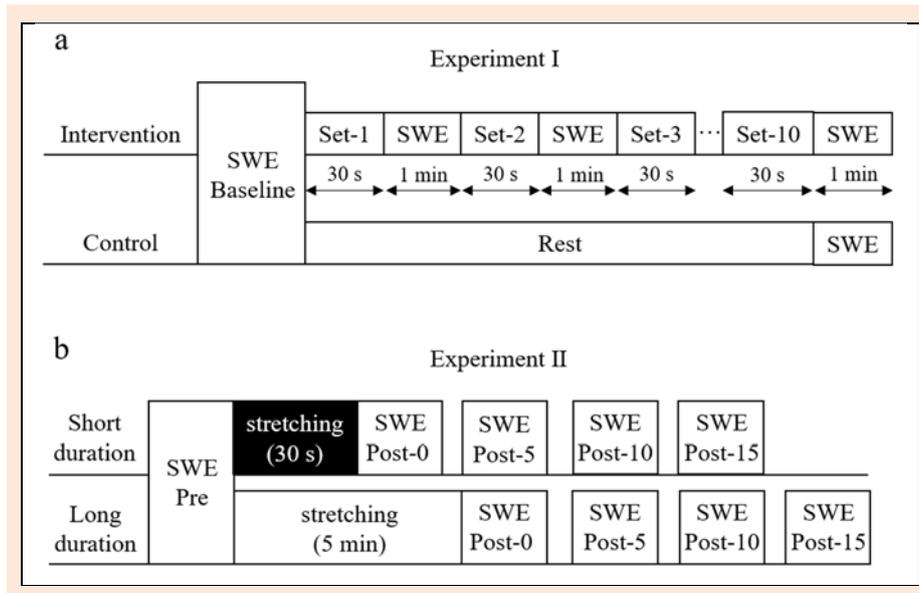
In experiment II, the prolonged effect of different durations of stretching on the shear modulus of the PMi was observed and compared. The short duration group performed 30 s  $\times$  1 set (= 30 s) stretching, whereas the long duration group performed 30 s  $\times$  10 sets (= 5 min) stretching at 1 min intervals. Assessments of shear modulus were performed before, immediately after, and at 5, 10, and 15 min post-stretching (Pre, Post-0, Post-5, Post-10, and Post-15, respectively) in both groups (Figure 2b). The stretching durations used in experiments I and II were set based on a systematic review and meta-analysis investigating the stretching effect on muscle functions (Behm et al., 2016; Simic et al., 2013).



**Figure 1.** (a) Initial posture for experimental set-up. This posture is constant in both experimental I and II; (b) Stretching procedure for PMi; (c) Measurement of PMi shear modulus; (d) B-mode (left) and shear wave elastography (right) images of PMi. PMA, pectoralis major muscle; PMi, pectoralis minor muscle.

### Stretching maneuver

The stretching maneuver is shown in Figure 1b. PMi stretching was performed as partner stretching by two licensed physiotherapists. In the initial posture, one examiner passively abducted the subjects' shoulder at 135°, and moved the shoulder to horizontal abduction and then externally rotated it within the range that the subjects could tolerate without pain. Another examiner held the subjects' trunk by hand to avoid trunk rotation. After each bout of



**Figure 2.** Experimental protocols I (a) and II (b). SWE, shear wave elastography.

stretching, the examiner placed the interventional limb back to the initial posture. The subjects were instructed to relax as much as they could and to respire as usual during the stretching maneuver. Note that this stretching maneuver is certain of lengthening the PMi effectively, based on a previous study (Umehara et al., 2017). The stretching maneuver was common in experiments I and II.

### Shear wave elastography

An ultrasound scanner (Aixplorer v6.4, Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer (4-15MHz, SuperLinear 15-4, Vermon, Tours, France) was used in SWE mode (musculoskeletal preset, penetration mode, smoothing level 5, persistence medium). The SWE technology generates shear waves into the individual tissue using the acoustic radiation force resulting from the push pulse. Then, it acquires the propagation velocity of the shear wave by the ultrafast-imaging technique. SWE provides a 2-dimensional map of the shear modulus on the B-mode image of the target tissue at 1 Hz with a spatial resolution of  $1 \times 1$  mm (Lacourpaille et al., 2012).

