

Effect of static stretching with different rest intervals on muscle stiffness

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19 **ABSTRACT (244/250 words)**

20 The aim of the study was to investigate the effect of static stretching (SS) with different rest intervals on muscle
21 stiffness. Fifteen healthy males participated in the study. Four bouts of thirty-second SS for the gastrocnemii were
22 performed at the maximal dorsiflexion using dynamometer with two different rest intervals between stretches,
23 namely 0 s (R0) and 30 s (R30). Each participant underwent both stretching protocols at least 48 hours apart in a
24 random order. Between each bout of SS, the ankle was moved to 20°-plantar-flexion in 3 s, held for each rest
25 interval time, and then returned to the stretching position in 3 s. The shear elastic modulus of the medial
26 gastrocnemius was measured before (PRE) and immediately after (POST) four bouts of SS to assess muscle
27 stiffness of the medial gastrocnemius. Two-way repeated measures analysis of variance (protocol× time) indicated
28 a significant interaction effect on the shear elastic modulus. The shear elastic modulus significantly decreased
29 after SS in both protocols [R0, PRE: 11.5 ± 3.3 kPa, POST: 10.0 ± 2.6 kPa, amount of change: 1.6 ± 0.9 kPa (13.0
30 ± 5.2 %); R30, PRE: 11.0 ± 2.8 kPa, POST: 10.2 ± 2.1 kPa, amount of change: 0.8 ± 1.3 kPa (6.0 ± 10.4 %)].
31 Furthermore, the SS with 0-s rest interval induced greater decrease in shear elastic modulus when compared to SS
32 with 30-s rest interval ($p = 0.023$). Thus, when performing SS to decrease muscle stiffness, rest intervals between
33 stretches should be minimized.

34

35 **1. Introduction**

36 Static stretching (SS) is an effective intervention to decrease the stiffness of a muscle or muscle-tendon unit (MTU)
37 and to improve the joint range of motion (ROM) (Kay et al., 2015; Konrad et al., 2017; Nakamura et al., 2011).
38 Since increased stiffness is considered a risk factor of musculoskeletal injuries, SS is often performed prior to
39 performance to prevent injuries (Herbert et al., 2011; McHugh and Cosgrave, 2010). Therefore, investigating the
40 acute effects of SS is important. With respect to an appropriate SS time to decrease MTU stiffness, a previous study
41 demonstrated that at least 2 min of SS was required to decrease the passive torque of gastrocnemii (Nakamura et al.,
42 2013). In clinical situations, SS is typically divided into multiple repetitions as opposed to being performed
43 continuously for a few minutes (Baechle, 1994), presumably to its ease to perform for therapists and high
44 compliance for patients. When SS is divided into multiple repetitions, it is necessary to consider the total stretching
45 time (i.e., SS time per repetition and number of repetitions) and rest interval time between repetitions. With respect
46 to the total time, a previous study indicated that SS for a constant total time with different time per repetition and
47 number of repetitions (i.e., 60 s \times 2 times, 30 s \times 4 times, and 10 s \times 12 times) causes similar effects corresponding
48 to decreases in stiffness of the gastrocnemii muscles (Nakamura et al., 2017). Conversely, few studies investigated
49 the influence of rest interval time between repetitions. Freitas et al. (2015) compared the improvement in ROM
50 between SS with and without rest interval, and concluded that SS without rest interval was more effective in terms
51 of improving ROM. However, the results of the aforementioned study indicated that the effects of ROM
52 improvement were different based on number of repetitions because the number of repetitions (i.e., total time) was

53 not consistent among participants. Therefore, it is important to investigate the influence of rest interval time between
54 repetitions while holding the total time of SS as a constant.

