

- 1 Changes in passive properties of the gastrocnemius muscle–tendon unit during a 4-week
- 2 routine static stretching program
- 3

#### 4 **Abstract**

5 **Context:** Static Stretching (SS) is commonly performed within a warm-up routine to  
6 increase the range of motion (ROM) of a joint and to decrease muscle stiffness.  
7 However, the time course of changes in **ankle** dorsiflexion (DF) ROM and muscle  
8 stiffness during a routine SS program is unclear.

9 **Objective:** The present study investigated changes in ankle DF ROM, passive torque at  
10 DF ROM, and muscle stiffness during a routine SS program performed three times  
11 weekly for 4 weeks.

12 **Design:** A quasi-randomized controlled trial design.

13 **Participants:** The subjects comprised 24 male volunteers (age  $23.8 \pm 2.3$  years; height  
14  $172.0 \pm 4.3$  cm; body mass  $63.1 \pm 4.5$  kg) randomly assigned to either a group  
15 performing a 4-week stretching intervention program (SS group) or a control group.

16 **Main Outcome Measures:** The DF ROM, passive torque, and muscle stiffness were  
17 measured during passive ankle dorsiflexion in both groups using a dynamometer and  
18 ultrasonography once weekly during the 4-week intervention period.

19 **Results:** In the SS group, DF ROM and passive torque at DF ROM significantly  
20 increased after 2, 3, and 4 weeks compared with the initial measurements. Muscle  
21 stiffness also decreased significantly after 3 and 4 weeks in the SS group. However,

22 there were no significant changes in the control group.

23 **Conclusions:** Based on these results, the SS program effectively increased DF ROM  
24 and decreased muscle stiffness. Furthermore, an SS program greater than 2 weeks  
25 duration effectively increased DF ROM and changed the stretch tolerance, and an SS  
26 program greater than 3 weeks in duration effectively decreased muscle stiffness.

27

28 Key words: time course, muscle stiffness, stretch tolerance, ultrasound

29

30 Stretching is commonly performed within a warm-up routine to increase joint flexibility,  
31 improve performance, and reduce injury risk. Numerous previous studies reported that  
32 static stretching (SS) increased the joint range-of-motion (ROM) both acutely<sup>1, 2</sup> and  
33 following routine SS<sup>3-5</sup>. Hamstring and plantar flexor muscle stretching increased knee  
34 extension and **ankle** dorsiflexion (DF) ROM both acutely and chronically, according to  
35 systematic literature reviews<sup>6, 7</sup>. Potentially, the joint ROM increase following SS may  
36 be caused by: decreased passive torque, muscle-tendon unit (MTU), and muscle  
37 stiffness; and changes in psychological factors such as pain and stretch tolerance<sup>8, 9</sup>.

38 Previous studies evaluating acute effect of SS reported that a 3- to 5-min  
39 duration decreased MTU and muscle stiffness<sup>9-13</sup>. In a study examining MTU stiffness  
40 over time following SS (constant-torque stretching) at 2, 4, and 8 min, the initial  
41 decrease in MTU stiffness dissipated in less than 10 min following a 2 min SS, but after  
42 4- and 8-min SS, the effect was maintained for 10 min<sup>14</sup>. We recently reported that  
43 decreased MTU and muscle stiffness were maintained for 10 min following a 5-min  
44 constant-angle SS session<sup>12</sup>, which is consistent with a prior study<sup>14</sup>. However, Mizuno  
45 et al. (2013) reported that the MTU and muscle stiffness decreases following a 5-min  
46 constant-angle SS disappeared within 10–15 min, whereas the increased ROM persisted  
47 for 30 min. These results suggested that the increased ROM immediately following SS

48 may be attributed to changes in both MTU viscoelasticity and stretch tolerance, and the  
49 ROM increase at 15–30 min after SS could be attributed only to a stretch tolerance  
50 change. These studies concluded that the retention time of the acute effects of SS was  
51 shorter for MTU viscoelasticity than for stretch tolerance.

