

1 **Abstract**

2 The minimum time required for Static stretching (SS) to change the passive properties
3 of the muscle–tendon unit (MTU), as well as the association between these passive
4 properties, remains unclear. This study investigated the time course of changes in the
5 passive properties of gastrocnemius MTU during 5 min of SS.

6 The subjects comprised 20 healthy males (22.0 ± 1.8 years). Passive torque as
7 an index of MTU resistance and myotendinous junction (MTJ) displacement as an index
8 of muscle extensibility were assessed using ultrasonography and dynamometer during 5
9 min of SS. Significant differences before and every 1 minute during SS were
10 determined using Scheffé's post hoc test. Relationships between passive torque and
11 MTJ displacement for each subject were determined using Pearson's product-moment
12 correlation coefficient.

13 Although gradual changes in both passive torque and MTJ displacement were
14 demonstrated over every minute, these changes became statistically significant after 2, 3,
15 4, and 5 minutes of SS compared with the values before SS. In addition, passive torque
16 after 5 minutes SS was significantly lower than that after 2 minutes SS. Similarly, MTJ
17 displacement after 5 minutes SS was significantly higher than that after 2 minutes SS. A
18 strong correlation was observed between passive torque and MTJ displacement for each

19 subject ($r = -0.886$ to -0.991).

20 These results suggest that SS for more than 2 minutes effectively increases

21 muscle extensibility, which in turn decreases MTU resistance.

22

23 **Introduction**

24 Limited joint mobility and decreased muscle flexibility are common problems in
25 clinical situations and athletic settings. Static stretching (SS) is a useful method for
26 preventing joint contracture and improving joint mobility and muscle flexibility.
27 Although recent studies have suggested that SS may not reduce the incidence of injury
28 (Thacker et al., 2004), and that SS may actually decrease muscle strength and
29 performance (Behm & Chaouachi, 2011; Simicet et al., 2012), many previous studies
30 have reported that the maximum range of motion (ROM) increased immediately after
31 SS (Boyce & Brosky, 2008; de Weijer et al., 2003; Depino et al., 2000; Halbertsma et
32 al., 1996; Hubley et al., 1984; O’Sullivan et al., 2009; Ryan et al., 2008b). The effects of
33 SS have been estimated using maximum ROM as an outcome measure in various
34 studies. However, the use of ROM for this purpose has several limitations. For example,
35 maximum ROM measurements are influenced by many factors, such as pain, stretch
36 tolerance, and reflex activation of the agonist muscle (McHugh et al., 1998; Sale et al.,
37 1982). Measuring passive torque and muscle–tendon unit (MTU) stiffness using a
38 dynamometer are effective approach for determining the resistance of MTU during
39 passive movements (Toft et al., 1989a; Magnusson et al., 1996b). Passive torque
40 represents the amount of resistance provided by the MTU at a given joint angle (Toft et

41 al., 1989a), while MTU stiffness is the shape of the torque-angle curve which
42 demonstrates the relationship between passive torque and joint angle (Magnusson et al.,
43 1996b). Recently, noninvasive methods of measuring the passive properties of muscles
44 and tendons, such as myotendinous junction (MTJ) displacement, have been developed
45 using ultrasonography during passive movement (Kay & Blazevich, 2009a; b; 2010;
46 Mizuno et al., 2011; Morse 2011; Morse et al., 2008; Nakamura et al., 2011; 2012).

47 Many studies have examined the acute effects of SS on passive torque and
48 MTU stiffness, which is an indicator of MTU resistance, using a dynamometer and have
49 reported that SS of various durations (1–42 min) (Duong et al., 2001; Fowles et al.,
50 2000; Herda et al., 2011; Herda et al., 2009; Magnusson et al., 1996b; Morse et al.,
51 2008; Nordez et al., 2006, Nordez et al., 2008; Ryan et al., 2008a; Ryan et al., 2009)
52 resulted in decreased passive torque or decreased MTU stiffness. On the other hand,
53 Halbertsma et al. (1996) reported that maximum ROM increased immediately after 5
54 min of SS, but no changes were observed in MTU stiffness. They also suggested that
55 increased stretch tolerance explained the influence of SS on maximum ROM. Similarly,
56 other studies have reported increased stretch tolerance but no decrease in MTU stiffness
57 after SS for 1–2 min (Magnusson et al., 2000; Magnusson et al., 1998; Magnusson et al.,
58 1996a; McNair et al., 2001; Muir et al., 1999). Duration of SS is a key issue for

59 decreasing MTU resistance, which has not yet reached agreement.

