

- 1 **Acute effects of stretching on passive properties of human gastrocnemius muscle–tendon unit:**
- 2 **Analysis of differences between hold–relax and static stretching**
- 3

## 4 **Abstract**

5 **Context:** Hold–relax stretching (HRS) and static stretching (SS) are commonly used to  
6 increase the range of motion (ROM) of a joint and to decrease muscle stiffness. However,  
7 whether there are differences between acute effects of HRS and SS on end ROM, passive  
8 torque, and muscle stiffness is unclear. In addition, any differences between the mechanisms  
9 by which HRS and SS lead to an increase in end ROM are also unclear.

10 **Objective:** The purpose of this study was to compare the acute effects of HRS and SS on the  
11 passive properties of the gastrocnemius muscle–tendon unit (MTU), end ROM, passive  
12 torque, and muscle stiffness in vivo and to investigate the factors involved in increasing end  
13 ROM.

14 **Design:** A cross-over experimental design.

15 **Participants:** Thirty healthy men with no history of neuromuscular disease or  
16 musculoskeletal injury involving the lower limbs ( $21.7 \pm 1.2$  years).

17 **Intervention:** Both HRS and SS of 30 s was repeated four times, lasting a total of 2 min.

18 **Main Outcome Measures:** End ROM, passive torque, and muscle stiffness were measured  
19 during passive ankle dorsiflexion using a dynamometer and ultrasonography before and  
20 immediately after HRS and SS.

21 **Results:** The results showed that end ROM and passive torque at the end ROM significantly  
22 increased immediately after both HRS and SS, whereas muscle stiffness significantly

23 decreased. In addition, the percent change in passive torque at end ROM upon use of the HRS  
24 technique was significantly higher than that after use of the SS technique. However, the  
25 percent change in muscle stiffness following SS was significantly higher than that with HRS.  
26 **Conclusion:** These results suggest that both HRS and SS can effectively decrease muscle  
27 stiffness of the gastrocnemius MTU. In addition, these results suggest that HRS induces a  
28 change in the passive torque at end ROM, i.e., sensory perception, rather than changing  
29 muscle stiffness.

30 Keywords: muscle stiffness, ultrasonography, proprioceptive neuromuscular facilitation  
31 stretching, passive torque, stretch tolerance.

32

## INTRODUCTION

Stretching exercises are commonly used in clinical and athletic settings and can be classified as static stretching (SS), dynamic stretching, ballistic stretching, and proprioceptive neuromuscular facilitation (PNF) stretching. PNF stretching and SS are the most popular type of exercises<sup>1</sup>. SS is a stretching technique in which the target muscle is elongated and held at the lengthened position for a certain period of time. Many studies have reported that joint range of motion (ROM) increases immediately after SS<sup>2-4</sup>.

PNF stretching techniques, which involve hold-relax stretching (HRS), are based on the work of Voss et al<sup>5</sup>. **HRS is a stretching technique comprising a combination of SS and isometric contraction of the agonist muscle performed in the elongated position.** The target muscle is stretched for a certain period. An isometric contraction at the lengthened position is then performed, followed by another set of SS<sup>6,7</sup>. Similar to SS, many studies have reported that ROM increases immediately after HRS<sup>6-8</sup>. **In addition, recent studies regarding acute effects have reported that HRS is more effective than SS for increasing ROM<sup>9-11</sup>.** Many of the previous studies have used ROM as an outcome measurement of flexibility for stretching exercises.

However, measurement of ROM is also influenced by psychological factors and

stretch tolerance, such as pain and stretch tolerance, in addition to the viscoelasticity of muscles, tendons, ligaments, and joint capsules<sup>12, 13</sup>. Therefore, an alternative approach is to measure the passive torque during passive stretching. The overall stiffness of the muscle–tendon unit (MTU) can be estimated by calculating the relationship between passive torque and joint angle<sup>14</sup>. Recent studies have shown that gastrocnemius muscle stiffness can be assessed by measuring the displacement of the myotendinous junction (MTJ) during passive ankle dorsiflexion using a dynamometer and ultrasonography and that muscle stiffness decreases after 3–5 min of SS<sup>15-18</sup>.