52 Other studies have similarly examined the chronic effect of SS. For example,  
53 previous studies reported that passive torque and MTU stiffness decreased after a 3- to  
54 6-week routine SS program<sup>15-18</sup>. In addition, stretch tolerance changed after a 2- to  
55 6-week routine SS program<sup>19-21</sup>. We reported that muscle stiffness decreased after 4  
56 weeks of routine SS<sup>22</sup>. However, the time course of changes in muscle stiffness and  
57 stretch tolerance immediately following SS were discordant<sup>9</sup>; thus, a discrepancy in the  
58 time course of muscle stiffness and stretch tolerance changes may also occur during a  
59 routine SS program. Furthermore, the ideal SS program duration required to change the  
60 ROM, muscle stiffness, and stretch tolerance is unclear.

61 This study investigated changes in the gastrocnemius MTU passive properties  
62 over time, including DF ROM, muscle stiffness, and stretch tolerance during a 4-week  
63 SS program. A previous study showed that the acute effects of SS on muscle stiffness  
64 dissipated faster than the stretch tolerance<sup>9</sup>. Therefore, we hypothesized that muscle  
65 stiffness changes caused by the routine SS program would occur later during the

66 program than the stretch tolerance changes.

67

68

## **Methods**

### **69 Study Design**

70 A quasi-randomized controlled trial design was used to investigate changes in ankle DF

71 ROM, passive torque at DF ROM, and muscle stiffness during a routine SS program

72 performed three times weekly for 4 weeks. The gastrocnemius MTU passive properties

73 (DF ROM, passive torque at DF ROM, and muscle stiffness) were measured at the

74 initial evaluation and once weekly over 4 weeks in both groups. As an *a priori* sample

75 size calculation, we calculated the sample size that was needed for split-plot analysis of

76 variance (ANOVA) [alpha error = 0.05, power = 0.80, effect size = 0.25 (middle)] using

77 G\*Power 3.1 software (Heinrich Heine University, Düsseldorf, Germany). The results

78 showed that the requisite number of subjects for this study was 11 for each group.

79 Considering a possible dropout, 12 participants were recruited for each group. After an

80 initial evaluation of MTU passive properties, participants were randomly allocated in a

81 1:1 ratio to either the SS group (N = 12) or the control group (N = 12) using the

82 alternation method. To control for immediate SS impacts, all procedures in the SS group

83 were performed at least 24 h after the last SS session<sup>22</sup>. The subjects were instructed not

84 to initiate any other stretching or strength training program during the experimental  
85 period.

86

## 87 **Participants**

88 Twenty-four healthy male volunteers who were non-athletes participated in this study

89 (age  $23.8 \pm 2.3$  years; height  $172.0 \pm 4.3$  cm; body mass  $63.1 \pm 4.5$  kg). Subjects with a

90 history of neuromuscular disease or lower extremity musculoskeletal injury were

91 excluded. All subjects participated in sports at a recreational level and had not been

92 involved in any regular resistance or flexibility training. Written informed consent was

93 obtained from all subjects. Subject demographics of each group are summarized in

94 Table 1. There were no significant demographic differences between the two groups

95 based on an unpaired *t*-test. In addition, this study was approved by the ethics

96 committee.

97

## 98 **Procedures**

### 99 **Assessment of DF ROM and passive torque at the DF ROM**

100 The subjects laid in a prone position on a dynamometer table (MYORET RZ-450,

101 Kawasaki Heavy Industries, Kobe, Japan) secured at the hips with adjustable lap belts.

102 The dominant knee was maintained in full extension, and the ipsilateral foot was  
103 securely attached to the dynamometer footplate with adjustable lap belts to prevent the  
104 heel that moving away from the footplate. The ankle was passively dorsiflexed at a  
105 constant 5°/s velocity beginning at a 30° plantar flexion until reaching the DF ROM. In  
106 this study, DF ROM was defined as the angle where subjects experienced discomfort  
107 without pain<sup>9, 11, 12, 14</sup>. The passive torque at ankle angles of 0°, 30° dorsiflexion, and DF  
108 ROM were measured during the procedure using a dynamometer. Passive torque at DF  
109 ROM served as the index of stretch tolerance; a passive torque increase at the DF ROM  
110 indicated modified stretch tolerance<sup>9</sup>.