60 Recently, with the use of ultrasonography, a technique to measure the
61 displacement of MTJ, which could be used as an indicator of muscle extensibility, was
62 enabled. Using this technique, several studies (Kay & Blazeovich, 2009 b; Mizuno et al.,
63 2011; Morse et al., 2008; Nakamura et al., 2011) reported that MTU resistance during
64 passive ankle dorsiflexion decreased after SS because of an increase in muscle
65 extensibility. However, the minimum amount of time required for SS to increase muscle
66 extensibility remains undefined. In addition, the influence of muscle extensibility on
67 MTU resistance during SS also remains unclear since these studies did not examine the
68 association between changes in passive torque, which is an indicator of MTU resistance,
69 and MTJ displacement, which is an indicator of muscle extensibility.

70 This study aimed to determine the minimum time required for SS to decrease
71 MTU resistance and muscle extensibility and to examine the association between the
72 two indicators, i.e., passive torque and MTJ displacement, during 5 min of SS.

73

74 **Methods**

75 **Subjects**

76 Twenty healthy males (age, 22.0 ± 1.8 years; height, 173.4 ± 5.9 cm; body mass, $64.8 \pm$

77 6.1 kg; and dorsiflexion ROM, $31.4^{\circ} \pm 4.4^{\circ}$) volunteered for the study. Subjects with a
78 history of neuromuscular disease or musculoskeletal injury involving their lower limbs
79 were excluded from the study. All subjects were fully informed of the procedures and
80 purpose of the study, following which written informed consent was obtained from all
81 of them.

82

83 Experimental protocol

84 The subjects were familiarized with the procedure and instructed to remain relaxed
85 during measurement. They were instructed to lie in the prone position on a
86 dynamometer table, with their hips secured by adjustable lap belts (MYORET RZ-450,
87 Kawasaki Heavy Industries, Kobe, Japan). The knee of the dominant leg was in full
88 extension and the foot of the same leg was attached securely to the footplate of the
89 dynamometer. The ankle was passively dorsiflexed at a constant velocity of $5^{\circ}/s$,
90 starting from 0° to the dorsiflexion ROM, and was held in the dorsiflexion ROM for 5
91 min. In this study, the dorsiflexion ROM was defined as the angle that the subjects
92 could achieve without discomfort or pain (Nakamura et al., 2011). Passive torque and
93 ultrasound images of the gastrocnemius muscle were obtained every 1 min during 5 min
94 of SS. Passive torque is the resistance of the entire MTU to the direction of

95 plantarflexion during the passive dorsiflexion (Toft et al., 1989a). Electromyography
96 (EMG) (TeleMyo2400; Noraxon USA, Inc., Scottsdale, AZ, USA) using surface
97 electrodes (Blue Sensor M, Ambu, Denmark) was used to confirm that subjects were
98 relaxed and ensure that there was no high EMG activity in the medial gastrocnemius
99 muscle. For the measurement of changes in the medial head of the gastrocnemius
100 muscle, surface electrodes were placed over the muscle belly. The original low EMG
101 signals processed using a band-pass filter at 20–500 Hz were amplified and collected at
102 a sampling rate of 1500 Hz. EMG activity was calculated using the root mean square
103 (RMS), and full wave rectification was performed using an RMS smoothing algorithm
104 with a window interval of 50 ms. EMG activity within 3 s was calculated during
105 isometric maximum voluntary contraction (MVC) of the ankle plantar flexors with the
106 ankle at 0°. EMG activity recorded during the tests was expressed as a percentage of
107 MVC.

