ABSTRACT
Regarding hamstring stretching methods, many studies have investigated the effect of stretching duration or frequency on muscle stiffness. However, the most effective stretching positions for hamstrings are unclear because it is impossible to quantify muscle elongation directly and noninvasively in vivo. Recently, a new ultrasound technology, ultrasonic shear wave elastography, has permitted noninvasive and reliable measurement of muscle shear elastic modulus, which has a strong linear relationship to the amount of muscle elongation. This study aimed to investigate the effect of hip internal and external rotation on shear elastic modulus of the lateral and medial hamstrings, respectively, during stretching in vivo using ultrasonic shear wave elastography. Twenty-three healthy men (age, 23.0 ± 2.1 years) for this study. To investigate the effect of hip rotation on the elongation of the medial and lateral hamstrings, shear elastic modulus of the biceps femoris (BF) and semitendinosus (ST) was measured at rest (a supine position with 90° knee flexion, 90° hip flexion, and hip neutral rotation) and in seven stretching positions (with 45° knee flexion and hip internal, external, and neutral rotation) using ultrasonic shear wave elastography. In both BF and ST, the shear elastic modulus in the rest position was significantly lower than that in all stretching positions. However, no significant differences were seen among stretching positions. Our results suggest that adding hip
rotation at a stretching position for the hamstrings may not have a significant effect on muscle elongation of the medial and lateral hamstrings.

Key Words: hamstrings, ultrasonic shear wave elastography, stretching, hip rotation
Introduction

Hamstring muscle strain is one of the most common sports injuries (Bishop and Fallon, 1999, Brooks et al., 2006, Ekstrand et al., 2011, Gabbe et al., 2006) and causes considerable lost time from training and competition (Brooks, Fuller, 2006, Ekstrand, Hagglund, 2011). Therefore, many studies have been performed to investigate an effective method to prevent hamstring muscle strain (Gabbe, Bennell, 2006, McHugh and Cosgrave, 2010, Witvrouw et al., 2003). Stretching has been used as one of the main methods for preventing hamstring muscle strain, supported by the finding that less flexibility of the hamstrings increases the risk of hamstring muscle strain (Witvrouw, Danneels, 2003). However, a recent systematic review on prevention of hamstring muscle strain found inadequate evidence for the preventive effect of stretching (Goldman and Jones, 2010). Nevertheless, limited evidence suggests that time for recovery to full function may be reduced by increased frequency of stretching (Mason et al., 2007). To clarify the value of stretching, many studies have investigated the impact of stretching on muscle flexibility with attention to stretching duration and frequency (Ben and Harvey, 2010, Magnusson et al., 2000, Ylinen et al., 2009). However, no studies have investigated effective stretching positions for improving flexibility of the hamstrings in vivo or vitro.
For hamstrings, which have knee flexion and hip extension moment arms, a stretching position with knee extended and hip flexed is generally selected. Medial hamstrings, which consist of the semitendinosus (ST) and semimembranosus, have hip internal rotation moment arms, and lateral hamstrings, which consist of the biceps femoris (BF), have hip external rotation moment arms (Dostal et al., 1986). Therefore, we hypothesized that the medial hamstrings could be stretched more by adding external rotation, and the lateral hamstrings could be stretched more by adding internal rotation.

No studies have investigated effective stretching positions in vivo because it is impossible to quantify muscle elongation directly and noninvasively in vivo. Recently, a new ultrasound technology, ultrasonic shear wave elastography, has permitted noninvasive and reliable measurement of muscle shear elastic modulus. Previous studies have reported a strong linear relationship between the shear elastic modulus measured by ultrasonic shear wave elastography and the amount of muscle elongation (Eby et al., 2013, Koo et al., 2014, Maisetti et al., 2012). Therefore, ultrasonic shear wave elastography is a very useful tool to estimate changes in muscle elongation in vivo. Nevertheless, no studies have investigated the most effective stretching positions using this apparatus.

This study aimed to investigate the effect of hip internal and external rotation
on shear elastic modulus of the lateral and medial hamstrings, respectively, during stretching in vivo using ultrasonic shear wave elastography.