55 Increased stiffness of the muscle or MTU has been reported to increase the risk of musculoskeletal injuries
56 (Watsford et al., 2010). It is noted that ROM is insufficient to assess passive mechanical properties because it is also
57 affected by pain and stretch tolerance (Weppeler and Magnusson, 2010). A few studies (Halbertsma and Göeken,
58 1994; Magnusson et al., 1996; McNair et al., 2001) have indicated that the increase in ROM induced by SS might
59 be due only to a change in stretch tolerance without change in passive mechanical properties (so-called ‘Sensory
60 Theory’ reviewed by Weppeler and Magnusson, 2010). Since SS is performed to change not only ROM but also the
61 passive mechanical properties of a muscle or MTU, the effects of SS on passive mechanical properties should be
62 distinguished from stretch tolerance. Hence, passive joint stiffness (which is determined as a slope of torque-angle
63 relationship (Magnusson et al., 1996)) and muscle stiffness (which is represented by shear elastic modulus) are used
64 as indices of stretching effects to assess passive mechanical properties. Passive joint stiffness reflects several factors
65 in addition to muscle stiffness such as the stiffness of joint capsules and ligaments (Maïsetti et al., 2012). Shear
66 elastic modulus measured via ultrasound shear wave elastography (SWE) non-invasively makes it possible to
67 quantitatively assess the muscle stiffness of an individual muscle. Therefore, shear elastic modulus is often used as
68 an index of stretching effect for several skeletal muscles (Ichihashi et al., 2016; Kusano et al., 2017; Xu et al., 2018).
69 Thus, we focused on the stiffness of individual muscles among the passive mechanical properties.

70 The aim of the present study involves investigating the effect of SS with different rest intervals on muscle

71 stiffness of the medial gastrocnemius. Our hypothesis is that SS with shorter rest intervals leads to a greater decrease
72 in muscle stiffness.

73

74 **2. Methods**

75 2.1. Participants

76 Fifteen healthy men (height, 171.4 ± 6.2 cm; mass, 66.7 ± 9.2 kg; age, 24.3 ± 3.0 years) participated in the study.

77 The sample size required for a two-way repeated measures analysis of variance (ANOVA) [effect size = 0.40 (large),

78 α error = 0.05, power = 0.80] was calculated in advance via G*power software (version 3.1.; Heinrich Heine

79 University, Düsseldorf, Germany), and the calculated sample size corresponded to 14. The effect size was

80 determined based on a previous study, which showed the effects of SS on muscle stiffness using two-way analysis

81 of variance (Akagi and Takahashi, 2014). All participants received an explanation about the study and provided

82 written informed consent. The study was approved by the ethics committee of Kyoto University Graduate School

83 and the Faculty of Medicine (R0233-3).

84

85 2.2. Experimental protocol

86 The experimental design was a cross-over design wherein each participant underwent both stretching protocols at

87 least 48 hours apart in a random order. The participants were instructed to maintain their regular physical activities,

88 avoiding unusual exercise between the two sessions. Thirty-second SS for the triceps surae, especially the

89 gastrocnemii in the right leg was repeated for four bouts in the following two protocols: 0-s rest interval (R0) and

90 30-s rest interval (R30) between each bout of SS. The shear elastic modulus of the medial gastrocnemius (MG) was
91 measured before (PRE) and immediately after (POST) four bouts of SS.

92 The participants lay prone on a dynamometer (BIODEX System 4, Biodex, USA) with the hip in a neutral
93 position (without any flexion/extension, adduction/abduction, or internal/external rotation) and the knee fully
94 extended, and the foot was attached securely to the footplate of the dynamometer (Fig.1a, b). To define the final
95 angle, the ankle was passively dorsiflexed at 5°/s starting from 30°-plantar-flexion to the maximal dorsiflexion angle
96 (Fig. 1b) that the participants achieved without discomfort or pain (Nakamura et al., 2014). The participants
97 themselves stopped the dynamometer via a remote button. The maximal dorsiflexion angle was defined as the final
98 angle in the study and used for all four bouts of SS.

99 Surface electromyography (EMG) (TeleMyo2400, Noraxon USA, Scottsdale, AZ, USA) on the lateral
100 gastrocnemius muscle belly was used to ensure that the muscle was inactive during SS and measurements of shear
101 elastic modulus. EMG data was calculated using full-wave rectification and the root-mean-square, with a window
102 interval of 50 ms. Then, the EMG activities during SWE measurements and SS were represented as a percentage of
103 the maximal EMG values during maximal voluntary contraction, which was performed after all other protocols.