108

109 Ultrasound measurements

110 B-mode ultrasonography (Famio Cube SSA-520A; Toshiba Medical Systems
111 Corporation, Tochigi, Japan) with an 8-MHz linear probe was used to determine the
112 displacement of the distal MTJ of the medial head of the gastrocnemius muscle during

113 passive ankle dorsiflexion. MTJ was identified as described by Maganaris and Paul
114 (1999). An acoustically reflective marker was placed between the skin and the probe to
115 ensure probe stability during measurements (Morse et al., 2008; Nakamura et al., 2011).
116 A custom-made fixation device was used to secure the probe to the skin. MTJ
117 displacement was defined as the distance between MTJ and the acoustically reflective
118 marker secured to the probe. Ultrasound images of the MTJ were quantified using
119 open-source digital measurement software (Image J, NIH, USA). For accurate
120 measurement, the MTJ was identified at the innermost edges of the fascia surrounding
121 the muscle, where it fuses with the tendon. MTJ displacement was measured between 0°
122 and the dorsiflexion ROM for ankle dorsiflexion (Fig. 1). MTJ displacement is a
123 measure of the degree of muscle extensibility during passive dorsiflexion (Nakamura et
124 al., 2012). In a previous study, high intraclass correlation coefficients demonstrated the
125 reliability of the MTJ displacement measurement procedures used in this study
126 (Nakamura et al., 2011).

127

128 Statistical analysis

129 Significant differences in passive torque and MTJ displacement measurements taken
130 before SS and every 1 min during SS were assessed using one-way repeated analysis of

131 variance (ANOVA). When a significant effect was found, the differences between
132 measurements taken before SS and those taken every 1 min during SS were determined
133 using Scheffé's post hoc test.

134 Relationships between passive torque and MTJ displacement for each subject
135 during 5 min of SS were determined using Pearson's product-moment correlation
136 coefficient. In addition, the rate of change in passive torque and MTJ displacement was
137 defined using the following formula: rate of change = (value before SS – value 5 min
138 after SS) / (value before SS) × 100. The relationship between rate of change in passive
139 torque and that in MTJ displacement was determined using Pearson's product-moment
140 correlation coefficient. Differences were considered statistically significant at an alpha
141 level of $P < 0.05$. Descriptive data were determined as means \pm SEM with 95%
142 confidence intervals.

143

144 **Results**

145 EMG activity

146 Two subjects exhibited obvious EMG activity ($>2\%$ MVC) in the medial head of the
147 gastrocnemius muscle during SS. Therefore, results were obtained from the remaining
148 18 subjects (22.0 ± 1.9 years) with low EMG activity during SS.

149

150 Passive torque

151 ANOVA indicated a significant effect of SS duration on passive torque. The post hoc
152 test indicated no significant difference between passive torque after 1 min of SS and that
153 before SS. However, passive torque after 2, 3, 4, and 5 min of SS was significantly
154 lower than that before SS (Table 1). In addition, passive torque after 4 and 5 min of SS
155 was significantly lower than that after 1 min of SS, and passive torque after 5 min of SS
156 was significantly lower than that after 2 min of SS.

157

158 MTJ displacement

159 ANOVA indicated a significant effect of SS duration on MTJ displacement. The post
160 hoc test indicated no significant difference between MTJ displacement after 1 min of SS
161 and that before SS. However, MTJ displacement after 2, 3, 4, and 5 min of SS was
162 significantly higher than that before SS (Table 1). In addition, MTJ displacement after 4
163 and 5 min of SS was significantly higher than that after 1 min of SS, and MTJ
164 displacement after 5 min of SS was significantly higher than that after 2 and 3 min of
165 SS.

166

167 Relationship between passive torque and MTJ displacement

168 Pearson's product-moment correlation coefficient indicated a strong correlation between
169 passive torque and MTJ displacement for each subject ($r = -0.886$ to -0.991 , $P < 0.05$).

170 A typical example of the relationship between passive torque and MTJ displacement is
171 shown Figure 2. In addition, Pearson's product-moment correlation coefficient indicated
172 a significant correlation between rate of change in passive torque and that in MTJ
173 displacement ($r = -0.708$, $P < 0.01$, Fig. 3).