**Methods**

**Subjects**

Twenty-three healthy men (age, 23.0 ± 2.1 years; height, 172.0 ± 4.7 cm; weight, 66.1 ± 7.1 kg) volunteered for this study. Subjects were non-athletes and had not been involved in any regular stretching or resistance training. Subjects with a history of neuromuscular disease or musculoskeletal injury involving the lower limbs were excluded. All subjects were fully informed of the procedures and purpose of the study. Written informed consent was obtained from all subjects. This study was approved by the ethics committee of Kyoto University Graduate School and the Faculty of Medicine (E-1162).

**Experimental protocol**

The subjects were placed in a supine position, and their pelvises were secured by a belt. The rest position (Rest) was defined as the position with 90° knee flexion, 90° hip flexion, and hip-neutral rotation. Stretching was performed in the following seven positions: 1) R0 (45° knee flexion and 90° hip flexion at hip-neutral rotation); 2) IR10 (adding 10° hip internal rotation to R0); 3) IR20 (adding 20° hip internal rotation to...
R0); 4) IR30 (adding 30° hip internal rotation to R0); 5) ER10 (adding 10° hip external rotation to R0); 6) ER20 (adding 20° hip external rotation to R0); 7) ER30 (adding 30° hip external rotation to R0). These positions were determined by measuring the joint angles using a goniometer and were manually maintained. Rest and R0 are shown in Fig. 1. Our study (Nakamura et al., 2013) reported that >2 min of stretching decreased muscle stiffness. Therefore, in this study, each position was maintained for <10 s to avoid the effects of changes in muscle stiffness. The order in which positions were measured was randomized to remove the effect of measurement time.

Measurement of shear elastic modulus

Shear elastic modulus of the BF and ST muscle bellies of the dominant leg was measured at the midpoint of the thigh from the greater trochanter to the lateral and medial epicondyles of the thighbone. These points were confirmed by palpation and marked prior to measurement. Shear elastic modulus of the BF and ST was measured using ultrasonic shear wave elastography (Axplorer; SuperSonic Imagine, Axi-en-Provence, France). This apparatus uses acoustic radiation force created by ultrasound beams to perturb muscle tissues by inducing shear waves that propagate within the muscle. As the shear waves propagate, they are captured by the ultrasound
transducer at an ultrafast frame rate. Shear wave propagation speed is estimated at each pixel using a cross-correlation algorithm. Shear elastic modulus (G) can be calculated using the shear wave speed (v) by the following equation:

\[ G = \rho v^2 \]

where \( \rho \) is the muscle mass density, which is assumed to be 1000 kg/m\(^3\). An ultrasound transducer (50 mm long SL-15-4 linear ultrasound transducer) was positioned on the marking points along the sagittal plane of the muscle fibers for BF and ST, which were confirmed by tracing several fascicles without interruption across the B-mode image. Shear wave elastography generated color-coded images with a scale from blue (low) to red (high) depending on the shear wave propagation speed (Fig. 2). The region of interest (ROI) was set near the center part of the muscle belly in the image. The mean shear wave propagation speed (m/s) of an 11-mm-diameter circle set near the center of the ROI was automatically calculated. The measurements of shear elastic modulus for ST and BF were performed once in each position. Measurement of shear elastic modulus was performed by an experienced measurer. The reliability of the shear elastic modulus measured by this apparatus has been confirmed in previous studies (Koo, Guo, 2014, Maisetti, Hug, 2012).
Measurement reliability

Measurements of shear elastic modulus were repeated twice, in different sessions, to assess reliability (8 healthy men; age 22.8 ± 1.8 years; height 172.8 ± 3.6 cm; body mass 67.0 ± 7.5 kg).

Priori sample size calculation

We calculated the sample size needed for one-way repeated measures analysis of variance (ANOVA) (alpha error = 0.05, power = 0.95, effect size = 0.25 [middle]), and the requisite number of subjects for this study was 23.

Statistical analysis

Statistical analysis was performed using SPSS (version 18.0, SPSS Japan INC., Tokyo, Japan). Measurement reliability was assessed using the intraclass correlation coefficient (ICC [1, 1]) with 95% confidence interval (CI), and the coefficients of variation were calculated. An ICC value of 0.40 is generally considered as poor reliability, 0.40–0.75 as moderate to good, and 0.75 as excellent reliability (Leong et al., 2013). For the shear elastic modulus of the BF and ST, one-way repeated measures analysis of variance (ANOVA) was used to determine the differences in positions. When a significant main effect was observed, the differences between positions were determined using Bonferroni’s post hoc test. Differences were considered statistically significant at an
alpha level of $P < 0.05$. In addition, after Bonferroni’s adjustment, differences were considered statistically significant at an alpha level of $P < 0.00625$.