104

105 2.3. Measurement of shear elastic modulus

106 The shear elastic modulus of MG was measured to assess muscle stiffness via an ultrasound SWE (Aixplorer,
107 SuperSonic Imagine, France) with a linear probe (4-15 MHz, SuperLinear 15-4, France) in Musculoskeletal (MSK)

108 preset. The measurements were performed in a neutral ankle position (0° plantar-flexion). The measurement site
109 was defined at a level corresponding to proximal 30% of the lower leg length from the popliteal crease to the lateral
110 malleolus in accordance with previous studies (Akagi and Takahashi, 2013; Nakamura et al., 2014). After
111 identifying MG on the ultrasound B-mode image, the measurement site was marked on the skin with a pen to ensure
112 that PRE and POST measurements are performed on the same site. The orientation of the probe was adjusted to the
113 longitudinal plane so that the muscle fascicles were clearly identified on the B-mode image (Hirata et al., 2015;
114 Maïsetti et al., 2012). The region of interest (ROI), 2.25 cm width × 1.75 cm depth, was set near the center of muscle
115 belly bulge of MG, and the image was then obtained. Two images were obtained at each PRE and POST time point.
116 The location of the ROI was kept constant for each participant. The probe was repositioned between capturing two
117 images at each time point. SWE measurements were completed within 1 min. SWE measurements were performed
118 by the same investigator for all participants.

119 After obtaining the images, a circle with a diameter of 10 mm was drawn at the center of ROI for
120 quantitative analysis (Fig.2). The shear elastic modulus value in the circle was calculated. The shear elastic modulus
121 value (G) is calculated from shear wave speed (V) using the following equation (Genisson et al., 2010).

$$122 \quad G \text{ (kPa)} = \rho V^2$$

123 where ρ is the muscle mass density (1000 kg/m³), and high values indicate high muscle stiffness (Koo et al., 2013).

124 The mean value of two images at each time was used in the following analysis.

125 To evaluate intra-rater reliability of measurements, the intraclass correlation coefficient (1,2) (ICC_{1,2}) with

126 95% confidence interval (CI) was calculated from the shear elastic modulus of the two measurements at each time
127 point. ICC_{1,2} values were 0.985 (95% CI: 0.956-0.995) and 0.970 (95% CI: 0.912-0.990) at PRE and POST,
128 respectively; therefore, good reliability was observed (Portney and Watkins, 2000).

129

130 2.4. Static stretching

131 The participants received SS in a prone position with the hip in a neutral position and the knee fully extended. The
132 final angle was maintained for 30 s and SS was repeated at this angle for four bouts, corresponding to a total of 2-
133 min SS. Immediately after each bout of SS, the ankle was manually moved to 20° plantar-flexion, at which the
134 passive force of triceps surae would be almost zero (Hirata et al., 2015), in 3 s. In the R0 protocol, the ankle was
135 immediately returned to the final angle in 3 s without being held at 20° plantar-flexion. In the R30 protocol, the
136 ankle was held at 20° plantar-flexion for 30 s and then returned to the final angle in 3 s (Fig.3). The investigator
137 carefully monitored these movements with a stopwatch to ensure that the ankle was moved in 3 s at each repetition.

138

139 2.5. Statistical analysis

140 Statistical analysis was performed via SPSS Statistics (version 22; IBM, Armonk, NY, USA). After confirming the
141 normal distribution of each variable via the Shapiro–Wilk test, the following analyses were performed. The final
142 angle, which was defined before each SS protocol, was compared via a paired t-test between the two protocols to
143 examine whether the angle at which SS was performed differed between protocols.

144 With respect to the shear elastic modulus values, a two-way repeated measures ANOVA based on two
145 factors [protocol (R0, R30) \times time (PRE, POST)] was performed. When a significant interaction effect was obtained,
146 a post-hoc test (paired t-test) was performed to examine the simple main effect of time. In addition, the change and
147 the percentage change in shear elastic modulus were calculated as follows:

148 change in shear elastic modulus = PRE-value – POST-value

149 percentage change in shear elastic modulus = (change in shear elastic modulus/PRE-value) \times 100

150 The change and the percentage change in shear elastic modulus were compared between two protocols via a paired
151 t-test. The statistical significance was set at 5%.

152

153 **3. Results**

154 The EMG activities of lateral gastrocnemius were $< 5\%$ of maximal voluntary contraction in all participants,
155 indicating that the muscle was almost inactive during SS and SWE measurements. The final angle corresponded to
156 $37.7 \pm 7.5^\circ$ in R0 and $39.2 \pm 6.9^\circ$ in R30. The results of a paired t-test did not indicate a significant difference
157 between protocols ($p = 0.289$), indicating that SS was performed at almost the same angle in the two protocols.