174

175 **Discussion**

176 Our findings revealed that passive torque was significantly lower after 2 min of SS than
177 before SS, although passive torque decreases gradually over every minute during SS
178 (Table 1). Since passive torque was used to represent the amount of resistance provided
179 by the entire MTU at a given joint angle (Toft et al., 1989a), this result suggests that SS
180 for more than 2 min effectively decreases the resistance of the entire MTU. Other
181 studies have found similar results for passive torque, reporting that SS for periods
182 shorter than 2 min did not decrease passive torque (Magnusson et al., 2000; Magnusson
183 et al., 1998; Magnusson et al., 1996a; McNair et al., 2001). In contrast, Ryan et al.
184 (2009) reported that two 30-s repetitions of SS (i.e., 1 min of SS) decrease the resistance

185 of the entire MTU. This discrepancy may be due to differences in SS measurement
186 procedures. Ryan et al. (2009) used constant-torque SS, which allowed some joint angle
187 movement, whereas 5-min constant-angle SS was used in our study. Herda et al. (2011)
188 recently suggested that 8-min constant-torque SS may be more effective in decreasing
189 the resistance of the entire MTU compared with the same duration of constant-angle SS.
190 They also suggested that constant-torque SS had more effects on the decreasing the
191 resistance of the entire MTU compared with constant-angle SS for the same duration.
192 Therefore, the constant-torque SS protocol used by Ryan et al. (2009) may have
193 decreased the resistance of the entire MTU in less time compared with the protocol used
194 in our study.

195 To our knowledge, this is the first report which studied the influence of SS
196 duration on both passive torque as an index of MTU resistance and MTJ displacement
197 as an index of the degree of muscle extensibility. Similar to the results for passive
198 torque, MTJ displacement was significantly greater after 2 min of SS than before SS,
199 although MTJ displacement increases gradually over every minute during SS (Table 1).
200 MTJ displacement is an indicator of degree of muscle extensibility, and an increase in
201 MTJ displacement at the same angle means an increase in muscle extensibility
202 (Nakamura et al., 2012). Therefore, this result suggests that SS for more than 2 min is

203 effective for increasing muscle extensibility. In addition, gradual changes in both
204 passive torque and MTJ displacement were observed during 5 min of SS. A significant
205 difference was observed in the two parameters between 2 min of SS and 5 min of SS.
206 The results of this study suggest that SS for 5 min may be more effective in decreasing
207 MTU resistance and increasing muscle extensibility compared with SS for 2 min.

208 With regard to the relationship between passive torque and MTJ displacement,
209 there were strong negative correlations between passive torque and MTJ displacement
210 for each subject ($r = -0.886$ to -0.991 , $P < 0.05$). In addition, a significant correlation
211 was found between the rate of change in passive torque and that in MTJ displacement (r
212 $= -0.708$, $P < 0.01$). This is the first report regarding the relationship between passive
213 torque and MTJ displacement, and these results suggest that a increase of muscle
214 extensibility contributes to a decrease of MTU resistance, which is consistent with the
215 results of previous studies (Kay & Blazevich, 2009 b; Mizuno et al., 2011; Morse et al.,
216 2008; Nakamura et al., 2011).

217 Regarding the mechanism of increased muscle extensibility, viscoelastic
218 materials cause plastic deformation due to external stress. This feature is called stress
219 relaxation, which refers to the decline in external stress over time when the MTU is
220 lengthened for a prolonged duration. Viscoelastic stress relaxation of muscles or tendons

221 has been demonstrated in vitro (Sanjeevi, 1982; Taylor et al., 1982; Taylor et al., 1990)
222 and in vivo (Duong et al., 2001; Fowles et al., 2000; Magnusson et al., 1995;
223 Magnusson et al., 1996c; McHugh et al., 1992; McNair et al., 2001; Toft et al., 1989b)
224 in the MTU of the hamstring and gastrocnemius. In this study, gradual changes in both
225 passive torque and MTJ displacement were observed, and a significant correlation was
226 observed between passive torque and MTJ displacement during 5 min of SS. Therefore,
227 viscoelastic stress relaxation of the gastrocnemius muscle may have contributed to
228 increase muscle extensibility, which may have decreased MTU resistance after SS.

