

Selective static stretching of rectus femoris alters motor unit firing behaviors of knee extensors

Running title

Motor unit behaviors after selective stretching

Authors

Tetsuya Hirono¹, Masahide Yagi¹, Zimin Wang^{1,2}, Haruka Sakata¹, Shogo Okada^{1,2}, Kaede Nakazato^{1,2}, Noriaki Ichihashi¹, Kohei Watanabe³

Affiliations

1. Human Health Sciences, Graduate School of Medicine, Kyoto University,
53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
2. Research Fellow of Japan Society for the Promotion of Science,
5-3-1 Kojimachi, Chiyoda-ku, Tokyo 102-0083, Japan
3. Laboratory of Neuromuscular Biomechanics, School of Health and Sport Sciences,
Chukyo University, 101 Tokodachi, Kaizu-cho, Toyota, Aichi, Japan

Corresponding author

Tetsuya Hirono, Ph.D., R.P.T.

Human Health Sciences, Graduate School of Medicine, Kyoto University,
53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Email: hirono.tetsuya.4r@kyoto-u.ac.jp

ORCID: 0000-0002-4337-6249

26 **Abstract**

27 Static stretching immediately affects various neuromusculoskeletal components. Among
28 quadriceps muscles, only the rectus femoris is stretched by hip extension and knee flexion.
29 The aim of this study was to investigate the motor unit (MU) firing behaviors of two synergistic
30 muscles after selective static stretching on only one side. Fourteen males (23.7 ± 2.4 yrs)
31 performed knee extension tasks before and after intervention: static stretching or control
32 conditions. The static stretching protocol consisted of passive hip joint extension and knee
33 joint flexion as selective stretching of the rectus femoris (RF) for 1 minute, repeated for 6 sets,
34 while 6-minute rest was applied as the control condition. MU firings of RF and the vastus
35 lateralis (VL) were detected using high-density surface electromyography, and the MU
36 recruitment threshold and firing rate were calculated during ramp-up contraction to 35% of
37 maximal voluntary contraction. RF stiffness, evaluated by shear wave elastography, was
38 significantly reduced after static stretching, and not VL stiffness (interaction: $p=0.037$). There
39 were main effects of time, but no interaction in the MU recruitment threshold of RF ($p=0.282$),
40 firing rates of either muscle (RF: $p=0.363$, VL: $p=0.557$), or maximal strength ($p = 0.362$),
41 suggesting that these variables were changed after both conditions. However, an interaction
42 was noted in the recruitment threshold of VL ($p = 0.018$), indicating that the decline in
43 recruitment threshold of VL was greater in the static stretching (from 24.9 ± 6.3 to
44 21.7 ± 6.1 %MVC) than control condition (from 24.4 ± 6.7 to 22.3 ± 6.1 %MVC). These findings
45 suggest that reducing muscle stiffness by static stretching in one muscle can enhance MU

46 recruitment in the other synergist while the change was slightly small.

47 **Keywords**

48 Discharge rate, High-density surface electromyography, Muscle stretching, Quadriceps

49 femoris, Shear wave elastography

50

51

Introduction

Static stretching (SS) is often performed as a warm-up exercise and conditioning muscular performance. The aim of SS is to increase flexibility of the muscle-tendon unit (Witvrouw et al., 2004) and prevent muscle strain injuries (Shehab et al., 2006; Takeuchi et al., 2024). SS can alter neural (Budini & Tilp, 2016; Mazzo et al., 2021; Trajano et al., 2020) as well as muscle (Behm et al., 2016) properties. Specifically, the prolonged static stretching can immediately reduce maximum voluntary contraction (MVC) strength (Behm et al., 2016; Kay & Blazevich, 2012) which may be caused by a decrease in muscle stiffness (Fowles et al., 2000) and/or modulation of the nervous system (Trajano et al., 2017; Trajano et al., 2013).

A previous study (Mazzo et al., 2021) investigated motor unit (MU) behaviors immediately after SS of the triceps surae using high-density surface electromyography (HDsEMG) and decomposition methods. Firing rates of the soleus and medial and lateral gastrocnemius significantly increased after repeated SS of the calf muscle-tendon unit, and the recruitment threshold significantly decreased (Mazzo et al., 2021). The results suggest that the modulation of MU firing behaviors could compensate for a reduction in the force-generating and transmission capabilities of the muscle–tendon unit. Another research that investigated MU behaviors immediately after SS of triceps surae reported that SS increased the firing rate and reduced motoneuronal persistent inward currents in soleus, which are facilitated by

neuromodulatory inputs, but the effects were not observed in gastrocnemius medialis (Trajano et al., 2020). Considering that the effect of SS can be different among synergistic muscles because they have different architecture which influences the adaptability of elastic properties (Fukaya et al., 2024), the changes in neuromuscular activations may be dependent on the changes in elastic properties of individual muscles. However, the previous studies did not evaluate the different effects of SS, such as changes in each muscle elastic property, on individual synergistic muscles. Synergistic muscles show characteristic neuromuscular activation, i.e., alternative muscle activation during sustained low-force contraction (Kouzaki et al., 2004), and compensation for fatigued muscles by unfatigued muscles (Akima et al., 2012; Kouzaki & Shinohara, 2006). Thus, we may consider that MU firing behaviors of synergistic muscles are controlled differently when the mechanical property of one of them is modulated. In the case where one muscle is stretched, the neuromuscular activation of other muscles may be adjusted to maintain constant force production and compensate for the reduced capacity of the subjected muscle. A decline of recruitment threshold and an increase in firing rate may also be observed in the motor units of non-stretched muscle, however, it remains unclear whether MU firing behaviors differ between synergistic muscles in the presence/absence of SS.