158 The shear elastic modulus values are listed in Table 1. The result of a two-way repeated measures ANOVA
159 indicated a significant interaction effect ($p = 0.023$, $F = 6.56$, effect size = 0.319). Post-hoc paired t-tests comparing
160 PRE and POST yielded uncorrected p-value of < 0.001 and 0.029 for R0 and R30 protocols, respectively.
161 Additionally, both the change and the percentage change in shear elastic modulus in R0 exceeded that in R30 ($p =$

162 0.023 and 0.031, respectively).

163

164 **4. Discussion**

165 In this study we investigated the effect of four bouts of 30-s SS with different rest intervals (0 s, 30 s) on muscle
166 stiffness of MG, and the results indicated that the decrease in muscle stiffness observed after SS with 0-s rest interval
167 exceeded the decrease in stiffness after SS with 30-s of rest between stretching intervals. To the best of our
168 knowledge, this is the first study that investigated the effect of rest interval duration between SS repetitions on
169 muscle stiffness given a constant total stretching time. In addition, the muscle stiffness of MG decreased after both
170 stretching protocols. This result is consistent with previous studies (Akagi and Takahashi, 2013; Kay et al., 2015).

171 The reason as to why the decrease in muscle stiffness was smaller in R30 when compared to that in R0 is
172 potentially related to the degree of recovery in muscle stiffness during rest interval between each bout of SS. Stress
173 relaxation is reported as a phenomena caused by SS, which corresponds to a gradual decline in the passive force on
174 the muscle during stretching at a constant angle (Taylor et al., 1990). In a manner similar to the gradual declines in
175 passive force during SS, it is known that the muscle force also gradually recovers after the muscle is released from
176 stretching (Duong et al., 2001). Duong et al., (2001) reported that the decrease in force due to 20-min SS recovered
177 2 min after SS by approximately 43%. Another study reported that the decrease in MTU stiffness due to 5 bouts of
178 1-min SS returned to the baseline within 10 min after SS (Mizuno et al., 2013). In this study, SS with longer rest
179 interval, such as that in R30, resulted in a smaller effect on the decrease in muscle stiffness owing to a certain

180 amount of recovery between SS repetitions, although this recovery did not cancel the SS effect of each repetition.

181 There are a few limitations in the study. First, the study only investigated the acute effect of SS, and thus
182 the prolonged or long-term effect is unclear. Second, all the participants were healthy young men. Future studies
183 should investigate the influence of rest interval time in SS for different populations and/or in long-term intervention.
184 Third, the shear elastic modulus was measured on one specific point of MG; therefore, these findings may not
185 necessarily apply to the whole MG. Moreover, we focused only on muscle stiffness and not tendon stiffness. Finally,
186 the effects on ROM or passive torque, that is, MTU stiffness remain unclear. This is because it was not possible to
187 measure them immediately after SS owing to the time spent resetting the experimental setup.

188 In conclusion, the study investigated the effect of four bouts of 30-s SS with different rest intervals,
189 namely 0 s and 30 s, on muscle stiffness of the medial gastrocnemius. The results indicated that SS with 0-s rest
190 interval decreased muscle stiffness to a greater extent although SS with 30-s rest interval also decreased muscle
191 stiffness. The results indicated that SS with a shorter rest interval may be more effective in decreasing muscle
192 stiffness. Clinically, SS with shorter rest intervals could be recommended to decrease muscle stiffness from the
193 viewpoint of injury prevention. Future studies are needed to investigate in greater detail the effect of rest interval
194 duration between stretching repetitions to support our findings.

195

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199

200 **Conflict of interest statement**

201 The authors declare that they have no conflict of interest.

202

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- 277

278 **Table**

279 Table 1. Shear elastic modulus values of the medial gastrocnemius in each protocol

	Shear elastic modulus (kPa)			Change in shear elastic modulus	Percentage change in shear elastic modulus (%)	Interaction effect
	PRE	POST	p-value of post-hoc test			
R0	11.5 ± 3.3	10.0 ± 2.6 **	p < 0.001	1.6 ± 0.9 †	13.0 ± 5.2 †	p = 0.023, F = 6.559 effect size = 0.319
R30	11.0 ± 2.8	10.2 ± 2.1 *	p = 0.029	0.8 ± 1.3	6.0 ± 10.4	

Values are expressed as mean ± standard deviation

PRE: before SS, POST: immediately after SS

R0: 0-s rest interval, R30: 30-s rest interval

Change in shear elastic modulus = PRE-value – POST-value

Percentage change in shear elastic modulus = (change in shear elastic modulus/PRE-value) × 100

* significant difference between time points (p < 0.05)

** significant difference between time points (p < 0.01)

† significant difference between protocols (p < 0.05)

281 **Figure captions**

282 **Fig.1** Experimental setup

283 (a) Participant is in a prone position with the hip in neutral position and the knee fully extended. In determining the
284 final angle, the participants pressed the remote button to stop the dynamometer.