Data acquisition for the shear modulus was conducted with the probe handheld by the examiner. The measurement site was defined as the midpoint between the coracoid process and the fourth rib-sternum junction (Figure 1c). The PMi was identified on the B-mode image and the probe was precisely orientated along the muscle fascicle line (Figure 1d). To measure the shear modulus, we instructed the subjects to hold their breath to prevent muscle elongation due to rib cage movement. The shear modulus was measured three times in each acquisition session. The measurement reproducibility in this experimental set-up was obtained from our previous study (e.g., the intraclass correlation coefficient (1,3) and its 95% confidence interval were 0.99 and 0.97-0.99) (Umehara et al., 2018).

### Image analysis

Data were processed using Aixplorer scanner software.

The mean values of the shear modulus in each image were obtained over the largest possible circular region of interest. Particular care was taken not to include artefacts and aponeurosis in the region of interest. Actually, the Aixplorer scanner calculated the Young's modulus. Since Young's modulus calculation requires the assumption of an isotropic material, this was not applicable for the muscle (Royer et al., 2011). Therefore, Young's modulus was divided by 3 to obtain the shear modulus of muscles (i.e.  $E = 3G = 3\rho V_s^2$ , where  $E$  is the Young's modulus,  $G$  is the shear modulus,  $V_s$  is the propagation velocity of the shear wave, and  $\rho$  is the muscle density, assumed to be  $1,000 \text{ kg/m}^3$ ) (Nordez et al., 2008). The mean values of three measurements were used for further analysis. For experiment II, the amount of change in the shear modulus was also calculated by subtracting the shear modulus before stretching (Pre) from each shear modulus after stretching (i.e., Post-0, Post-5, Post-10, Post-15).

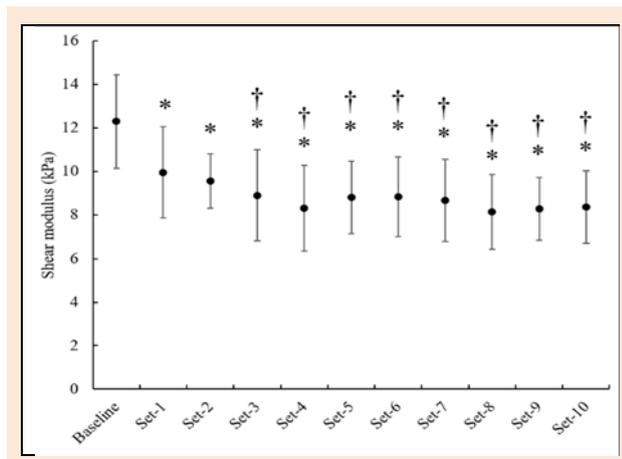
### Statistical analyses

All statistical analyses were performed using IBM SPSS statistics software (version 22; IBM, Armonk, NY, USA). The data were screened for normal distribution using the Shapiro-Wilk test.

For experiment I, the time course of the shear modulus of PMi during stretching was assessed in the interventional limb through a one-way analysis of variance (ANOVA) with repeated measures [acquisition sessions (Baseline, and Set-1 through Set-10)]. When the sphericity assumption in ANOVA with repeated measures as Mauchly's test was violated, a Geisser-Greenhouse correlation was used. When appropriate, multiple comparisons with Holm adjustment were conducted as a post-hoc test to compare the shear modulus of Baseline with that of each set. Furthermore, the shear moduli were compared between sets and a significant difference was found with Baseline at first, and afterwards, by multiple comparisons with Holm adjustment. For the control limb, the shear modulus

before and after the rest period was compared using a paired *t*-test. Moreover, the changes in the shear modulus between Baseline and Set-10 for the interventional limb and between before and after the rest period for the control limb were calculated. The changes were compared between both limbs using a two-sample *t*-test after Leven's test.