Ultrasound shear wave elastography may be useful for assessing the effects of SS on synergistic muscles selectively. Focusing on quadriceps femoris muscles, the rectus femoris

(RF) is a unique bi-articular muscle crossing the hip and knee joints. Passive SS consisting of hip joint extension and knee joint flexion reduces the stiffness of only RF, and not the vastus medialis or lateralis (VL) (Nakamura et al., 2020). The purpose of the present study was to examine MU firing modulation of RF and VL immediately after selective SS for RF. It was hypothesized that a decline in the MU recruitment threshold and/or an increase in the MU firing rate of RF or VL would occur in order to compensate for reduced RF stiffness

Materials and Methods

Participants

Fourteen young healthy male adults (23.7 ± 2.4 years, 168.5 ± 2.9 cm, and 62.1 ± 7.5 kg) were enrolled. Their activity levels were sedentary to recreationally active but did not have a habit of stretching muscles. They did not have any physical problems, such as neurological or musculoskeletal disorders. After the purpose and procedures were explained, participants provided informed written consent to participate in this study. The study protocol was approved by a local ethics committee (R3944) and conducted in accordance with the Declaration of Helsinki.

Experimental design

A crossover design was employed in the present study. The participants visited the laboratory twice, with an interval of more than 7 days. Intervention of SS and the control condition were administered in random order. The time-course is presented in Figure 1. All measurements were conducted involving the right thigh. The participants were seated on a chair with attached dynamometer and performed several submaximal knee extension isometric contractions as a warm up. The limbs and joints were in precisely the same position between the two sessions which was verified by checking the dynamometer position and knee angle via electro-goniometer, which was followed by the knee extension tasks, consisting of maximal voluntary isometric and submaximal ramp-up contractions. During the contraction tasks, HDsEMG was recorded from RF and VL. Subsequently, ultrasound shear wave elastography was measured to evaluate RF and VL stiffness. Immediately after the intervention, ultrasound shear wave elastography, submaximal contraction tasks with HDsEMG, and MVC were measured again.

Knee extension task

The participants were seated on a chair with a dynamometer (Biodex system 4; Biodex Medical Systems, Inc., Shirley, NY, USA), while their trunk and pelvis were fixed using non-elastic belts,

132 maintaining hip and knee joint flexion at 80 and 90 degrees, respectively. The dynamometer
133 axis was aligned to the knee axis followed by submaximal isometric knee extensions as a
134 warmup. The participants then performed two MVCs of knee extension at 90 degrees of
135 knee flexion, with 90-seconds of rest in-between. The highest value of torque was
136 determined as the MVC torque, which was used to scale submaximal isometric ramp-up
137 contraction tasks.

138

139 After each participant's thigh was shaved and the skin over RF and VL was cleaned with
140 alcohol or water, grid electrodes were attached to both muscle bellies. A semi-disposable
141 adhesive grid of 64 electrodes (13 rows and 5 columns, with one missing electrode in the
142 corner) with a 1-mm diameter and an 8-mm inter-electrode distance (GR08MM1305, OT
143 Bioelettronica, Turin, Italy) and conductive paste (AC Cream, Spes Medica, Italy) were used
144 to record HDsEMG from RF and VL. The centers of grids were placed at the 2/3 point from
145 the anterior superior iliac spine and superior edge of the patella for RF and at the 2/3 point
146 from the greater trochanter and superior and lateral edges of the patella for VL. The long
147 axis direction of the grid followed the muscle fibre direction, and a reference electrode was
148 placed on the tibial tuberosity (WS2.1, OT Bioelettronica, Turin, Italy). Based on the MVC
149 evaluated before interventions, participants performed submaximal ramp-up contractions to
150 35% of MVC, consisting of 17.5-second increasing and 13-second sustained phases, and 70%
151 of MVC, consisting of 14-second increasing and 5-second sustained phases. Monopolar

surface EMG signals were bandpass filtered from 10 to 500 Hz, amplified by a factor of 256, sampled at 2,000 Hz, and converted to digital form by a 16-bit analog-to-digital converter (Sessantaquattro, OT Bioelettronica, Turin, Italy) and software (OTBioLab+ v.1.5.5.0). The torque signal from the dynamometer was synchronized with HDsEMG signals on this analog-to-digital converter. The exerted and target torques were shown on a monitor in real-time as visual feedback. To obtain the HDsEMG from the two muscles, ramp-up contractions at each intensity were performed twice for RF and VL because the device can collect data from only one muscle during a task.

Recorded monopolar signals were imported to DEMUSE software (ver. 6.1; The University of Maribor, Slovenia) in MATLAB (R2023a, MathWorks GK, Tokyo, Japan). The signals were differentiated between adjacent electrodes in the longitudinal direction, and 59-channel differentiated signals were decomposed into single MU firings using a validated algorithm with the Convolution Kernel Compensation (CKC) technique in DEMUSE software (Holobar et al., 2009; Holobar et al., 2014; Holobar & Zazula, 2007). Physiological irregular firings, such as below 4 or over 30 pulses per second, and individual MU firing rates showing a coefficient of variation of over 30% were discarded (Adam & De Luca, 2005; Hirono, Igawa, et al., 2024; Hirono et al., 2022). High-frequency firings such as doublets seldom occur during the present interval where the firing rate was calculated: they occur most often during initial force production or with increasing contraction velocity (Bawa & Calancie, 1983; Van Cutsem et al.,

1998), and could additionally cause a statistical anomaly; therefore, we removed high-frequency firings with over 30 pulses per second. Decomposed MU firings were visually inspected by a trained investigator. MU action potential wave forms were checked, and only MUAPs with a reliable discharge pattern, e.g. clearly propagated action potentials, were entered into the analysis. Detected MUs were tracked between before (PRE) and after (POST) SS or the control condition using the CKC technique in DEMUSE software, where the same MU filters were transferred to PRE and POST data (Francic & Holobar, 2021; Hirono, Igawa, et al., 2024; Hirono et al., 2022). During ramp-up to 35% of the MVC task, recruitment thresholds of individual MUs were defined as the torque level where the initial firing was identified. The sustained phase was not analyzed for calculation of the firing rate; it was used only to ensure reliable MU identification in decomposition analysis. The MU firing rate was defined as an average firing rate during the ramp-up phase between 30 and 35% of MVC. Representative data from one participant is presented in Figure 2. Unfortunately, few MUs were detected in the ramp-up to 70% of the MVC task, especially from RF. Specifically, the numbers of detected MUs from RF were 0 in SS and 2 (collected from one participant) under control conditions, and the numbers from VL were 13 (collected from 8 participants, with a range from 0 to 3 per participant) under the SS condition and 16 (collected from 6 participants, with a range from 0 to 6 per participant) under the control condition. Therefore, all MU data in the 70% of MVC task were discarded from the current analysis.