In addition, the passive torque at end ROM was defined as a stretch tolerance<sup>10, 13, 18, 19</sup>, and an increase in the passive torque at end ROM was defined as a modification of stretch tolerance. Many studies have reported that the passive torque at end ROM increases after SS<sup>10, 18, 20</sup> and HRS<sup>6, 8-11</sup>, which suggests that a change in stretch tolerance occurs after stretching.

Previous studies have compared the acute effects of HRS and SS on end ROM and stretch tolerance. However, to the best of our knowledge, there has been no study examining the acute effect of HRS on muscle stiffness and comparing the acute effects on muscle stiffness between HRS and SS. Because decreased muscle stiffness can lead to improvements

in athletic performance or prevention of injury<sup>21-23</sup>, a clear understanding of the differences in the effects of HRS and SS on muscle stiffness is important in clinical and athletic settings.

In addition, any differences between the mechanisms by which HRS and SS lead to an increase in end ROM are also unclear. Therefore, this study aimed to compare the acute effects of HRS and SS on passive properties of gastrocnemius MTU in vivo and to investigate the factors involved in increasing end ROM. We hypothesized that both HRS and SS could increase end ROM, but that the underlying mechanisms for these effects may differ, with the effect of SS influenced by the decrease in muscle stiffness and that of HRS influenced by the modified stretch tolerance.

## **METHODS**

### **Study Design**

A cross-over experimental design was used to compare the acute effects of HRS and SS on passive properties of gastrocnemius MTU in vivo and to investigate the factors related to increasing end ROM. All participants visited the laboratory on two occasions separated by at least 1 week but no more than 2 weeks to take into account the influence of the measurements and the minimize the carry over effect. Each participant performed HRS once and SS once but in a random order. The subjects were instructed not to begin any other stretching program during the experimental period. All measurements were performed prior to (PRE) and

immediately after (POST) HRS and SS. The subjects were familiarized with the procedure and were instructed to remain relaxed throughout the measurement period.

## **Participants**

Thirty men volunteered for this study (age,  $21.7 \pm 1.2$  years; height,  $170.0 \pm 5.3$  cm; body mass,  $62.4 \pm 7.8$  kg). Participants with a history of neuromuscular disease or musculoskeletal injury involving the lower limbs were excluded. All participants were fully informed of the procedures and purpose of the study and gave their written informed consent. This study was approved by the ethics committee.

## **Procedures**

### **Measurements of end ROM and passive torque**

The subjects were instructed to lie in the prone position on a dynamometer table (MYORET RZ-450, Kawasaki Heavy Industries, Kobe, Japan) with their hips securely held in place with an adjustable lap belt (Fig 1). The knee of the dominant leg was kept in full extension, and the foot of the same leg was attached securely to the dynamometer footplate with adjustable lap belts. The ankle was passively dorsiflexed at a constant velocity of  $5^\circ/\text{s}$ , starting from  $30^\circ$  plantarflexion to end dorsiflexion ROM. In this study, end dorsiflexion ROM was defined as the angle achieved by the joint when the point of discomfort, but not pain was reached<sup>16-18</sup>.

Passive plantarflexion torque was measured using a dynamometer in a similar manner as end ROM was measured. Passive torque at end ROM was defined as a stretch tolerance, and an increase in the passive torque at end ROM was defined as a modification of stretch tolerance during stretching<sup>10, 13, 18, 19</sup>.

#### **Measurement of muscle stiffness**

B-mode ultrasonography (Famio Cube SSA-520A; Toshiba Medical Systems Corporation, Tochigi, Japan) was used to determine the displacement of MTJ of medial gastrocnemius (MG) during passive ankle dorsiflexion. MTJ was identified and visualized as a continuous sagittal plane on the ultrasound image using an 8-MHz linear-array probe. An acoustically reflective marker was placed on the skin under the ultrasound probe to confirm that the probe did not move during the measurements<sup>16</sup>. We defined MTJ displacement as the distance between MTJ and an acoustically reflective marker. A custom-made fixation device was used to secure the probe to the skin. Ultrasound images of MTJ were quantified using an open-source digital measurement software (Image J, National Institutes of Health, Bethesda, Maryland, USA). To accurately measure MTJ, it was identified at the inner-most edges of the fascia surrounding the muscle where it fuses with the tendon. MTJ displacement was measured between 0° and 30° of ankle dorsiflexion. According to a previous study<sup>24</sup>, movement of the dynamometer was stopped at 0° and 30°, and at these angles, the



measurement of MTJ by ultrasonography and that of passive torque by dynamometer were synchronously performed. Muscle stiffness was defined as the value obtained by dividing the change in passive torque (between 0° and 30°) by the MTJ displacement<sup>17</sup>.