111

### 112 **Muscle stiffness assessment**

113 Myotendinous junction (MTJ) displacement at the gastrocnemius muscle medial head  
114 during passive ankle dorsiflexion was determined using B-mode ultrasonography  
115 (Famio Cube SSA-520A; Toshiba Medical Systems Corporation, Tochigi, Japan). MTJ  
116 was visualized on a continuous sagittal plane ultrasound image using an 8-MHz  
117 linear-array probe. An acoustically reflective marker was placed on the skin under the  
118 ultrasound probe to confirm that the probe remained stable during measurement. The  
119 MTJ displacement was defined as the distance between the MTJ and the reflective



120 marker. A customized fixation device secured the probe to the skin. Ultrasound MTJ  
121 images were quantified using open-source digital measurement software (Image J,  
122 National Institutes of Health, Bethesda, MD, USA). To ensure accuracy, the MTJ was  
123 identified at the inner fascial edge surrounding the muscle at its fusion to the tendon;  
124 displacement was measured during 0° and 30° ankle dorsiflexion. Muscle stiffness was  
125 calculated by dividing the passive torque change during 0–30° ankle dorsiflexion by the  
126 MTJ displacement<sup>12</sup>.

127

### 128 **Surface electromyography (EMG)**

129 Electromyography (EMG) (TeleMyo2400; Noraxon USA Inc., Scottsdale, AZ, USA)  
130 confirmed that the subjects were relaxed and muscles were inactive during passive **ankle**  
131 dorsiflexion. Surface electrodes (Blue Sensor M, Ambu, Denmark) at a 2.0-cm  
132 interelectrode distance were placed on the medial and lateral gastrocnemius muscle  
133 bellies<sup>13</sup>.

134 An EMG was recorded from the muscle bellies while the subjects performed an  
135 isometric maximum voluntary contraction (MVC), obtained during maximal isometric  
136 plantar flexion with the ankle at 0°. Strong verbal encouragement was provided during  
137 the contraction to promote maximal effort. EMG activity was calculated from the root

138 mean square (RMS), and a full wave rectification was performed using an RMS  
139 smoothing algorithm at a 50-ms window interval. EMG activity recorded during passive  
140 **ankle** dorsiflexion was expressed as a percentage of MVC. The EMG sampling rate was  
141 1500 Hz.

142

### 143 **Static stretching (SS) program**

144 Subjects in the SS group were placed in a prone position with the knee extended, similar  
145 to conditions during the DF ROM and passive torque measurements. During SS, the  
146 ankle was passively dorsiflexed, starting from 30° of plantar flexion to the DF ROM,  
147 and was held at the DF ROM for 30 s, e.g, constant-angle stretching method. We  
148 previously confirmed that an SS greater than 2 min significantly decreased muscle  
149 stiffness<sup>23</sup>. Therefore, the 30-s maneuver was repeated four times, 2 min in total. A  
150 previous study reported that stretching exercises performed three times weekly were  
151 sufficient to improve ROM compared to stretching once weekly<sup>5</sup>. Therefore, the SS  
152 maneuver was performed three times weekly over a 4-week period. The sessions were  
153 conducted every 2 or 3 days. Subjects in the control group did not receive any  
154 intervention.

155

156

**157 Measurement reliability**

158 All measurements were performed by the same experienced examiner. We selected  
159 seven subjects (age,  $23.8 \pm 1.1$  years; height,  $172.7 \pm 4.9$  cm; body mass,  $65.2 \pm 2.8$  kg)  
160 from the control group and adopted the initial and 1 week data for the reliability  
161 analysis.

162

**163 Statistical analysis**

164 SPSS (version 17.0; SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses.  
165 Measurement reliability was assessed using the intraclass correlation coefficient (ICC [1,  
166 1]). The Shapiro–Wilk test was performed to evaluate the normality of the data, and the  
167 assumption was met for almost all variables, suggesting the use of a parametric analysis.