Measurements of individual muscle stiffness

An ultrasound shear wave elastography device (Aixplorer v.12.2.0, SuperSonic Imagine, Aix-en-Provence, France) with a 2- to 10-MHz linear probe (SL10-2; SuperSonic Imagine) was used to calculate the shear elastic modulus, an estimation of individual muscle stiffness. During shear wave elastography measurements, participants' right hip was set to 0 degrees of flexion/extension (neutral position) and the knee was flexed at 90 degrees, whereas the contralateral hip and knee were flexed at 90 degrees by an investigator. According to a guideline on the use of shear wave elastography for soft tissue (Cipriano et al., 2022): musculoskeletal and penetration modes were employed as follows: frequency, 2 Hz; gain, 90%; opacity, 100%; persistence, high; smoothing, 5; and range, 0–267 kPa. Measurement sites for the RF and VL were the midpoint between the anterior superior iliac spine and superior edge of the patella and midpoint between the greater trochanter and superior and lateral edges of the patella, respectively. Both sites were positioned proximal to the grid electrodes. Confirming the muscle belly in the B-mode transverse image, the transducer was held parallel to the muscle fascicle. In shear wave mode, the shear elastic modulus was obtained three times in each muscle without pressing the probe strongly on the skin. The region of interest was set as a square of 1.5 x 1.5 cm, and the shear elastic modulus was calculated as the mean value of the region. If an artifact, the void area, or non-muscle tissue was included in the region, it was excluded, and shear elastic modulus values were computed

for manually drawn areas as wide as possible using the Q-boxTM trace function. The shear elastic modulus (G) was calculated using the following equation, making use of shear wave velocity (V), and ρ represents the density of muscle (1000 kg/m³): $G \text{ (kPa)} = \rho V^2$ (Gennisson et al., 2010). A higher shear elastic modulus indicates greater stiffness of the muscle (Koo et al., 2013). A single investigator measured all shear wave elastography images, and other investigators analyzed the values from stored images to blind them to the data. From the same ultrasound images, pennation angles of RF and VL were analyzed using Image J (ver. 1.54 g; National Institutes of Health, USA). If any fascicle or aponeurosis was unclear, the images were excluded.

Static stretching or control condition

Under the SS condition, participants lay in the chair of the dynamometer with the backrest fully reclined to a supine position. Their left hip was maximally flexed and fixed by a belt and the right lower limb was moved by a certified physical therapist. The right hip was passively extended until the point of starting to feel discomfort or pain, followed by passive flexion of their knee until they began to feel discomfort or pain (Fig. 1). This posture was maintained for one minute, the hip and knee angles were measured using a goniometer. The stretching duration of over 60 s could significantly reduce muscle strength immediately (Behm et al., 2016). The 1-minute SS was repeated 6 times with 10-second rest intervals. Under the

control condition, participants rested in a supine position with the hip in a neutral position and the knee at 90-degree of flexion for 6 minutes.

Statistical analysis

Statistical analyses were performed using statistical software (SPSS v.29.0, IBM Japan Inc., Tokyo, Japan). Linear mixed model analyses of variance (ANOVA), with participant and MU as random factors and condition and time as fixed factors, and with an unstructured variance-covariance matrix, were performed to compare the changes in the MU recruitment threshold and firing rate at PRE and POST under both conditions, in each muscle. Linear mixed model ANOVA was also performed to compare the MVC torque, shear elastic modulus and pennation angle between PRE and POST under both conditions in each muscle, with participant as a random factor and condition and time as fixed factors using an unstructured variance-covariance matrix. If an interaction was identified, post-hoc tests were conducted. Additionally, the same mixed model ANOVA was employed to compare changes in hip and knee angles during SS. If a main effect was identified, post-hoc tests with Bonferroni correction were performed. Significance was set as $p < 0.05$. If a significant difference is observed, Cohen's d effect sizes was also calculated.

Results

252

253 *Joint angles during static stretching*

254

255 The average and standard deviation values of hip extension and knee flexion angle are shown
256 in Table 1. ANOVA revealed a main effect of time in both joint angles, indicating that both
257 angles significantly increased (hip joint angle: $p < 0.01$, knee joint angle: $p < 0.01$).

258

259 *Shear elastic modulus of RF and VL*

260

261 ANOVA for shear elastic modulus of RF indicated an interaction ($p = 0.037$) and a main effect
262 of time ($p = 0.032$), as shown in Figure 3. Post-hoc tests revealed a significantly lower value
263 at POST than PRE under the SS condition (PRE: 20.0 ± 7.5 kPa, POST: 17.6 ± 6.8 kPa, $p =$
264 0.012 , Cohen's $d = 0.33$), but not control condition (PRE: 16.9 ± 7.7 kPa, POST: 17.4 ± 7.9 kPa,
265 $p = 0.425$). ANOVA for the shear elastic modulus of VL did not indicate any significance for
266 interaction ($p = 0.378$) nor a main effect of time ($p = 0.114$). Shear elastic moduli of VL were
267 9.0 ± 2.4 kPa at PRE and 10.4 ± 3.4 kPa at POST under the SS condition, whereas they were
268 8.3 ± 3.4 kPa at PRE and 9.1 ± 3.6 kPa at POST under the control condition. Regarding
269 pennation angles of RF or VL, there were no interactions (RF: $p = 0.985$, VL: $p = 0.103$) or
270 main effect of time (RF: $p = 0.499$, VL: $p = 0.446$).