For experiment II, the prolonged effect of stretching on the shear modulus of PMi was assessed using a two-way ANOVA for a split-plot design [groups (short, long) × times (Pre, Post-0, Post-5, Post-10, and Post-15)]. When the interaction and main effects of the time factor were found, post hoc tests were performed using multiple comparisons with a Bonferroni correlation *t*-test to compare the shear modulus of Pre and Post-0 through Post-15 in each group. For the group comparison, the amount of change in the shear modulus calculated by subtracting the Pre-value was compared between the short and long duration groups through a two-way ANOVA for a split-plot design [groups (short, long) × times (Post-0, Post-5, Post-10, Post-15)]. Effect sizes (*r*) were calculated for all paired comparisons. The effect sizes *r* of 0.1, 0.3, and 0.5 were considered as small, medium, and large, respectively (Cohen, 1988). In addition, a post hoc power analysis was performed for the main results by using the G\*power software (Heinrich Heine University, Duesseldorf, Germany). An alpha level of 0.05 was used in all statistical tests.



**Figure 3.** Shear modulus of pectoralis minor muscle during repeated stretching. The black dots and error bars represent mean values and standard deviation respectively. The asterisk means that the shear

modulus is significantly lower than that of Baseline. The dagger indicates that the shear modulus is significantly lower than that of Set-1.

## Results

### Experiment I

The PMi shear modulus decreased steeply after the first bout of stretching and then gradually (Figure 3). ANOVA found a significant main effect of the acquisition session ( $p < 0.001$ ,  $F = 16.018$ , partial  $\eta^2 = 0.457$ ). Post-hoc tests showed that all shear moduli from Set-1 through Set-10 were significantly lower than those of Baseline (Table 1). Although there was no difference between Set-1 and Set-2, furthermore, the shear moduli from Set-3 to Set-10 were significantly lower than that of Set-1 (Table 1); Set-1 was the first time point with a significant difference compared to Baseline. However, no significant differences were found in the shear modulus of Set-3 compared to Set-4 to Set-10. For the control limb, there was no significant difference in the shear modulus before and after rest period ( $p = 0.391$ , before:  $10.2 \pm 2.4$  kPa, after:  $9.6 \pm 2.2$  kPa). For the limb comparison, Leven's test result showed the equal variance in the limbs ( $p = 0.89$ ,  $F = 0.019$ ), and the two-sample *t*-test result indicated that the change in the shear modulus of the intervention was significantly greater than that of the control limb ( $p < 0.001$ ,  $r = 0.67$ , intervention limb:  $-3.9 \pm 2.6$  kPa, control limb:  $0.5 \pm 2.3$  kPa).

A post hoc power analysis was conducted to confirm whether our design has enough power to detect the main effect of acquisition time, which is a factor that primarily influences the shear modulus. The post hoc power analysis indicated that the observed power was 1.00.

### Experiment II

The changes in the shear modulus over time are shown in Figure 4a. The two-way ANOVA revealed a significant group-time interaction ( $p = 0.029$ ,  $F = 2.926$ , partial  $\eta^2 = 0.173$ ) and the main effect of the time factor ( $p < 0.001$ ,  $F = 20.724$ , partial  $\eta^2 = 0.597$ ). In the short duration group, post-hoc tests showed that there were no significant differences in the shear modulus between Pre and Post5, Post10, and Post15. The shear modulus of Post-0 was significantly lower than that of Pre (Table 2). In the long duration group, the shear moduli of all times were significantly lower than those of Pre (Table 2). For between group comparison,

**Table 1.** Acute effect on shear modulus through Baseline to Set-10 in experiment I.

Variables	vs Baseline				vs Set-1		
	Shear modulus (kPa)	P value	95% confidence interval	ES (r)	P value	95% confidence interval	ES (r)
Baseline	12.2±2.1	-	-	-	-	-	-
Set-1	9.9±2.1	< 0.001*	1.2 – 3.4	0.72	-	-	-
Set-2	9.5±1.2	< 0.001*	1.7 – 3.7	0.78	0.25	-0.3 – 1.1	0.25
Set-3	8.8±2.1	< 0.001*	2.5 – 4.2	0.88	0.03†	0.2 – 1.8	0.53
Set-4	8.3±1.9	< 0.001*	2.8 – 5.1	0.86	< 0.001†	0.9 – 2.3	0.75
Set-5	8.7±1.6	< 0.001*	2.3 – 4.6	0.82	0.02†	0.3 – 1.9	0.56
Set-6	8.8±1.8	< 0.001*	2.4 – 4.5	0.84	0.04†	0.1 – 2.0	0.49
Set-7	8.6±1.8	< 0.001*	2.3 – 4.9	0.79	0.01†	0.4 – 2.0	0.61
Set-8	8.1±1.7	< 0.001*	3.0 – 5.2	0.86	< 0.001†	1.0 – 2.5	0.75
Set-9	8.2±1.4	< 0.001*	2.7 – 5.2	0.84	0.003†	0.8 – 2.5	0.69
Set-10	8.3±1.6	< 0.001*	2.6 – 6.4	0.82	0.007†	0.7 – 2.4	0.65