168 Differences between the SS and control groups for all variables relative to the initial  
169 evaluation were assessed with an unpaired *t*-test. Split-plot ANOVA and one-way  
170 repeated ANOVA compared the SS and control groups over time and the initial  
171 evaluation vs. data at 1, 2, 3, and 4 weeks. When one-way repeated ANOVA indicated a  
172 significant effect associated with time, the Dunnett's multiple comparison test was  
173 employed to determine the change time course compared with the initial evaluation.

174 Differences were considered statistically significant at an alpha level of  $p < 0.05$ .

175 Descriptive data are shown as mean  $\pm$  standard deviation.

176

## 177 **Results**

### 178 **Reliability assessment**

179 Measurement reliability assessments are summarized in Table 2. The ICC (1, 1) was  
180 0.836 (95% confidence interval [CI]; 0.464–0.960) for DF ROM, 0.942 (95% CI;  
181 0.782–0.986) for passive torque at DF ROM, and 0.941 (95% CI; 0.779–0.986) for  
182 muscle stiffness.

183

### 184 **DF ROM, passive torque at DF ROM, and muscle stiffness changes over time**

185 There were no significant differences between the two experimental groups in all  
186 variables relative to the initial evaluation. The DF ROM, passive torque at DF ROM,  
187 and muscle stiffness changes over time in both groups are shown in Table 3. The  
188 split-plot ANOVA indicated that there were significant group  $\times$  time interaction effects  
189 for DF ROM, passive torque at DF ROM, and muscle stiffness ( $F = 20.6, p < 0.01, \eta_p^2 =$   
190  $0.483; F = 5.88, p < 0.01, \eta_p^2 = 0.211; and F = 11.0, p < 0.01, \eta_p^2 = 0.334, respectively$ ).

191 There was also a significant time effect on DF ROM, passive torque at DF ROM, and

192 muscle stiffness in the SS group, but there was no significant time effect on the  
193 variables in the control group.

194 In the SS group, the DF ROM significantly increased after 2 weeks ( $p < 0.05$ ),  
195 3 weeks ( $p < 0.01$ ), and 4 weeks ( $p < 0.01$ ). Similarly, the passive torque at DF ROM  
196 significantly increased after 2 weeks ( $p < 0.05$ ), 3 weeks ( $p < 0.01$ ), and 4 weeks ( $p <$   
197  $0.01$ ). In addition, muscle stiffness significantly decreased after 3 ( $p < 0.05$ ) and 4  
198 weeks ( $p < 0.05$ ).

199

## 200 **EMG activity**

201 The GM and LG EMG activities were  $<2\%$  MVC, which confirmed a lack of contractile  
202 contribution to the DF ROM, passive torque, and muscle stiffness.

203

204

## Discussion

205 We investigated the gastrocnemius MTU passive property changes during a 4-week  
206 routine SS program. The major study finding was that the DF ROM and passive torque  
207 at DF ROM changes occurred earlier than the muscle stiffness change during the routine  
208 SS program. Although previous studies investigated the acute impact of SS on passive  
209 properties<sup>9, 10, 12, 14</sup>, this is the first known study demonstrating the time course of MTU

210 passive property changes during a 4-week routine SS program in vivo.

211 Our study revealed two-way ANOVA (group  $\times$  time) interactions in the DF  
212 ROM and passive torque at DF ROM. In addition, the multiple comparison test  
213 indicated that DF ROM and passive torque at DF ROM in the SS group significantly  
214 increased after 2–4 weeks compared with the initial evaluation, with no significant  
215 changes in the control group. These results suggest that a 2-week or longer SS program  
216 effectively increases the DF ROM, which is consistent with previous studies<sup>3-5</sup>. The  
217 passive torque at DF ROM, which indicated stretch tolerance, also increased after 2  
218 weeks of the SS program. These results suggest that a 2-week or longer SS program  
219 may be required to change DF ROM and stretch tolerance. Although the mechanism of  
220 stretch tolerance change after routine SS program is unknown, afferent input from  
221 muscles and joints during stretching inhibits signals from nociceptive fibers, which may  
222 increase pain thresholds<sup>24-26</sup>.