271

272 *Motor unit firing behaviors*

273

274 The detected numbers of tracked MUs from RF were 14 and 19 under the SS and control
275 conditions, respectively. The numbers of tracked MUs from VL were 69 and 93 under the SS
276 and control conditions, respectively. The results regarding the recruitment threshold and
277 firing rate during the ramp-up contraction are presented in Figure 4. ANOVA for the MU
278 recruitment threshold of RF and ANOVAs for MU firing rates of RF and VL revealed main
279 effects of time (all: $p < 0.001$), but not an interaction (recruitment threshold of RF: $p = 0.282$,
280 firing rate of RF: $p = 0.363$, firing rate of VL: $p = 0.557$), indicating that the recruitment threshold
281 significantly decreased and firing rate significantly increased after both conditions. ANOVA
282 for recruitment threshold of VL revealed an interaction ($p = 0.018$) and a main effect of time (p
283 < 0.001). Post-hoc tests revealed significantly lower values regarding the recruitment
284 threshold of VL at POST than at PRE under both conditions (from 24.9 ± 6.3 to 21.7 ± 6.1 %MVC
285 under the SS condition; from 24.4 ± 6.7 to 22.3 ± 6.1 %MVC under the control condition, both p
286 < 0.001). The interaction indicated that the reduction of recruitment threshold of VL was
287 greater under SS condition than that under control condition.

288

289 *Maximum voluntary knee extension torque*

290

291 ANOVA revealed a main effect of time ($p < 0.001$), but not an interaction ($p = 0.362$), indicating

that the maximal knee extension strength significantly decreased after both conditions (Figure 5). MVC torque decreased from PRE (187.4 ± 22.0 Nm) to POST (153.3 ± 28.8 Nm) under the SS condition, but similarly decreased from PRE (186.1 ± 29.1 Nm) to POST (156.2 ± 24.1 Nm) under the control condition.

Discussion

The present study investigated the effects of selective SS of RF on MU firing behaviors of RF and VL. We also confirmed the effects of selective SS using ultrasound shear wave elastography, which can be used to estimate individual muscle stiffness. Our results showed that selective SS could reduce RF stiffness, and it reduced the MU recruitment threshold of VL, even though VL stiffness was not affected. The changes in MU firing behaviors were also observed under the control condition, suggesting that the changes could be partially explained by muscle fatigue caused by repeating submaximal contractions, but we observed an interaction that revealed that the recruitment threshold in VL was decreased to a greater extent after selective SS compared with the control condition. To our knowledge, this is the first study to investigate the differences in MU firing behaviors between synergistic muscles consisting of stretched and non-stretched muscles after selective SS.

SS induces viscoelastic stress relaxation of the muscle, (i.e., decreases muscle stiffness, (Magnusson, 1998)) since only RF is a bi-articular muscle crossing the hip and knee joint among quadriceps femoris muscles, passive movement including hip extension can stretch RF selectively (Nakamura et al., 2020). A strong merit regarding the present study is that it confirms the alteration of individual muscle stiffness using shear wave elastography. Passive knee joint flexion movement following hip joint extension can be effective for stretching only RF because VL crosses the knee joint only. Indeed, the knee angle was similar between the SS protocol and control; thus, the influence on VL stiffness did not differ between the present SS and control conditions. Additionally, pennation angles of RF and VL were not changed by the interventions in the present study, suggesting that SS could impact mechanical properties of RF, not caused by changes in muscle architecture. Since the interaction and the significant decrease in RF stiffness were observed, the current SS method was confirmed as selective SS for RF. A previous study reported similar changes in RF stiffness following static stretching ($-10.5 \pm 18.9\%$), in which the same shear wave elastography device was used (Takeuchi et al., 2021). Therefore, although the present change in RF stiffness was small ($d=0.33$) and the percent change was $-11.5 \pm 12.5\%$, the stiffness change is similar to the previous report and this change could be considered reliable. However, because the stiffness of other muscles could be changed more by static stretching (Fukaya et al., 2024), the present change in RF stiffness could potentially limit the physiological relevance of the findings.

332

333 Immediately after selective SS, MU firing behaviors were altered in the present study. The
334 results for the recruitment threshold of VL showed an interaction, suggesting that the reduction
335 in recruitment threshold was greater after SS than after the control trial. We analyzed only
336 tracked MUs; therefore, the same MUs were recruited at a lower intensity after SS. The
337 reduction in recruitment threshold suggests that high-threshold MUs are activated earlier
338 during contraction (Martinez-Valdes et al., 2020; ter Haar Romeny et al., 1982). A previous
339 study (Del Vecchio et al., 2019) suggested that the latency between neural activation and force
340 generation could be associated with the reduction in the recruitment threshold. As the delay
341 is modulated by differences in MU recruitment (Del Vecchio et al., 2018), it may be considered
342 that the present decrease in muscle stiffness could cause the reduction in recruitment
343 threshold to adapt the change in neural drive and force generation. As the same reduction
344 was observed after fatiguing exercise, MU recruitment threshold significantly decreases to
345 exert the same absolute torque (Hirono et al., 2022). In the current results, the VL
346 recruitment threshold decreased more markedly after SS, suggesting that the recruitment
347 strategy could be modified to exert the same absolute torque as that at PRE. In a previous
348 study investigating the effects of SS on MU firing behaviors of the triceps surae (Mazzo et al.,
349 2021), the MU recruitment thresholds decreased after repeated SS to compensate for the
350 reduction in muscle stiffness. The previous work supported our results as they also observed
351 a reduction of the recruitment threshold, however they also reported the MU firing rate

increased (Mazzo et al., 2021), which was contrary to our results whereby an interaction was not observed in the MU firing rate in either muscle. Either strategy, the decrease in recruitment threshold or increase in firing rate, could be a method to compensate for the reduction of muscle stiffness. Although an interaction for RF was not observed, the effect size of recruitment threshold of RF was greater under the SS condition than under the control condition. The motor units in RF could be influenced by the SS for RF, similar to findings in previous literature (Mazzo et al., 2021), but the small sample size prevents the clear conclusion regarding the motor unit behaviors in RF. SS alters the elastic deformation of connective tissue related to maximal force production capacities of the muscle-tendon unit (Behm & Chaouachi, 2011), leading to a reduction of the maximal voluntary muscle contraction capacity related to the modulation of neuromuscular excitation-contraction coupling efficiency (Trajano et al., 2013). Therefore, these neuromuscular adaptations may contribute to the change in the MU recruitment strategy.

