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

1 Introduction

 $\mathbf{2}$ 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 3 4 considerable lost time from training and competition (Brooks, Fuller, 2006, Ekstrand, Hagglund, 2011). Therefore, many studies have been performed to investigate an $\mathbf{5}$ 6 effective method to prevent hamstring muscle strain (Gabbe, Bennell, 2006, McHugh $\overline{7}$ 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 8 9 flexibility of the hamstrings increases the risk of hamstring muscle strain (Witvrouw, 10 Danneels, 2003). However, a recent systematic review on prevention of hamstring muscle strain found inadequate evidence for the preventive effect of stretching 11 (Goldman and Jones, 2010). Nevertheless, limited evidence suggests that time for 12recovery to full function may be reduced by increased frequency of stretching (Mason et 1314al., 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 15(Ben and Harvey, 2010, Magnusson et al., 2000, Ylinen et al., 2009). However, no 1617studies have investigated effective stretching positions for improving flexibility of the hamstrings in vivo or vitro. 18

19	For hamstrings, which have knee flexion and hip extension moment arms, a
20	stretching position with knee extended and hip flexed is generally selected. Medial
21	hamstrings, which consist of the semitendinosus (ST) and semimembranosus, have hip
22	internal rotation moment arms, and lateral hamstrings, which consist of the biceps
23	femoris (BF), have hip external rotation moment arms (Dostal et al. , 1986). Therefore,
24	we hypothesized that the medial hamstrings could be stretched more by adding external
25	rotation, and the lateral hamstrings could be stretched more by adding internal rotation.
26	No studies have investigated effective stretching positions in vivo because it is
27	impossible to quantify muscle elongation directly and noninvasively in vivo. Recently, a
28	new ultrasound technology, ultrasonic shear wave elastography, has permitted
29	noninvasive and reliable measurement of muscle shear elastic modulus. Previous studies
30	have reported a strong linear relationship between the shear elastic modulus measured
31	by ultrasonic shear wave elastography and the amount of muscle elongation (Eby et al.,
32	2013, Koo et al. , 2014, Maisetti et al. , 2012). Therefore, ultrasonic shear wave
33	elastography is a very useful tool to estimate changes in muscle elongation in vivo.
34	Nevertheless, no studies have investigated the most effective stretching positions using
35	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.

39

40 Methods

41 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).

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50 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 56rotation to R0); 6) ER20 (adding 20° hip external rotation to R0); 7) ER30 (adding 30° 57hip external rotation to R0). These positions were determined by measuring the joint 5859angles 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 60 muscle stiffness. Therefore, in this study, each position was maintained for <10 s to 61 avoid the effects of changes in muscle stiffness. The order in which positions were 6263 measured was randomized to remove the effect of measurement time.

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65 Measurement of shear elastic modulus

Shear elastic modulus of the BF and ST muscle bellies of the dominant leg was 66 measured at the midpoint of the thigh from the greater trochanter to the lateral and 67 medial epicondyles of the thighbone. These points were confirmed by palpation and 68 marked prior to measurement. Shear elastic modulus of the BF and ST was measured 69 ultrasonic shear wave elastography (Axiplorer; SuperSonic 70using Imagine, Axi-en-Provence, France). This apparatus uses acoustic radiation force created by 7172ultrasound beams to perturb muscle tissues by inducing shear waves that propagate 73within 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 ρ is the muscle mass density, which is assumed to be 1000 kg/m³. An ultrasound 78transducer (50 mm long SL-15-4 linear ultrasound transducer) was positioned on the 79marking points along the sagittal plane of the muscle fibers for BF and ST, which were 80 confirmed by tracing several fascicles without interruption across the B-mode image. 81 82Shear wave elastography generated color-coded images with a scale from blue (low) to 83 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 84 shear wave propagation speed (m/s) of an 11-mm-diameter circle set near the center of 85the ROI was automatically calculated. The measurements of shear elastic modulus for 86 87 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 88 modulus measured by this apparatus has been confirmed in previous studies (Koo, Guo, 89 90 2014, Maisetti, Hug, 2012).

92	Measurement reliability
93	Measurements of shear elastic modulus were repeated twice, in different sessions, to
94	assess reliability (8 healthy men; age 22.8 \pm 1.8 years; height 172.8 \pm 3.6 cm; body
95	mass 67.0 ± 7.5 kg).
96	
97	Priori sample size calculation
98	We calculated the sample size needed for one-way repeated measures analysis of
99	variance (ANOVA) (alpha error = 0.05, power = 0.95, effect size = 0.25 [middle]), and
100	the requisite number of subjects for this study was 23.
101	
102	Statistical analysis
103	Statistical analysis was performed using SPSS (version 18.0, SPSS Japan INC., Tokyo,
104	Japan). Measurement reliability was assessed using the intraclass correlation coefficient
105	(ICC [1, 1]) with 95% confidence interval (CI), and the coefficients of variation were
106	calculated. An ICC value of 0.40 is generally considered as poor reliability, 0.40-0.75 as
107	moderate to good, and 0.75 as excellent reliability (Leong et al. , 2013). For the shear
108	elastic modulus of the BF and ST, one-way repeated measures analysis of variance
109	(ANOVA) was used to determine the differences in positions. When a significant main
110	effect was observed, the differences between positions were determined using
111	Bonferroni's post hoc test. Differences were considered statistically significant at an