### **Surface electromyography**

Electromyography (EMG; TeleMyo2400; Noraxon USA, Inc., Scottsdale, AZ, USA) was used to confirm that the subjects were relaxed and to ensure that muscles were inactive during passive dorsiflexion. Surface electrodes (Blue Sensor M, Ambu, Denmark) with a 2.0-cm interelectrode distance were placed on certain portions of muscle bellies of the following muscles: MG, lateral gastrocnemius (LG). The EMG sampling rate was 1500 Hz.

EMG activity was recorded from MG and LG while the subjects were performing an isometric maximum voluntary contraction (MVC). MVC of MG and LG were obtained during maximal isometric plantar flexion the ankle at 0° using the dynamometer. Strong verbal encouragement was provided during the contraction to promote maximal effort. EMG activity was calculated using root mean square (RMS), and full wave rectification was performed using an RMS smoothing algorithm with a window interval of 50 ms. EMG activity recorded during the measurements was expressed as a percentage of MVC.

## HRS and SS

Both HRS and SS were performed using the dynamometer in the prone position with the knee extended, similar to the measurements of end ROM and passive torque. In HRS, initially, the ankle was passively dorsiflexed at a constant velocity of 5°/s, starting from 30° plantar flexion to end dorsiflexion ROM, and was held at the end angle for 15 s. The subjects were then instructed to perform MVC of the plantar flexors for 5 s in the same position. After this contraction, the ankle was held at the end angle for an additional 10 s. After each 30-s HRS stretch, the ankle was returned to 30° plantar flexion. This application of HRS for 30 s was repeated four times, for a total time of 2 min.

During SS, the ankle was passively dorsiflexed, starting from 30° plantar flexion to end dorsiflexion ROM and was held at the end angle for 30 s. This SS technique of 30 s was repeated four times, lasting a total of 2 min. In both HRS and SS techniques, the angles of stretching were the same for each stretching. In addition, for both the HRS and SS techniques, we used constant angle stretching, which is routinely used to stretch MTU<sup>17, 18, 25</sup>, to standardize stretching intensity, which is routinely used to stretch MTU<sup>17, 18, 25</sup>. We previously confirmed that the SS protocol with stretching for more than 2 min significantly decreases muscle stiffness<sup>26</sup>. Therefore, we adopted the SS and HRS protocols with 2-min stretching durations in total.

166

**167 Reliability of the measurements**

168 All measurements were repeated twice on different days to assess test–retest reliability (10  
169 healthy men; age,  $21.8 \pm 1.2$  years; height,  $172.0 \pm 3.4$  cm; body mass,  $63.2 \pm 8.9$  kg). The  
170 measurements were performed with at least a 1-week interval, but not longer than a 2-week  
171 interval, between the two tests.

172

**173 Statistical analyses**

174 SPSS (version 17.0; SPSS Japan INC., Tokyo, Japan) was used for statistical analyses. For all  
175 variables, significance of differences between HRS and SS techniques at PRE was assessed  
176 using the unpaired t-test. Significant differences between PRE and POST were determined for  
177 both the HRS and SS techniques using the paired t-test with Bonferroni correction. In  
178 addition, the percent change between the PRE and POST conditions was calculated to clarify  
179 the differences in the effects of HRS and SS on passive properties (end ROM, muscle  
180 stiffness, and stretch tolerance): percent change = (PRE value – POST value) / (PRE value) ×  
181 100. Because the Shapiro–Wilk tests showed that the percent change was not normally  
182 distributed, differences in the rates of change between the HRS and SS groups were  
183 determined using the Mann–Whitney U test.

184 Differences were considered statistically significant at an alpha level of  $P < 0.05$ .

The reliability of end ROM, passive torque at end ROM, and muscle stiffness measurements were examined using the intra-class correlation coefficient (ICC). On the basis of the reliability coefficients, the standard error of measurement (SEM) was calculated ( $SEM = SD \sqrt{1 - ICC}$ ) for each measurement. Descriptive data are shown as mean  $\pm$  standard deviation (SD).