285 (b) The foot on the right is attached securely to the footplate of the dynamometer. The axis of the ankle joint
286 corresponds to the axis of the dynamometer rotation.

287

288 **Fig.2** Typical example of shear wave elastography image

289 The region of interest (ROI) was set near the center of the muscle belly bulge of the medial gastrocnemius. A 10
290 mm circle was drawn at the center of the ROI. Shear elastic modulus was calculated from the mean shear wave
291 speed in the circle.

292

293 **Fig.3** Experimental protocol of stretching

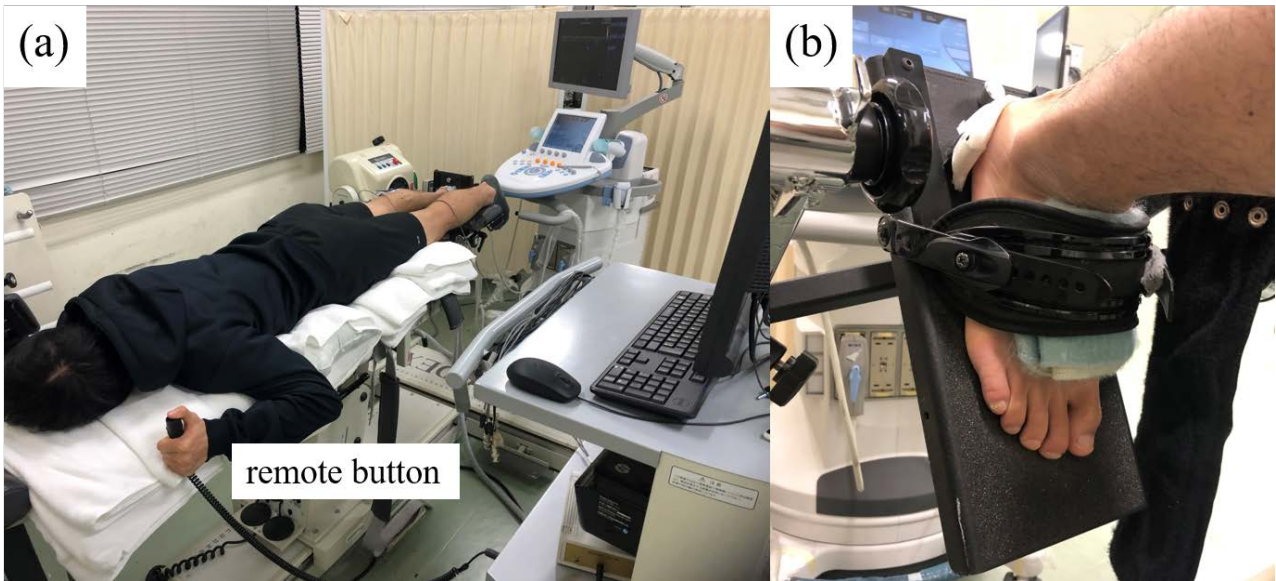
294 Maximal dorsiflexion angle was defined prior to the stretching.