The asterisk means that the shear modulus is significantly lower than that of baseline. The dagger indicates that the shear modulus is significantly lower than that of Set-1. ES: effect size.

**Table 2. Prolonged effect on shear modulus from Pre to Post-15 in experiment II.**

Variables	Short duration				Long duration		
	Shear modulus (kPa)	P value	95% confidence interval	ES (r)	P value	95% confidence interval	ES (r)
Pre	12.5±1.2	-	-	-	13.2±1.9	-	-
Post-0	10.3±1.3	0.02*	0.8 – 3.4	0.83	8.1±1.5	0.001*	3.2 – 6.8
Post-5	10.2±1.1	0.06	0.5 – 3.9	0.77	8.7±1.5	< 0.001*	2.2 – 6.6
Post-10	9.9±1.9	0.07	0.6 – 4.5	0.75	8.6±1.0	< 0.001*	3.3 – 5.8
Post-15	10.8±1.8	0.06	0.4 – 2.9	0.76	8.7±1.8	0.004*	2.4 – 6.4

The asterisk means that the shear modulus is significantly lower than that of Pre. ES: effect size.

neither significant interaction ( $p = 0.724$ ,  $F = 0.443$ , partial  $\eta^2 = 0.031$ ) nor the main effect of time ( $p = 0.590$ ,  $F = 0.645$ , partial  $\eta^2 = 0.044$ ) was found. However, there was a significant main effect of group ( $p = 0.013$ ,  $F = 8.053$ , partial  $\eta^2 = 0.365$ ) and the amount of change in the shear modulus in the long duration group was significantly greater than that in the short duration group (Figure 4b).

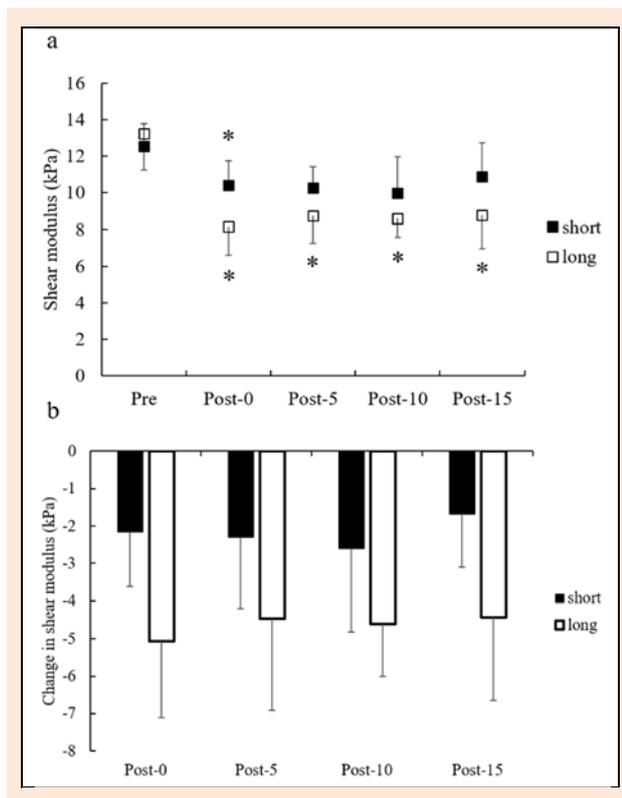
For the post hoc power analysis, the power for the interaction and main effect of times in the first two-way ANOVA were determined to be 0.71 and 1.00, respectively. Moreover, in the second two-way ANOVA, the observed power for the main effect of group was 0.75.