223 In the evaluation of effects of routine SS program on muscle stiffness, there  
224 was a two-way ANOVA (group  $\times$  time) interaction observed. Furthermore, a multiple  
225 comparison test revealed that muscle stiffness significantly decreased after 3 and 4  
226 weeks in the SS group. These results suggest that a SS program greater than 3 weeks  
227 effectively decreases muscle stiffness. The underlying mechanism of this change is

228 unknown, but previous studies reported that the decreased muscle stiffness acutely and  
229 chronically following an SS program might be associated with alterations in the  
230 properties of intramuscular connective tissue properties rather than muscle fiber  
231 lengthening<sup>11, 12, 22</sup>. Therefore, the muscle stiffness decrease after 3 weeks of routine SS  
232 may also reflect a change in intramuscular connective tissue flexibility. Our results  
233 showed that muscle stiffness significantly decreased after 3 and 4 weeks, with no  
234 change observed during the initial 1–2 weeks. There may be a dose-response  
235 relationship between the SS duration and the MTU stiffness response<sup>14</sup>. Therefore, a 1-  
236 to 2-week SS program may be insufficient to decrease muscle stiffness, and an SS  
237 program lasting at least 3 weeks may be necessary using the current study protocol.

238         Our results showed a discrepancy the between muscle stiffness and stretch  
239 tolerance changes during the 4-week routine SS program. In particular, more than 2  
240 weeks of routine SS increased passive torque at DF ROM, which indexes stretch  
241 tolerance, but more than 3 weeks of routine SS was required to decrease muscle  
242 stiffness. These results show that the stretch tolerance changed earlier than muscle  
243 stiffness during a routine SS program, which confirms our hypothesis, though the  
244 underlying mechanism is unclear. As for the acute effect of SS, Mizuno et al. (2013)  
245 reported that the acute benefits of 5-min SS on muscle stiffness persisted for a shorter

246 time than the stretch tolerance benefits. Potentially, the stretch tolerance change  
247 occurred earlier than the muscle stiffness decrease during the routine SS program.  
248 Decreased muscle stiffness can be beneficial in improving athletic performance or  
249 preventing injury<sup>27, 28</sup>; further study is needed to clarify the long-term effects of routine  
250 SS not only on passive properties, such as DF ROM and muscle stiffness, but also on  
251 improving performance and preventing injury. Notably, under the current study protocol  
252 of 2-min SS, three times weekly over a 4-week period, it is unclear whether the same  
253 decreased muscle stiffness could be realized under an SS program with longer single  
254 sessions combined with a shorter program duration. Additional study determining the  
255 ideal SS program duration and intervention frequency maximizing the muscle stiffness  
256 decrease is needed.

257         Our results showed that there was a discrepancy in the time course of muscle  
258 stiffness and stretch tolerance changes during a routine SS program, i.e., the stretch  
259 tolerance changed earlier than muscle stiffness during a routine SS program. In addition,  
260 decreased muscle stiffness can be beneficial in improving athletic performance or  
261 preventing injury<sup>27, 28</sup>. Therefore, taken together, it was suggested that it is necessary to  
262 perform the routine SS program to cause a decrease in muscle stiffness in order to  
263 improve the athletic performance or prevent injury.



264 This study had some limitations. First, the examiner taking measurements was  
265 not blinded to the group. Therefore, a bias in the results cannot be completely  
266 discounted. Second, we have not investigated the time course of changes in passive  
267 properties during a detraining period after 4 weeks of static stretching program.  
268 Therefore, further research is required to determine the prolonged effect of SS program  
269 on passive properties.