295 Static stretching for 30 s at the final angle was repeated for four bouts.

296 PF: plantar-flexion

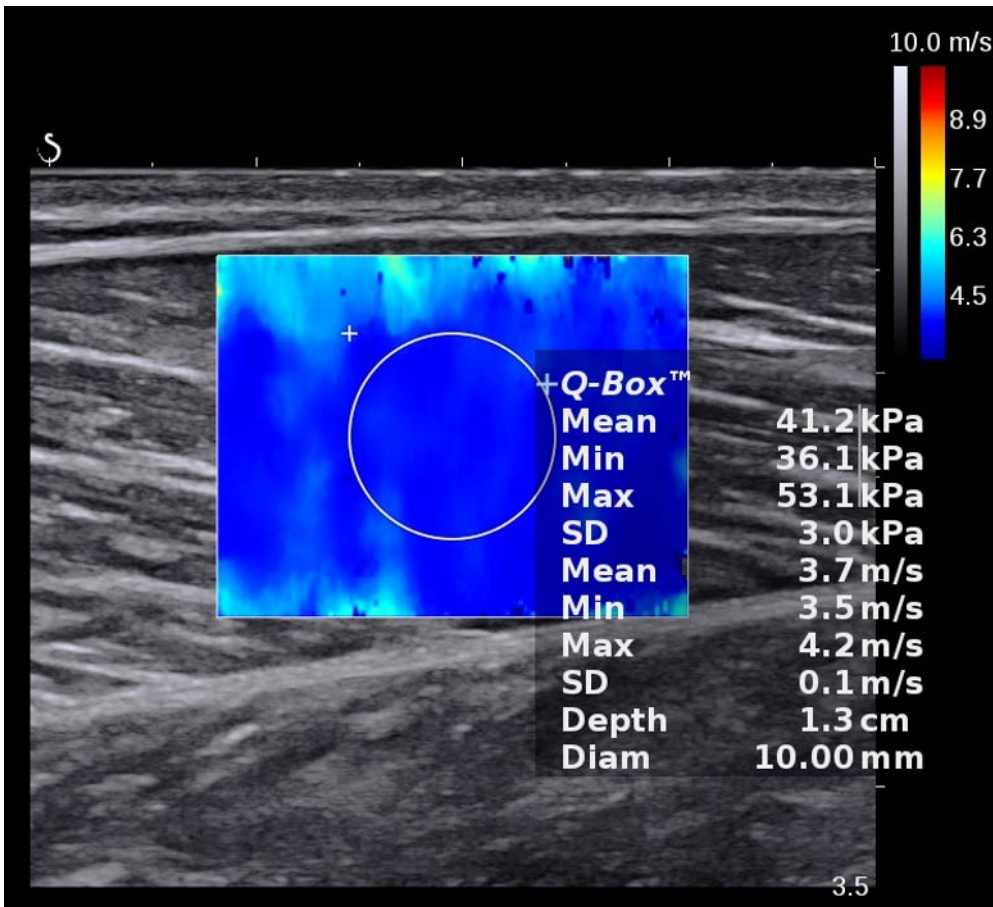
297

298 Fig.1



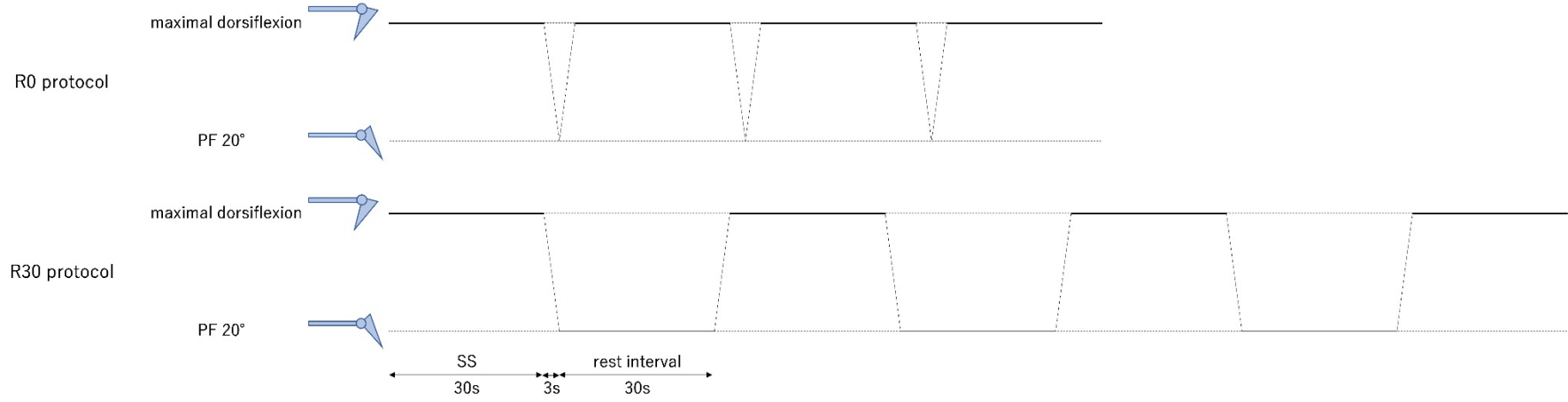
299

300 Fig.2



301

302 Fig.3



303