## Discussion

The current study explored the responses of shear modulus in stretching PMi using SWE through experiments I and II. Three main findings can be highlighted from the current study: (i) the shear modulus of the PMi immediately decreased after a bout (=30 s) of stretching, (ii) the shear modulus further decreased after Set-3 (= 90 s) of stretching, and (iii) different prolonged effects on the shear modulus resulting from the different stretching durations. To our knowledge, this is the first study to report the responses of shear modulus to stretching in the PMi using SWE. These findings fully support our hypotheses for experiments I and II.

For experiment I, the shear modulus of PMi immediately decreased after a bout (30 s) of stretching. The conclusion regarding the change in the shear modulus after stretching is consistent with many studies where an immediate decrease in the muscle shear modulus implies a decrease in muscle stiffness (Freitas et al., 2015; Hirata et al., 2016; Ikeda et al., 2020; Nakamura et al., 2014; Umegaki et al., 2015). Certain possible mechanisms are responsible for the reduction in muscle stiffness. One possible mechanism is known as stress-relaxation. If a viscoelastic material such as a muscle is stretched and then held at a constant length, the stress at that length gradually declines (Taylor et al., 1990). Interestingly, stress-relaxation is demonstrated more graphically for the first several seconds and then levels off (McNair et al., 2001; Taylor et al., 1990). Another possible mechanism is the connective tissue surrounding the muscle fibers (Morse et al., 2008; Nakamura et al., 2011). Connective tissue, particularly the perimysium, is considered a major extracellular contributor to muscle stiffness (Purslow, 1989). Lengthening deformation of the connective tissue within the muscle belly has been suggested to influence passive muscle stiffness (Gajdosik, 2001). The other mechanism is the muscle slack angle. Previous study indicated that the shift of the muscle slack angle (i.e., muscle slack length) results in muscle stiffness reduction after static stretching (Hirata et al., 2016). Therefore, the acute decrease in the PMi shear modulus was caused by the possible mechanisms underlying the muscle stiffness reduction after stretching.

Of interest is that the stretching duration that immediately changes the muscle stiffness may differ in the target muscle. The stretching durations for 3 min, 2 min, 1 min, and 20 s were needed to change the muscle stiffness of the hamstrings (Nakamura et al., 2019), the gastrocnemius (Nakamura et al., 2013), the iliacus (Nojiri et al., 2019),



**Figure 4. (a) Shear modulus before (Pre), and immediately- (Post-0), 5 minutes later- (Post-5), 10 minutes later- (Post-10), 15 minutes later- (Post-15) after stretching.** The filled and empty diamonds represent the mean values of Pre through to Post-15 in the short and long duration groups respectively. The error bar indicates standard deviation. The asterisk means that the shear modulus is significantly lower than that of Pre. **(b) Amount of change in shear modulus from Pre.** The black and white bar represent the short and long duration groups respectively. The error bar indicates standard deviation. There is significant main effect of group, and the change in shear modulus of the long duration group is significantly larger than that of the short one at all times.

and infraspinatus (Kusano et al., 2017), respectively. One of the factors that determine the stretching duration could be the muscle volume, more precisely, the cross-sectional area of the muscle. It is possible that the larger the cross-sectional area of the muscle, the smaller the force per unit area of the muscle when the muscle is stretched at the same intensity. Similarly, an *in vitro* study indicated a positive correlation between the rate of increase in shear modulus per unit load and the reciprocal of cross-sectional area (Koo et al., 2013). Taken together, the stretching duration required to change muscle stiffness is likely to depend on the cross-sectional area of the muscle. It is plausible that the relatively short duration of stretching decreased the shear modulus of PMi with a small cross-sectional area (Ward et al., 2009) compared with the lower limb muscles (Herzberg et al., 1999).

The shear moduli of Set-3 (= 90 s) significantly decreased compared to that of Set-1 (= 30 s), which means that the muscle stiffness further decreased at Set-3 and afterward. This suggests a carry-over effect of stretching repetitions on muscle stiffness. The carry-over effect was indicated in a previous study. Nakamura et al. (2013) reported that both passive torque of the ankle joint and myotendinous junction displacement of the gastrocnemius changed after 2 min of static stretching and further lowered or increased after 5 min. Nojiri et al. (2019) also found that the stiffness of the iliacus muscle declined at 1 min and at further 5 min of static stretching. The carry-over effect of stretching repetitions in the current study was more early found compared with those reported in previous studies. This difference may result from the cross-sectional area of the muscles as mentioned above. In shoulder rehabilitation and sport practice, where the increase in stiffness of the PMi is seen as a problem, the carry-over effect of stretching repetition would be relevant in terms of the extent of decrease in muscle stiffness.