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271

272

### **Conclusion**

273 This study investigated the change in the gastrocnemius MTU passive properties, the  
274 DF ROM, muscle stiffness, and stretch tolerance, during a 4-week routine SS program.  
275 Our results showed that the changes in muscle stiffness and stretch tolerance occur at  
276 different speeds during the 4-week routine SS program. In particular, these results  
277 suggest that a SS program greater than 2 weeks effectively increases DF ROM and  
278 changes stretch tolerance, and a SS program greater than 3 weeks is needed to decrease  
279 muscle stiffness.

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284

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389



390 Table 1. Subject demographics.

391 <sup>a</sup>SS group: performed a routine static stretching (SS) maneuver.

392

393 Table 2. Reliability assessment for DF ROM, passive torque at DF ROM, and muscle

394 stiffness.

395 Data presented as mean  $\pm$  standard deviation

396 DF, dorsiflexion; ROM, range-of-motion; ICC, intraclass correlation coefficient; CI,

397 confidence interval

398

399 Table 3. Passive property changes of the gastrocnemius muscle–tendon unit over time.

400 \*  $p < 0.05$ , \*\*  $p < 0.01$ ; significantly different from the initial measurement.

401 SS, static stretching; DF, dorsiflexion; ROM, range-of-motion.

402

403

|                | SS group <sup>a</sup><br>(N = 12) | control group<br>(N = 12) | p-value  |
|----------------|-----------------------------------|---------------------------|----------|
| Age (years)    | 23.9 ± 3.0 (21–33)                | 23.6 ± 1.0 (22–26)        | p = 0.23 |
| Height (cm)    | 171.4 ± 4.4 (163–183)             | 172.7 ± 4.0 (163–180)     | p = 0.89 |
| Body mass (kg) | 61.9 ± 5.1 (50–70)                | 64.3 ± 3.3 (57–70)        | p = 0.33 |

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|                          | Test 1     | Test 2     | ICC (1, 1) | 95% CI      |
|--------------------------|------------|------------|------------|-------------|
| DF ROM                   | 34.9 ± 2.7 | 35.0 ± 2.6 | 0.836      | 0.464–0.960 |
| Passive torque at DF ROM | 40.7 ± 8.5 | 40.1 ± 8.2 | 0.942      | 0.782–0.986 |
| Muscle stiffness         | 37.8 ± 6.7 | 38.3 ± 6.5 | 0.941      | 0.779–0.986 |

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|                    | DF ROM (°)                           |               | Passive Torque at DF ROM<br>(Nm)     |               | Muscle Stiffness (Nm/cm)             |               |
|--------------------|--------------------------------------|---------------|--------------------------------------|---------------|--------------------------------------|---------------|
|                    | SS group                             | Control group | SS group                             | Control group | SS group                             | Control group |
| Initial            | 34.8 ± 3.8                           | 37.6 ± 5.6    | 39.3 ± 5.0                           | 40.6 ± 10.5   | 38.7 ± 9.2                           | 39.9 ± 8.9    |
| 1 week             | 37.3 ± 4.2                           | 36.8 ± 5.1    | 48.0 ± 10.0                          | 41.6 ± 9.8    | 37.1 ± 8.6                           | 41.1 ± 6.8    |
| 2 weeks            | 39.6 ± 3.1*                          | 37.4 ± 5.2    | 51.8 ± 10.5*                         | 42.1 ± 10.8   | 37.2 ± 8.4                           | 39.0 ± 8.0    |
| 3 weeks            | 40.7 ± 3.9**                         | 37.3 ± 4.9    | 53.8 ± 10.9**                        | 41.1 ± 7.2    | 30.4 ± 7.0*                          | 41.0 ± 7.1    |
| 4 weeks            | 43.9 ± 4.5**                         | 36.8 ± 4.7    | 58.3 ± 10.7**                        | 41.9 ± 9.2    | 29.6 ± 6.8*                          | 41.5 ± 7.5    |
| <u>Effect size</u> | <u><math>\eta_p^2 = 0.483</math></u> |               | <u><math>\eta_p^2 = 0.211</math></u> |               | <u><math>\eta_p^2 = 0.334</math></u> |               |

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