**Results**

Reliability of shear elastic modulus is shown in Table 1. The ICC (1, 1) was 0.966–0.998 for shear elastic modulus of BF and 0.959–0.995 for shear elastic modulus of the semitendinosus ST at all positions. The results of shear elastic modulus of the BF and ST are shown in Table 2, presented as mean ± SD (standard deviation). For both BF and ST, one-way ANOVA indicated significant main effects (BF: $F = 9.69, P < 0.05$, ST: $F = 9.37, P < 0.05$) of positions. The post hoc test indicated that the shear elastic modulus in Rest was significantly lower than that in all stretching positions. However, no significant differences were seen among the stretching positions in both BF and ST.

**Discussion**

The ICC (1, 1) was 0.966–0.998 for shear elastic modulus of BF and 0.959–0.995 for shear elastic modulus of ST at all positions, and the ICC for both BF and ST was greater than 0.75. Therefore, we consider the data in this study to be reliable. We investigated the effect of hip internal and external rotation on shear elastic modulus of the medial and lateral hamstrings during stretching using ultrasonic shear wave elastography. To
the best of our knowledge, this is the first report examining effective stretching positions of the hamstrings in vivo.

In this study, the shear elastic modulus of the BF and ST in Rest was significantly lower than that in all stretching positions. This result suggested that the medial and lateral hamstrings could be stretched at 45° knee flexion and 90° hip flexion regardless of hip rotation angle. However, no significant differences among stretching positions were found for BF or ST. Therefore, adding hip rotation at a stretching position may have less effect on muscle elongation in the medial and lateral hamstrings.

We hypothesized that the medial hamstrings could be stretched more by adding external rotation and the lateral hamstrings could be stretched more by adding internal rotation. However, the results of this study did not support the hypothesis. The moment arm can be calculated by dividing the amount of elongation of muscle tendon unit (MTU) by the changes in joint angle (tendon excursion methods) (Maganaris et al., 2000). Therefore, MTU would be more elongated as moment arm and changes in joint angle become greater. In both BF and ST, the moment arm of hip internal and external rotation was considerably smaller than that of knee flexion (Buford et al., 1997, Dostal, Soderberg, 1986). Moreover, in this study, change in hip rotation angle (maximally 30°) was smaller than the change in knee extension angle (from 90° to 45°). Therefore, the effect
of hip rotation may be lesser than that of knee extension.

Some limitations of this study must be noted. First, the amount of elongation of the medial and lateral hamstrings could not be directly measured using shear elastic modulus. Although previous studies have reported a strong linear relationship between the shear elastic modulus measured by ultrasonic shear wave elastography and the amount of muscle elongation (Eby, Song, 2013, Koo, Guo, 2014, Maisetti, Hug, 2012), it may not be adequately evaluated by a slight change in muscle elongation. Second, in this study, only the effect of hip rotation on the shear elastic modulus of BF and ST was investigated. It is very important to develop an effective stretching position for improving flexibility of the hamstring muscles to prevent hamstring muscle strain. Further research is required to investigate the most effective stretching position besides hip rotation for the hamstrings.

Conclusion

We investigated the effect of hip internal and external rotation on the shear elastic modulus of the BF and ST using ultrasonic shear wave elastography. Our results suggest that adding hip rotation at a stretching position for the hamstrings may not have a significant effect on muscle elongation of the medial and lateral hamstrings.
References

Ben M, Harvey LA. Regular stretch does not increase muscle extensibility: a randomized controlled trial. Scandinavian journal of medicine & science in sports. 2010;20:136-44.


Goldman EF, Jones DE. Interventions for preventing hamstring injuries. The Cochrane database of systematic reviews. 2010:CD006782.