## RESULTS

Reliability assessments for end ROM, passive torque at end ROM, and muscle stiffness are shown in Table 1. ICC (1, 1) was between 0.891 and 0.957 ( $P < 0.01$ ), and SEM was between 0.4 and 1.4.

Changes in variables between PRE and POST in HRS and SS techniques are shown in Table 2. There were no significant differences in any variable between HRS and SS techniques at PRE. In both techniques, POST values of end ROM and passive torque at end ROM were significantly higher than PRE values. Furthermore, for both techniques the POST value of muscle stiffness was significantly lower than the PRE value.

A comparison of the percent changes between PRE and POST conditions induced by the HRS and SS techniques is shown in Table 3. There was no significant difference in the percent change in the end ROM between the HRS and SS techniques. The percent change in

muscle stiffness in the SS technique was significantly higher than that in the HRS technique, whereas the percent change in passive torque at the end ROM in the HRS technique was significantly higher than that in the SS technique. There were no significant differences in the PRE and POST EMG activities of MG and LG, which were <2.0% MVC during all the measurements in both techniques (table 2).

## DISCUSSION

This study investigated the acute effects of 2 min of HRS and SS on passive properties of gastrocnemius MTU. The major findings of this study was that the percent change in passive torque at end ROM in HRS technique was significantly higher than that in SS technique, whereas that in muscle stiffness in SS was significantly higher than that in HRS. These results suggest that HRS affects the stretch tolerance, rather than muscle stiffness, in contrast to SS, which is consistent with our hypothesis. To the best of our knowledge, this is the first report to compare the acute effects of HRS and SS on passive properties of gastrocnemius MTU, including end ROM, muscle stiffness, and stretch tolerance.

Our results showed that POST value of end ROM was significantly higher than PRE value in both techniques. In addition, POST value of muscle stiffness was significantly lower than PRE values in both techniques. These results suggest that an increase in end ROM is a

reflection of a decrease in muscle stiffness, which is consistent with the results of other studies examining the acute effects of SS<sup>15-18</sup>. The previous study<sup>18</sup> suggested that the effect of stretching on end ROM may be related to decreases in muscle stiffness and a modification in stretch tolerance. We measured passive torque at end ROM, as a stretch tolerance, and POST value of passive torque at end ROM was significantly higher than PRE value in both techniques. This result suggests that an increase in end ROM is also related to a modification in stretch tolerance, which is consistent with the results of previous studies examining the acute effects of SS<sup>10, 18, 20</sup> and HRS<sup>6, 8-11</sup>. Therefore, we concluded that decreased muscle stiffness and a modification in stretch tolerance occurring after 2 min of HRS and SS could contribute to an increase in end ROM<sup>18</sup>.

However, Magnusson et al.<sup>10</sup> reported that 90 s of HRS increased ROM without a corresponding decrease in passive torque, suggesting that the increase in end ROM was because of a modification in stretch tolerance rather than changes in the passive properties of MTU<sup>10</sup>. In addition, many other studies<sup>6, 8, 9, 11</sup> have concluded that the increase in end ROM is predominantly because of a modification in stretch tolerance using HRS durations less than 90 s. These results do not agree with our results showing that the decrease in muscle stiffness also contributes toward increasing end ROM. We consider that this discrepancy may be because of differences in HRS duration. In this study, HRS duration was 2 min (four

repetitions of a 30-s HRS technique), which was comparatively longer than previous studies<sup>6, 8, 9, 11</sup>. In addition, this discrepancy may be due to differences in the target muscle for HRS, which was the gastrocnemius MTU in our study and the hamstring MTU in the previous study<sup>10</sup>. Furthermore, the method used for HRS also may have contributed to the difference in the results of our study and the previous study<sup>10</sup>. Specifically, the contraction duration in our study was 20 s (4 times  $\times$  5 s), whereas that in the previous study<sup>10</sup> was 6 s. Therefore, in addition to a modification in stretch tolerance, muscle stiffness may also change after HRS technique in this study.