In experiment II, the short duration group only showed a decrease in the PMi shear modulus immediately after stretching, while the long duration group showed a decrease in the PMi shear modulus after the stretching and maintained it for 15 min. The rapid recovery of the PMi shear modulus observed in the short duration group may be attributed to the viscoelastic recoil, a muscle mechanical property. This phenomenon is one of the natural behaviors, and elements contributing to muscle elasticity may stabilize the muscle force-generating capacity (Fowles et al., 2000). No studies have investigated viscoelastic recoil in individual muscles around the shoulder joint, although a previous study found that the decreased shear modulus of the gastrocnemius muscle returned to the pre-stretching value 20min after stretching (Taniguchi et al., 2015). Taking advantage of SWE in evaluating individual muscle stiffness is of use for exploration of the viscoelastic recoil in biomechanics research and prescription of stretching in sports settings. Further studies are needed to focus on the other muscles around the shoulder joint.

Our results show a greater decrease in the PMi shear modulus and a prolonged phase in the long duration group. This implies dose-dependency for the change and prolonged effect on muscle stiffness after stretching. Matsuo et al. (Matsuo et al., 2013) reported that the passive torque

after static stretching for 60 s, 180 s, and 300 s is lower than that after the stretching for 20 s. The stiffness after 300 s of stretching was also lower than that after the stretching of 20 s. Ryan et al. (2008) investigated the prolonged effect of stretching with different durations on musculotendinous stiffness and showed that musculotendinous stiffness after 4 min and 8 min stretching returned to baseline within 20 min, while musculotendinous stiffness for 2 min stretching returned to baseline within 10 min. Our results show a dose-dependent change in the shear modulus of the PMi, supporting the previous studies in which the passive torque and the musculotendinous stiffness were used. Keeping the muscle stiffness of PMi low probably makes the treatment and movement practice more effective in rehabilitation and sport practice.

Our findings have notable limitations. First, whether the stretching duration for 30 s is the minimum time to decrease the shear modulus of the pectoralis minor is unclear. A previous study used a stretching minimum duration of 20 s for the infraspinatus muscle (Kusano et al., 2017). We decided on a stretching duration of 30 s because of its practical use in rehabilitation and sport practice, although the minimum stretching duration could be shorter than 30 s for PMi. Second, it is possible that muscle activity could have affected the shear modulus because we could not monitor the muscle activity of the PMi, which is a deep muscle, during stretching. A recent study indicated that even 2% of maximal voluntary contraction increased the shear modulus (Le Sant et al., 2019). Actually, there was a trend to increase the shear modulus at Set-5 to Set-7, although statistical significance was not found. Third, we did not quantify neuromuscular modulation after muscle stretching. Spinal reflex excitability is well known to be inhibited after muscle stretching (Budini and Tilp, 2016). Further study is warranted to determine whether the inhibition of spinal reflex excitability modulates the muscle shear modulus. Finally, the post hoc power analysis revealed insufficient power for two-way ANOVA in experiment II. However, a type II error could not occur because the significant interaction and main effect of interest were found in the statistical analysis.

## Conclusion

In conclusion, the current study revealed three main findings in terms of the shear modulus of the PMi: (i) the shear modulus of the PMi immediately decreased after a bout (=30 s) of stretching, (ii) the shear modulus of the PMi further decreased after 3set (=90 s) stretching; (iii) the decrease in the shear modulus of the PMi was maintained for 15 min after the 5 min stretching, whereas the decrease in the shear modulus was only noted immediately after the 30 s stretch. The time-course and prolonged effect of stretching are relevant for an efficient intervention in rehabilitation and sports settings.

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### Key points

- Mechanical responses of the pectoralis minor muscle to stretching over time were examined.
- Shear modulus was used as a surrogate of muscle stiffness.
- Shear modulus of the pectoralis minor decreased after a bout (= 30s) of stretching.
- Shear modulus further decreased after 3set (= 90s) of stretching.
- Different prolonged effect resulted from the different stretching durations.

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