Skeletal muscle consists of contractile and noncontractile components including series and parallel elastic components, such as aponeurosis morphology and connective tissue. They can contribute to passive stiffness of the muscle (Gajdosik, 2001) and also to force transmission capacity along the series of elastic components (Nordez et al., 2009). On performing SS for a muscle-tendon unit, the series elastic component could change (Nakamura et al., 2011; Purslow, 1989), leading to lower-level compliance of force

transmission. Since Golgi tendon organ mechanoreceptors can detect changes in tendon mechanical stress generated by muscle contraction and Ib afferents inhibit motoneurons (Davies et al., 1995), the afferent system could be attenuated by the reduction of muscle-tendon unit stiffness following SS. Indeed, a previous study investigating the relationship between tendon stiffness and the MU firing rate (Contreras-Hernandez et al., 2024) showed that the resting stiffness of the Achilles tendon was correlated with the MU firing rate of the triceps surae. In the present study, selective SS reduced RF muscle stiffness; following SS, MU firing behaviors might be modified by the biomechanical changes in the series of elastic components and afferent programs.

The present results were based on relatively low-intensity contractions involving the detection of a number of MUs in both muscles; thus, MU behaviors during high-intensity contraction remain unclear. During high-intensity contraction, it is difficult to detect a large number of MUs (Francic & Holobar, 2021); indeed, few MUs were detected during ramp-up contraction to 70% of MVC in the present study. Mazzo et al. (2021) reported significant changes in MU firing behaviors during low-intensity contraction but not during moderate-intensity contraction. Another study (Contreras-Hernandez et al., 2024), investigating correlations between tendon stiffness and the MU firing rate during 10 and 40% of MVC, showed that a significant correlation was only observed during 10% of MVC. Combined with those previous reports, a load-dependent relationship between MU firing behaviors and muscle-tendon stiffness is

present. Although we did not measure MU firing behaviors during high-intensity contraction, this relationship may influence any changes following selective SS.

Additionally, our results showed an increase in MU firing rate and a decrease in the recruitment threshold after both the SS and control. One reason may be the relaxation of muscle after 6-minute SS and resting. In relaxation, MU persistent inward currents are decreased (Mesquita et al., 2022). Sufficient warm-up increases the input-output gain (Lee et al., 2024), suggesting that resting could reduce the efficiency of the pathway from neural input to force generation due to the changes in muscle viscosity and/or stiffness. Considering these reports, it is necessary for either the MU firing rate to increase or recruitment threshold to decrease at POST to exert the same absolute torque as PRE due to not only SS condition but also control condition, as noted in the present study.

In clinical settings, enhancing the excitability of the vasti muscles may be appropriate for patients. Force distribution is observed among synergistic muscles (Hug et al., 2015), and this distribution could influence the magnitude of joint force in a simulation study (Lewis et al., 2007). A previous study reported that knee osteoarthritis patients with the capacity to exert a higher vasti muscle force had thicker medial femur cartilage, and a higher force of the vastus lateralis was negatively correlated with knee pain (Yagi et al., 2022). For such patients, the selective SS protocol could influence modulation of neural drive contribution among

quadriceps, with it offering a possible prescription. In other cases, a previous study reported that patellofemoral cartilage stresses are related to vastus medialis force (Pal et al., 2019). For both patients and athletes, selective SS could be useful to alter the recruitment strategies among synergistic muscles. However, the implications of selective SS are still unclear for clinical settings and sports training as a modulation of neural drive. Future studies should focus on the effects of modulating the neuromuscular strategy in patients in clinical settings.

There were several limitations of the present study. One was the small sample size of detected MUs from RF. RF has a unique muscle architecture (Hasselman et al., 1995; Sung et al., 2003), which might be the cause of the small number of detected MUs. The limited sample size may lead to an insufficient statistical power to detect differences in MU firing behaviors. Indeed, the effect sizes of recruitment threshold and firing rate of RF were greater under the SS condition than under the control condition. It is difficult to conclude the modulation of RF motor unit behaviors clearly in the present study. Another limitation was we only investigated MU behaviors during low-intensity isometric contractions, although we were interested in and conducted a high-intensity contraction task, few MUs were detected. Therefore, it is unclear whether such an MU firing strategy could be observed during high-intensity contractions or during dynamic muscle actions. Third, submaximal tasks were repeated several times, which could have influenced the current results. Since we used a device that can collect data on only one muscle during a task, ramp-up contraction tasks were

repeated twice for RF and VL. Additionally, we did not analyze data from the ramp-up task to 70% of MVC as the number of detected MUs was too small. Taking the protocol order and tasks into consideration, repeated tasks at 70% of MVC may have led to muscle fatigue, which could be a reason why the reduction in maximal strength did not differ between the conditions. This may partially explain the changes in MU behaviors under the control condition. Fourth, the present data included only male participants because a greater number of MUs can be detected in male subjects (Hirono, Okudaira, et al., 2024). Sex differences in muscle stiffness and MU behaviors were reported in previous studies (Granata et al., 2002; Lulic-Kuryllo & Inglis, 2022; Wang et al., 2015). Although we initially included 6 female subjects, the numbers of detected MUs were 0 and 6 from RF and VL, respectively, being insufficient for further analysis. If the female data included, the main findings were not different from the present results, which is reported in supplementary material (Supplementary Appendix 1). It is unclear whether our findings would be applicable to females.