The decrease in muscle stiffness after SS may be associated with a change in the properties of intramuscular connective tissues rather than muscle fiber lengthening<sup>16, 17</sup>. Although the detailed mechanism underlying the decrease in muscle stiffness after HRS is not known, the acute effects of HRS on the properties of intramuscular connective tissue, such as endomysium, perimysium, and epimysium, may also contribute to the decrease in muscle stiffness after HRS and SS

In this study, our results showed that there was no significant difference in the change in end ROM between HRS and SS techniques, which is consistent with previous studies<sup>27, 28</sup>. In contrast, previous studies<sup>9-11, 29, 30</sup> have reported that the acute effect of HRS on end ROM

was greater than that of SS, which is inconsistent with our results. This discrepancy may be because of differences in target muscles. We examined the effects of HRS and SS on gastrocnemius MTU, whereas previous studies<sup>9-11</sup> examined these effects on hamstring MTU. Further study is required to clarify differences in effects between stretching maneuvers on various target muscles.

The second major finding of this study was that there was a greater increase in passive torque at end ROM in HRS technique compared with SS technique, whereas there was a greater decrease in muscle stiffness in SS technique compared with HRS technique. We consider that the decrease in muscle stiffness in SS technique was greater than that in HRS technique because of the stretching duration. The subjects were instructed to perform MVC of the plantar flexors for 5 s between stretching maneuvers in HRS technique. Therefore, the target muscle was elongated for a total of 100 s in HRS technique, whereas the muscle was elongated for a total of 120 s in SS technique. The lengthening deformation of intramuscular connective tissue (e.g., endomysium, perimysium, and epimysium) may also contribute to the decrease in muscle stiffness<sup>16, 17</sup>. In HRS technique, shortened muscle fiber during a voluntary isometric contraction leads to deflection (“slack” in intramuscular connective tissue), which may hamper the decrease in muscle stiffness<sup>31</sup>. Therefore, our findings suggest that the effect on muscle stiffness in HRS is lower than that in SS because of differences in



stretch duration between HRS and SS. In addition, previous studies<sup>32, 33</sup> reported that the tendon stiffness decreases after an isometric contraction. Therefore, there was the possibility that the decrease in tendon stiffness during HRS technique was greater compared with SS technique. Because we did not measure the tendon stiffness in both techniques, further study is needed to clarify the effects of HRS and SS techniques on this outcome. Decreased muscle stiffness can be beneficial in improving athletic performance or preventing injury<sup>21-23</sup>. Therefore, because SS technique might be more beneficial in improving athletic performance or preventing injury, further study is needed to clarify the effects of HRS and SS not only on passive properties, such as end ROM and muscle stiffness, but also on improving performance and preventing injury.

Our results showed that there was a greater increase in passive torque at end ROM in HRS technique compared with SS technique. Regarding the mechanism of a modification in stretch tolerance, afferent input from muscles and joints during stretching may inhibit signals from nociceptive fibers, which may increase pain thresholds<sup>8, 10, 11</sup>. In addition, it is possible that sensory afferents affect interneuron release of enkephalins, which could help reduce transmission of nociception in the dorsal horn during stretching, thereby increasing the pain threshold. The analgesic effects achieved by increasing the pain threshold may have altered the stretch tolerance. Our results also suggest that HRS could increase pain thresholds, i.e., a

modification in stretch tolerance, to greater levels than those achievable with SS. It is possible that the greater modification in stretch tolerance may be because of a voluntary contraction in HRS technique<sup>8, 10</sup>. Compared with SS, HRS technique, which places stronger loads on MTU by a voluntary contraction, may increase pain thresholds. With respect to the contraction intensity in HRS, a previous study<sup>35</sup> suggested that max isometric contractions may not be required for firing sensory afferents or for inducing the anti-nociceptive signals. Therefore, further study is required to more closely examine contraction intensity.

This study had some limitations. First, the examiner performing the measurements was not blinded to the groups. Second, we examined only the acute effects of SS and HRS on the passive properties. Thus, we did not examine the prolonged effects after more than a few days or the effects of a stretching training program that lasts several weeks. Therefore, the results may not apply to long-term stretching programs.

## **Conclusion**

Our results suggest that both HRS and SS can increase end ROM, which may be because of the decreases in the muscle stiffness and modified stretch tolerance during the stretch application. In addition, compared to SS, HRS may have a greater effect on the alteration of stretch tolerance rather than the decrease in muscle stiffness.