229 In this study, the time course of changes in passive properties of the
230 gastrocnemius MTU were investigated during 5 min of SS. Recent studies reported that
231 5 min of SS increased MTU and muscle flexibility, and that this effect was maintained
232 for 10 min after SS (Nakamura et al., 2011). However, the effect disappeared 15 min
233 after SS (Mizuno et al., 2011). The changes in MTU properties observed in this study
234 may also be reversible. In a study examining ROM and muscle fascicle length,
235 O'Sullivan et al. (2012) reported that eccentric training was effective in increasing
236 flexibility. Incorporating other exercise regimens, for example, eccentric, concentric,
237 and isometric contractions with SS may be worthy to study.

238

239 Strengths and limitations

240 This study revealed the minimum amount of time required for SS to decrease MTU
241 resistance and increase muscle extensibility. These data may be effectively used in
242 clinical situations and athletic settings. However, some limitations of this study must be
243 noted. First, the assessor who analyzed MTJ displacement was not blinded to the time
244 of image procurement. Second, the subjects in this study were healthy young men.
245 Similar acute effects of SS cannot always be expected in elderly individuals and patients
246 with limited ROM or contracture. In addition, because differences at 1 min may be
247 statistically significant in a larger sample, future studies should consider including
248 larger samples to confirm this possibility. Third, this study examined the minimum
249 amount of time required for SS to decrease MTU resistance and increase muscle
250 extensibility in only the gastrocnemius muscle; other human muscles needs to be
251 investigated in the future. In addition, further research is required to clarify the
252 minimum amount of time required for SS to increase the extensibility of the
253 gastrocnemius muscle and other muscles in elderly individuals and patients with limited
254 ROM or contracture.

255

256 Conclusions

257 In this study, gradual changes in both passive torque and MTJ displacement reached the
258 significance level after 2 min. Therefore, SS for more than 2 min is recommended to
259 decrease MTU resistance and increase the degree of muscle extensibility.

260

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- 384

385 Table 1. Passive torque and MTJ displacement during 5 min of static stretching

	Passive torque (Nm)	MTJ displacement (cm)
before SS	49.4 ± 2.9 (43.7 to 55.2)	0.90 ± 0.06 (0.79 to 1.01)
1 minute	42.9 ± 2.6 (37.9 to 47.9)	1.13 ± 0.07 (1.01 to 1.26)
2 minutes	37.5 ± 2.4 (32.7 to 42.3)**	1.21 ± 0.07 (1.07 to 1.35)*
3 minutes	36.8 ± 2.4 (32.0 to 41.6)**	1.25 ± 0.07 (1.10 to 1.39)**
4 minutes	35.9 ± 2.4 (31.1 to 40.7)** ##	1.30 ± 0.07 (1.15 to 1.45)** ##
5 minutes	33.3 ± 2.3 (28.8 to 37.9)** ## \$\$	1.35 ± 0.08 (1.20 to 1.50)** ## \$\$†

386

387 Data are means ± SEM (95% confidence intervals). * $P < 0.05$, ** $P < 0.01$; significant388 difference from values before SS. # $P < 0.05$, ## $P < 0.01$; significant difference from389 1-min values. \$\$ $P < 0.01$; significant difference from 2-min values. † $P < 0.05$;

390 significant difference from 3-min values. SS: static stretching; MTJ: myotendinous

391 junction.

392

393 Figure 1

394 Ultrasound image showing measurements taken to determine MTJ displacement from
395 0° to the dorsiflexion ROM. An acoustically reflective marker (X) is placed between the
396 skin and the ultrasonic probe to ensure probe stability during measurement. The
397 distance between X and MTJ (MTJ displacement) is measured every 1 min during 5
398 min of SS. MTJ: myotendinous junction; ROM: range of motion; GM: gastrocnemius
399 muscle.