In conclusion, SS consisting of passive hip extension and knee flexion could reduce the shear elastic modulus of only RF, and not VL. Immediately after both SS and control conditions, MVC strength decreased, and MU firing behaviors may have been altered. Especially, MUs of VL, whose stiffness was not decreased, were recruited during lower-intensity contraction to compensate for the reduction in RF stiffness; therefore, these results suggest that SS could alter the neuromuscular strategy.

Acknowledgments

We would like to thank Dr. Masamichi Okudaira for helping with designing this study. We also appreciate all study participants.

Grant

The work was supported by Tobe Maki Foundation (22-JC-003). This work was also financially supported by a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows (24K20528).

Disclosures

There are no competing interests, financial or otherwise, to report regarding this manuscript.

Data Availability Statement

Data will be made available upon reasonable request.

Author contributions

T.H., M.Y., N.I., and K.W. conceived and designed the study; T.H., Y.M., Z.W., H.S., S.O., and K.N. performed data acquisition; T.H., Z.W., and H.S. analyzed the data; T.H., Y.M., N.I., and K.W. interpreted the results of experiments; T.H. prepared figures; T.H. drafted the manuscript; All authors edited and revised the manuscript; All authors approved the final version of the manuscript.

References

- Adam, A., & De Luca, C. J. (2005, Jul). Firing rates of motor units in human vastus lateralis muscle during fatiguing isometric contractions. *J Appl Physiol* (1985), 99(1), 268-280. <https://doi.org/10.1152/japplphysiol.01344.2004>
- Akima, H., Saito, A., Watanabe, K., & Kouzaki, M. (2012, Jul). Alternate muscle activity patterns among synergists of the quadriceps femoris including the vastus intermedius during low-level sustained contraction in men. *Muscle Nerve*, 46(1), 86-95. <https://doi.org/10.1002/mus.23268>
- Bawa, P., & Calancie, B. (1983, Jun). Repetitive doublets in human flexor carpi radialis muscle. *J Physiol*, 339, 123-132. <https://doi.org/10.1113/jphysiol.1983.sp014707>
- Behm, D. G., Blazeovich, A. J., Kay, A. D., & McHugh, M. (2016, Jan). Acute effects of muscle stretching on physical performance, range of motion, and injury incidence in healthy active individuals: a systematic review. *Appl Physiol Nutr Metab*, 41(1), 1-11. <https://doi.org/10.1139/apnm-2015-0235>
- Behm, D. G., & Chaouachi, A. (2011, Nov). A review of the acute effects of static and dynamic stretching on performance. *Eur J Appl Physiol*, 111(11), 2633-2651. <https://doi.org/10.1007/s00421-011-1879-2>
- Budini, F., & Tilp, M. (2016, Jul 1). Changes in H-reflex amplitude to muscle stretch and lengthening in humans. *Rev Neurosci*, 27(5), 511-522. <https://doi.org/10.1515/revneuro-2016-0001>
- Cipriano, K. J., Wickstrom, J., Glicksman, M., Hirth, L., Farrell, M., Livinski, A. A., Esfahani, S. A., Maldonado, R. J., Astrow, J., Berrigan, W. A., Piergies, A. M. H., Hobson-Webb, L. D., & Alter, K. E. (2022, Aug). A scoping review of methods used in musculoskeletal soft tissue and nerve shear wave elastography studies. *Clin Neurophysiol*, 140, 181-195. <https://doi.org/10.1016/j.clinph.2022.04.013>
- Contreras-Hernandez, I., Arvanitidis, M., Falla, D., Negro, F., & Martinez-Valdes, E. (2024, Oct 1). Achilles tendon morpho-mechanical parameters are related to triceps surae motor unit firing properties. *J Neurophysiol*, 132(4), 1198-1210. <https://doi.org/10.1152/jn.00391.2023>
- Davies, P., Petit, J., & Scott, J. J. (1995, Aug 28). The dynamic response of Golgi tendon organs to tetanic contraction of in-series motor units. *Brain Res*, 690(1), 82-91. [https://doi.org/10.1016/0006-8993\(95\)00592-e](https://doi.org/10.1016/0006-8993(95)00592-e)
- Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., & Farina, D.

(2019, Apr). The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *J Physiol*, 597(7), 1873-1887. <https://doi.org/10.1113/JP277250>

Del Vecchio, A., Ubeda, A., Sartori, M., Azorin, J. M., Felici, F., & Farina, D. (2018, Nov 1). Central nervous system modulates the neuromechanical delay in a broad range for the control of muscle force. *J Appl Physiol* (1985), 125(5), 1404-1410. <https://doi.org/10.1152/jappphysiol.00135.2018>

Fowles, J. R., Sale, D. G., & MacDougall, J. D. (2000, Sep). Reduced strength after passive stretch of the human plantarflexors. *J Appl Physiol* (1985), 89(3), 1179-1188. <https://doi.org/10.1152/jappl.2000.89.3.1179>

Francic, A., & Holobar, A. (2021). On the Reuse of Motor Unit Filters in High Density Surface Electromyograms Recorded at Different Contraction Levels. *IEEE Access*, 9, 115227-115236. <https://doi.org/10.1109/access.2021.3104762>

Fukaya, T., Morishita, K., Yokoi, Y., Takeuchi, K., & Nakamura, M. (2024, Dec). Acute and chronic effects of static stretching of different target muscles on shear elastic modulus: A narrative review. *J Electromyogr Kinesiol*, 79, 102939. <https://doi.org/10.1016/j.jelekin.2024.102939>