Table 1

Reliability of shear elastic modulus

<table>
<thead>
<tr>
<th>Position</th>
<th>BF ICC</th>
<th>95% CI</th>
<th>CV (%)</th>
<th>ST ICC</th>
<th>95% CI</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.993</td>
<td>0.967–0.999</td>
<td>3.5</td>
<td>0.959</td>
<td>0.783–0.990</td>
<td>4.6</td>
</tr>
<tr>
<td>R0</td>
<td>0.982</td>
<td>0.897–0.996</td>
<td>4.3</td>
<td>0.991</td>
<td>0.956–0.998</td>
<td>2.8</td>
</tr>
<tr>
<td>IR10</td>
<td>0.993</td>
<td>0.964–0.999</td>
<td>2.6</td>
<td>0.984</td>
<td>0.918–0.997</td>
<td>2.6</td>
</tr>
<tr>
<td>IR20</td>
<td>0.990</td>
<td>0.950–0.998</td>
<td>3.0</td>
<td>0.993</td>
<td>0.946–0.998</td>
<td>2.2</td>
</tr>
<tr>
<td>IR30</td>
<td>0.995</td>
<td>0.961–0.998</td>
<td>2.2</td>
<td>0.995</td>
<td>0.967–0.999</td>
<td>1.9</td>
</tr>
<tr>
<td>ER10</td>
<td>0.966</td>
<td>0.840–0.993</td>
<td>3.3</td>
<td>0.985</td>
<td>0.921–0.997</td>
<td>4.4</td>
</tr>
<tr>
<td>ER20</td>
<td>0.996</td>
<td>0.948–0.998</td>
<td>2.6</td>
<td>0.985</td>
<td>0.898–0.996</td>
<td>3.9</td>
</tr>
<tr>
<td>ER30</td>
<td>0.998</td>
<td>0.940–0.998</td>
<td>2.8</td>
<td>0.995</td>
<td>0.959–0.998</td>
<td>2.6</td>
</tr>
</tbody>
</table>

ICC: intraclass correlation coefficient (1, 1)
95% CI: 95% confidence interval
CV: coefficients of variation
Table 2

Shear elastic modulus of the biceps femoris and semitendinosus in each position.

<table>
<thead>
<tr>
<th>Position</th>
<th>BF (kPa)</th>
<th>ST (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>20.1 ± 7.9</td>
<td>13.9 ± 4.4</td>
</tr>
<tr>
<td>R0</td>
<td>67.8 ± 21.5**</td>
<td>49.7 ± 15.5**</td>
</tr>
<tr>
<td>IR10</td>
<td>74.6 ± 24.1**</td>
<td>50.3 ± 17.5**</td>
</tr>
<tr>
<td>IR20</td>
<td>71.9 ± 22.6**</td>
<td>46.6 ± 12.7**</td>
</tr>
<tr>
<td>IR30</td>
<td>74.9 ± 22.6**</td>
<td>46.7 ± 14.5**</td>
</tr>
<tr>
<td>ER10</td>
<td>64.2 ± 24.3**</td>
<td>50.4 ± 17.9**</td>
</tr>
<tr>
<td>ER20</td>
<td>67.7 ± 26.2**</td>
<td>55.5 ± 16.9**</td>
</tr>
<tr>
<td>ER30</td>
<td>65.0 ± 23.8**</td>
<td>56.3 ± 22.2**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (standard deviation).

**p < 0.01. Significant difference from Rest

BF: biceps femoris, ST: semitendinosus

95% CI: 95% confidence interval

Rest: position with 90° knee flexion, 90° hip flexion, and hip-neutral rotation

R0: position with 45° knee flexion and 90° hip flexion at hip-neutral rotation
IR10: position with 45° knee flexion and 90° hip flexion at 10° hip internal rotation

IR20: position with 45° knee flexion and 90° hip flexion at 20° hip internal rotation

IR30: position with 45° knee flexion and 90° hip flexion at 30° hip internal rotation

ER10: position with 45° knee flexion and 90° hip flexion at 10° hip external rotation

ER20: position with 45° knee flexion and 90° hip flexion at 20° hip external rotation

ER30: position with 45° knee flexion and 90° hip flexion at 30° hip external rotation
Rest: the position with 90° knee flexion, 90° hip flexion, and hip-neutral rotation

R0: the position with 45° knee flexion and 90° hip flexion at hip-neutral rotation
Representative image of the biceps femoris measured by shear wave elastography

A. Rest

Rest: position with 90° knee flexion, 90° hip flexion, and hip-neutral rotation

B. R0

R0: position with 45° knee flexion and 90° hip flexion at hip-neutral rotation