

Title	Changes in Passive Properties of the Gastrocnemius Muscle-Tendon Unit During a 4-Week Routine Static Stretching Program.
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- 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 ± 2.3 years; height
14 172.0 ± 4.3 cm; body mass 63.1 ± 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^{1, 2} and
33 following routine SS³⁻⁵. 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^{6, 7}. 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^{8, 9}.

38 Previous studies evaluating acute effect of SS reported that a 3- to 5-min
39 duration decreased MTU and muscle stiffness⁹⁻¹³. 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 min¹⁴. We recently reported that
43 decreased MTU and muscle stiffness were maintained for 10 min following a 5-min
44 constant-angle SS session¹², which is consistent with a prior study¹⁴. 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 program¹⁵⁻¹⁸. In addition, stretch tolerance changed after a 2- to
55 6-week routine SS program¹⁹⁻²¹. We reported that muscle stiffness decreased after 4
56 weeks of routine SS²². However, the time course of changes in muscle stiffness and
57 stretch tolerance immediately following SS were discordant⁹; 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 tolerance⁹. 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 session²². 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 ± 2.3 years; height 172.0 ± 4.3 cm; body mass 63.1 ± 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^{9, 11, 12, 14}. 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 tolerance⁹.

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 displacement¹².

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 bellies¹³.

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 stiffness²³. 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 weekly⁵. 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 ± 1.1 years; height, 172.7 ± 4.9 cm; body mass, 65.2 ± 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^{9, 10, 12, 14}, 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 studies³⁻⁵. 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 thresholds²⁴⁻²⁶.

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^{11, 12, 22}. 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 response¹⁴. 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^{27, 28}; 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^{27, 28}. 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.

270

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.

280

281

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284

285

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388

389

390 Table 1. Subject demographics.

391 ^aSS 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 ^a (N = 12)	control group (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

404

	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

405

406

	DF ROM (°)		Passive Torque at DF ROM (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>$\eta_p^2 = 0.483$</u>		<u>$\eta_p^2 = 0.211$</u>		<u>$\eta_p^2 = 0.334$</u>	

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