Gajdosik, R. L. (2001, Feb). Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clin Biomech (Bristol)*, 16(2), 87-101. [https://doi.org/10.1016/s0268-0033\(00\)00061-9](https://doi.org/10.1016/s0268-0033(00)00061-9)

Gennisson, J. L., Deffieux, T., Mace, E., Montaldo, G., Fink, M., & Tanter, M. (2010, May). Viscoelastic and anisotropic mechanical properties of in vivo muscle tissue assessed by supersonic shear imaging. *Ultrasound Med Biol*, 36(5), 789-801. <https://doi.org/10.1016/j.ultrasmedbio.2010.02.013>

Granata, K. P., Wilson, S. E., & Padua, D. A. (2002, Apr). Gender differences in active musculoskeletal stiffness. Part I. Quantification in controlled measurements of knee joint dynamics. *J Electromyogr Kinesiol*, 12(2), 119-126. [https://doi.org/10.1016/s1050-6411\(02\)00002-0](https://doi.org/10.1016/s1050-6411(02)00002-0)

Hasselmann, C. T., Best, T. M., Hughes, C. t., Martinez, S., & Garrett, W. E., Jr. (1995, Jul-Aug). An explanation for various rectus femoris strain injuries using previously undescribed muscle architecture. *Am J Sports Med*, 23(4), 493-499. <https://doi.org/10.1177/036354659502300421>

Hirono, T., Igawa, K., Okudaira, M., Takeda, R., Nishikawa, T., & Watanabe, K. (2024, Mar 1). Time-of-day effects on motor unit firing and muscle contractile properties in humans. *J Neurophysiol*,

131(3), 472-479. <https://doi.org/10.1152/jn.00368.2023>

Hirono, T., Kunugi, S., Yoshimura, A., Holobar, A., & Watanabe, K. (2022, Sep 17). Acute changes in motor unit discharge property after concentric versus eccentric contraction exercise in knee extensor. *J Electromyogr Kinesiol*, 67, 102704. <https://doi.org/10.1016/j.jelekin.2022.102704>

Hirono, T., Okudaira, M., Takeda, R., Ueda, S., Nishikawa, T., Igawa, K., Kunugi, S., Yoshimura, A., & Watanabe, K. (2024, Jun). Association between physical fitness tests and neuromuscular properties. *Eur J Appl Physiol*, 124(6), 1703-1717. <https://doi.org/10.1007/s00421-023-05394-y>

Holobar, A., Farina, D., Gazzoni, M., Merletti, R., & Zazula, D. (2009, Mar). Estimating motor unit discharge patterns from high-density surface electromyogram. *Clin Neurophysiol*, 120(3), 551-562. <https://doi.org/10.1016/j.clinph.2008.10.160>

Holobar, A., Minetto, M. A., & Farina, D. (2014, Feb). Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric. *J Neural Eng*, 11(1), 016008. <https://doi.org/10.1088/1741-2560/11/1/016008>

Holobar, A., & Zazula, D. (2007). Multichannel Blind Source Separation Using Convolution Kernel Compensation. *IEEE Transactions on Signal Processing*, 55(9), 4487-4496. <https://doi.org/10.1109/tsp.2007.896108>

Hug, F., Tucker, K., Gennisson, J. L., Tanter, M., & Nordez, A. (2015, Jul). Elastography for Muscle Biomechanics: Toward the Estimation of Individual Muscle Force. *Exerc Sport Sci Rev*, 43(3), 125-133. <https://doi.org/10.1249/JES.0000000000000049>

Kay, A. D., & Blazevich, A. J. (2012, Jan). Effect of acute static stretch on maximal muscle performance: a systematic review. *Med Sci Sports Exerc*, 44(1), 154-164. <https://doi.org/10.1249/MSS.0b013e318225cb27>

Koo, T. K., Guo, J. Y., Cohen, J. H., & Parker, K. J. (2013, Aug 9). Relationship between shear elastic modulus and passive muscle force: an ex-vivo study. *J Biomech*, 46(12), 2053-2059. <https://doi.org/10.1016/j.jbiomech.2013.05.016>

Kouzaki, M., & Shinohara, M. (2006, Sep). The frequency of alternate muscle activity is associated with the attenuation in muscle fatigue. *J Appl Physiol* (1985), 101(3), 715-720. <https://doi.org/10.1152/japplphysiol.01309.2005>

Kouzaki, M., Shinohara, M., Masani, K., & Fukunaga, T. (2004, Dec). Force fluctuations are modulated

by alternate muscle activity of knee extensor synergists during low-level sustained contraction. *J Appl Physiol* (1985), 97(6), 2121-2131. <https://doi.org/10.1152/jappphysiol.00418.2004>

Lee, J., Park, D., Lee, J. Y., & Park, J. (2024, Apr). Effect of Warm-Up Exercise on Functional Regulation of Motor Unit Activation during Isometric Torque Production. *J Hum Kinet*, 92, 29-41. <https://doi.org/10.5114/jhk/185157>

Lewis, C. L., Sahrmann, S. A., & Moran, D. W. (2007). Anterior hip joint force increases with hip extension, decreased gluteal force, or decreased iliopsoas force. *J Biomech*, 40(16), 3725-3731. <https://doi.org/10.1016/j.jbiomech.2007.06.024>

Lulic-Kuryllo, T., & Inglis, J. G. (2022, Oct). Sex differences in motor unit behaviour: A review. *J Electromyogr Kinesiol*, 66, 102689. <https://doi.org/10.1016/j.jelekin.2022.102689>

Magnusson, S. P. (1998, Apr). Passive properties of human skeletal muscle during stretch maneuvers. A review. *Scand J Med Sci Sports*, 8(2), 65-77. <https://doi.org/10.1111/j.1600-0838.1998.tb00171.x>

Martinez-Valdes, E., Negro, F., Farina, D., & Falla, D. (2020, Jun). Divergent response of low- versus high-threshold motor units to experimental muscle pain. *J Physiol*, 598(11), 2093-2108. <https://doi.org/10.1113/JP279225>