112	alpha level of $P < 0.05$. In addition, after Bonferroni's adjustment, differences were
113	considered statistically significant at an alpha level of $P < 0.00625$.
114	
115	Results
116	Reliability of shear elastic modulus is shown in Table 1. The ICC (1, 1) was 0.966–
117	0.998 for shear elastic modulus of BF and 0.959–0.995 for shear elastic modulus of the
118	semitendinosus ST at all positions. The results of shear elastic modulus of the BF and
119	ST are shown in Table 2, presented as mean \pm SD (standard deviation). For both BF and
120	ST, one-way ANOVA indicated significant main effects (BF: F = 9.69, $P < 0.05$, ST: F =
121	9.37, $P < 0.05$) of positions. The post hoc test indicated that the shear elastic modulus in
122	Rest was significantly lower than that in all stretching positions. However, no
123	significant differences were seen among the stretching positions in both BF and ST.
124	
125	Discussion
126	The ICC (1, 1) was 0.966–0.998 for shear elastic modulus of BF and 0.959–0.995 for
127	shear elastic modulus of ST at all positions, and the ICC for both BF and ST was greater
128	than 0.75. Therefore, we consider the data in this study to be reliable. We investigated
129	the effect of hip internal and external rotation on shear elastic modulus of the medial
130	and lateral hamstrings during stretching using ultrasonic shear wave elastography. To

the best of our knowledge, this is the first report examining effective stretchingpositions of the hamstrings in vivo.

In this study, the shear elastic modulus of the BF and ST in Rest was 133134significantly 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 135regardless of hip rotation angle. However, no significant differences among stretching 136 positions were found for BF or ST. Therefore, adding hip rotation at a stretching 137position may have less effect on muscle elongation in the medial and lateral hamstrings. 138139We hypothesized that the medial hamstrings could be stretched more by adding external 140 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 141 be calculated by dividing the amount of elongation of muscle tendon unit (MTU) by the 142changes in joint angle (tendon excursion methods) (Maganaris et al., 2000). Therefore, 143144MTU 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 145146 considerably smaller than that of knee flexion (Buford et al., 1997, Dostal, Soderberg, 1471986). 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 148

149 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 150the medial and lateral hamstrings could not be directly measured using shear elastic 151152modulus. Although previous studies have reported a strong linear relationship between the shear elastic modulus measured by ultrasonic shear wave elastography and the 153amount of muscle elongation(Eby, Song, 2013, Koo, Guo, 2014, Maisetti, Hug, 2012), it 154may not be adequately evaluated by a slight change in muscle elongation. Second, in 155this study, only the effect of hip rotation on the shear elastic modulus of BF and ST was 156157investigated. It is very important to develop an effective stretching position for improving flexibility of the hamstring muscles to prevent hamstring muscle strain. 158Further research is required to investigate the most effective stretching position besides 159hip rotation for the hamstrings. 160

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162 Conclusion

163 We investigated the effect of hip internal and external rotation on the shear elastic

164 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

166 significant effect on muscle elongation of the medial and lateral hamstrings.

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239 Table 1

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

240 Reliability of shear elastic modulus

241 ICC: intraclass correlation coefficient (1, 1)

242 95% CI: 95% confidence interval

243 CV: coefficients of variation

244

245

247 Table 2

Position	BF (kPa)	ST (kPa)					
Rest	20.1 ± 7.9	13.9 ± 4.4					
R0	$67.8 \pm 21.5^{**}$	$49.7 \pm 15.5^{**}$					
IR10	$74.6 \pm 24.1^{**}$	$50.3 \pm 17.5^{**}$					
IR20	$71.9 \pm 22.6^{**}$	$46.6 \pm 12.7^{**}$					
IR30	$74.9 \pm 22.6^{**}$	$46.7 \pm 14.5^{**}$					
ER10	$64.2 \pm 24.3^{**}$	$50.4 \pm 17.9^{**}$					
ER20	$67.7 \pm 26.2^{**}$	$55.5 \pm 16.9^{**}$					
ER30	$65.0 \pm 23.8^{**}$	56.3 ± 22.2**					

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

- 249 Values are expressed as mean \pm SD (standard deviation).
- 250 **p < 0.01. Significant difference from Rest
- 251 BF: biceps femoris, ST: semitendinosus
- 252 95% CI: 95% confidence interval
- 253 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

255	IR10: posit	tion	with	45°	knee	flexion	and	90°	hip	flexion	at	10°	hip	internal	rotation
256	IR20: posit	tion	with	45°	knee	flexion	and	90°	hip	flexion	at	20°	hip	internal	rotation
257	IR30: posit	tion	with	45°	knee	flexion	and	90°	hip	flexion	at	30°	hip	internal	rotation
258	ER10: posi	ition	with	45°	knee	flexion	and	90°	hip	flexion	at	10°	hip	external	rotation
259	ER20: posi	ition	with	45°	knee	flexion	and	90°	hip	flexion	at	20°	hip	external	rotation
260	ER30: posi	tion	with 4	45° 1	knee f	lexion a	nd 9	0° hi	ip fle	exion at	30 [°]	° hip	exte	ernal rota	ation
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273 Fig. 1



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





- 282 Fig. 2
- 283 Representative image of the biceps femoris measured by shear wave elastography
- 284 **A.** Rest



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

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287 **B.** R0



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

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