400 Figure 2

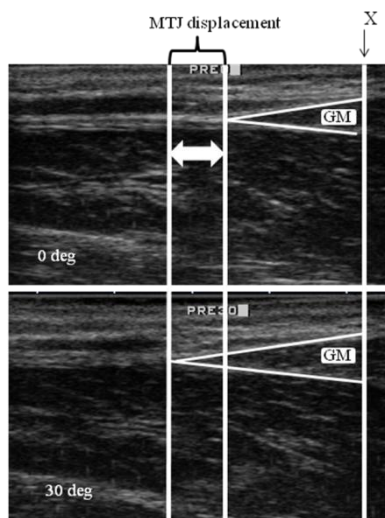
401 A typical example of the relationship between passive torque and MTJ displacement
402 during 5 min of SS. The line is drawn by linear regression ($r = -0.972$, $P < 0.01$).

403 Figure 3

404 Correlation coefficient between the rate of change in passive torque and that in MTJ
405 displacement. The line is drawn by linear regression ($r = -0.708$, $P < 0.01$).

406

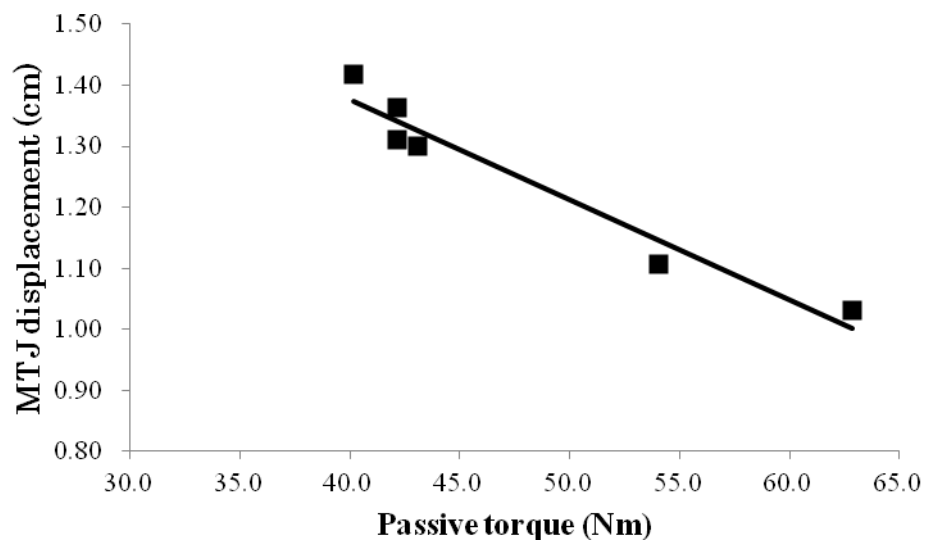
407 Figure 1



408

409

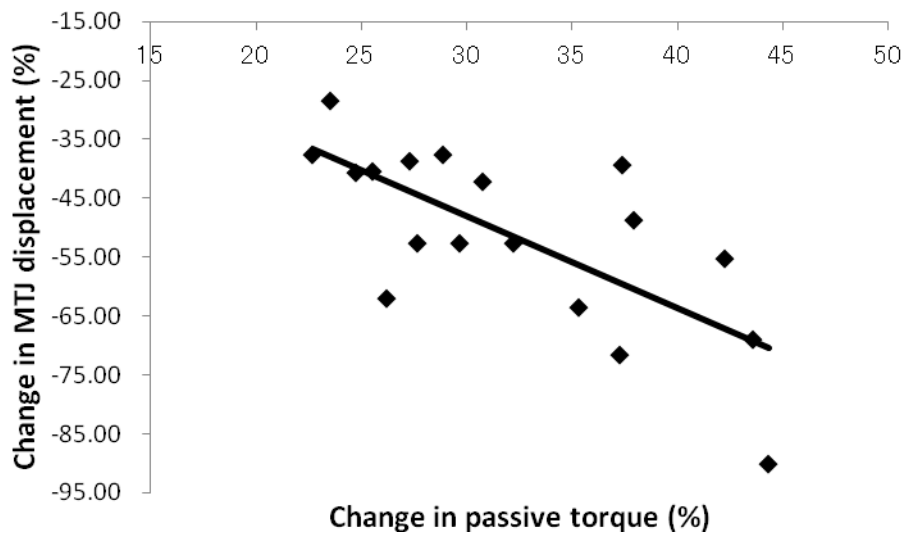
410 Figure 2



411

412

413 Figure 3



414

415