318

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417 Figure 1 Experimental set-up of passive dorsiflexion test



418  
419 The ankle of the dominant leg was attached securely to the dynamometer footplate by  
420 adjustable lap belts to prevent the heel that moving away from the footplate.

421

422 **Table 1 Reliability assessment for end ROM, passive torque at end ROM, muscle**

423 **stiffness**

	Test 1	Test 2	ICC (1, 1) (95% CI)	Difference <sup>a</sup> (95% CI)	<u>SEM</u>
End ROM	32.1 ± 2.8	32.6 ± 2.5	0.891 (0.642–0.971)	0.5 ± 1.2 (–0.3–1.3)	<u>1.4</u>
passive torque at end ROM	43.2 ± 9.7	42.1 ± 7.9	0.893 (0.648–0.972)	–1.1 ± 4.4 (–4.3–2.0)	<u>0.9</u>
muscle stiffness	38.1 ± 5.2	36.5 ± 4.5	0.957 (0.846–0.989)	–1.6 ± 4.5 (–4.9–1.6)	<u>0.4</u>

424 Data are means ± standard deviation

425 a: Difference = Test 2 value – Test 1 value

426

427 **Table 2 Changes between PRE and POST values in both HRS and SS techniques**

Outcome	Technique	PRE	POST	P value	Difference <sup>a</sup>
					(95% CI)
End ROM (°)	HRS	33.7 ± 2.7	39.5 ± 3.1**	<u>P &lt; 0.025</u>	<u>5.8±1.5 (5.2-6.3)</u>
	SS	33.8 ± 3.0	39.1 ± 3.2**	<u>P &lt; 0.025</u>	<u>5.3±1.3 (4.8-5.8)</u>
Passive torque at end ROM (Nm)	HRS	41.7 ± 10.7	47.2 ± 10.5**	<u>P &lt; 0.025</u>	<u>5.6±3.5 (4.2-6.9)</u>
	SS	41.5 ± 9.0	43.5 ± 9.9**	<u>P &lt; 0.025</u>	<u>2.0±2.3 (1.1-2.8)</u>
Muscle stiffness (Nm/cm)	HRS	36.1 ± 13.3	31.6 ± 10.2**	<u>P &lt; 0.025</u>	<u>-4.5±4.7 (-6.3- -2.7)</u>
	SS	37.3 ± 17.1	25.3 ± 12.1**	<u>P &lt; 0.025</u>	<u>-12.0±9.2 (-15.4- -8.5)</u>
MG EMG activity (%MVC)	HRS	1.6 ± 0.7	1.6 ± 0.7	P = 0.45	<u>0.0±0.4 (-0.1-0.1)</u>
	SS	1.7 ± 0.7	1.8 ± 0.7	P = 0.62	<u>0.1±0.4 (-0.1-0.2)</u>
LG EMG activity (%MVC)	HRS	1.5 ± 1.0	1.4 ± 0.9	P = 0.94	<u>-0.1±0.4 (-0.2-0.1)</u>
	SS	1.6 ± 0.9	1.7 ± 0.9	P = 0.41	<u>0.0±0.5 (-0.1-0.2)</u>
TA EMG activity (%MVC)	HRS	1.2 ± 0.8	1.1 ± 0.8	P = 0.78	<u>0.0±0.5 (-0.2-0.2)</u>
	SS	1.3 ± 1.0	1.2 ± 0.8	P = 0.20	<u>-0.1±0.6 (-0.3-0.1)</u>

428 \*\*: P &lt; 0.05; significant difference in change between PRE and POST

429 Data are means ± standard deviation

430 a: Difference = POST value – PRE value

431

**Table 3 Comparisons of the rates of change between HRS and SS techniques**

Rate of change (%)	HRS <u>technique</u>	SS <u>technique</u>	P value
End ROM	$17.2 \pm 4.4$	$15.8 \pm 4.2$	$P = 0.26$
Passive torque at the end ROM	$14.5 \pm 11.8$	$4.7 \pm 6.3$	$P < 0.01$
Muscle stiffness	$-10.9 \pm 10.2$	$-30.4 \pm 14.3$	$P < 0.01$