Mazzo, M. R., Weinman, L. E., Giustino, V., McLagan, B., Maldonado, J., & Enoka, R. M. (2021, Sep). Changes in neural drive to calf muscles during steady submaximal contractions after repeated static stretches. *J Physiol*, 599(18), 4321-4336. <https://doi.org/10.1113/JP281875>

Mesquita, R. N. O., Taylor, J. L., Trajano, G. S., Skarabot, J., Holobar, A., Goncalves, B. A. M., & Blazeovich, A. J. (2022, Jun). Effects of reciprocal inhibition and whole-body relaxation on persistent inward currents estimated by two different methods. *J Physiol*, 600(11), 2765-2787. <https://doi.org/10.1113/JP282765>

Nakamura, M., Ikezoe, T., Takeno, Y., & Ichihashi, N. (2011, Nov). Acute and prolonged effect of static stretching on the passive stiffness of the human gastrocnemius muscle tendon unit in vivo. *J Orthop Res*, 29(11), 1759-1763. <https://doi.org/10.1002/jor.21445>

Nakamura, M., Sato, S., Murakami, Y., Kiyono, R., Yahata, K., Sanuki, F., Yoshida, R., Fukaya, T., & Takeuchi, K. (2020). The Comparison of Different Stretching Intensities on the Range of Motion and Muscle Stiffness of the Quadriceps Muscles. *Front Physiol*, 11, 628870. <https://doi.org/10.3389/fphys.2020.628870>

- Nordez, A., Gallot, T., Catheline, S., Guevel, A., Cornu, C., & Hug, F. (2009, Jun). Electromechanical delay revisited using very high frame rate ultrasound. *J Appl Physiol (1985)*, 106(6), 1970-1975. <https://doi.org/10.1152/jappphysiol.00221.2009>
- Pal, S., Besier, T. F., Gold, G. E., Fredericson, M., Delp, S. L., & Beaupre, G. S. (2019, Feb). Patellofemoral cartilage stresses are most sensitive to variations in vastus medialis muscle forces. *Comput Methods Biomech Biomed Engin*, 22(2), 206-216. <https://doi.org/10.1080/10255842.2018.1544629>
- Purslow, P. P. (1989). Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *J Biomech*, 22(1), 21-31. [https://doi.org/10.1016/0021-9290\(89\)90181-4](https://doi.org/10.1016/0021-9290(89)90181-4)
- Shehab, R., Mirabelli, M., Gorenflo, D., & Fethers, M. D. (2006, May). Pre-exercise stretching and sports related injuries: knowledge, attitudes and practices. *Clin J Sport Med*, 16(3), 228-231. <https://doi.org/10.1097/00042752-200605000-00007>
- Sung, D. H., Jung, J. Y., Kim, H. D., Ha, B. J., & Ko, Y. J. (2003, Jul). Motor branch of the rectus femoris: anatomic location for selective motor branch block in stiff-legged gait. *Arch Phys Med Rehabil*, 84(7), 1028-1031. [https://doi.org/10.1016/s0003-9993\(03\)00029-7](https://doi.org/10.1016/s0003-9993(03)00029-7)
- Takeuchi, K., Nakamura, M., Fukaya, T., Nakao, G., & Mizuno, T. (2024, Jul 4). Stretching intervention can prevent muscle injuries: a systematic review and meta-analysis. *Sport Sciences for Health*. <https://doi.org/10.1007/s11332-024-01213-9>
- Takeuchi, K., Sato, S., Kiyono, R., Yahata, K., Murakami, Y., Sanuki, F., Yoshida, R., & Nakamura, M. (2021). High-Intensity Static Stretching in Quadriceps Is Affected More by Its Intensity Than Its Duration. *Front Physiol*, 12, 709655. <https://doi.org/10.3389/fphys.2021.709655>
- ter Haar Romeny, B. M., Denier van der Gon, J. J., & Gielen, C. C. (1982, Nov). Changes in recruitment order of motor units in the human biceps muscle. *Exp Neurol*, 78(2), 360-368. [https://doi.org/10.1016/0014-4886\(82\)90054-1](https://doi.org/10.1016/0014-4886(82)90054-1)
- Trajano, G. S., Nosaka, K., & Blazevich, A. J. (2017, Aug). Neurophysiological Mechanisms Underpinning Stretch-Induced Force Loss. *Sports Med*, 47(8), 1531-1541. <https://doi.org/10.1007/s40279-017-0682-6>
- Trajano, G. S., Seitz, L., Nosaka, K., & Blazevich, A. J. (2013, Jul 15). Contribution of central vs. peripheral factors to the force loss induced by passive stretch of the human plantar flexors. *J Appl Physiol (1985)*, 115(2), 212-218. <https://doi.org/10.1152/jappphysiol.00333.2013>

- Trajano, G. S., Taylor, J. L., Orssatto, L. B. R., McNulty, C. R., & Blazeovich, A. J. (2020, Nov 5). Passive muscle stretching reduces estimates of persistent inward current strength in soleus motor units. *J Exp Biol*, 223(Pt 21). <https://doi.org/10.1242/jeb.229922>
- Van Cutsem, M., Duchateau, J., & Hainaut, K. (1998, Nov 15). Changes in single motor unit behaviour contribute to the increase in contraction speed after dynamic training in humans. *J Physiol*, 513 (Pt 1)(Pt 1), 295-305. <https://doi.org/10.1111/j.1469-7793.1998.295by.x>
- Wang, D., De Vito, G., Ditroilo, M., Fong, D. T., & Delahunt, E. (2015, Jun). A comparison of muscle stiffness and musculoarticular stiffness of the knee joint in young athletic males and females. *J Electromyogr Kinesiol*, 25(3), 495-500. <https://doi.org/10.1016/j.jelekin.2015.03.003>
- Witvrouw, E., Mahieu, N., Danneels, L., & McNair, P. (2004). Stretching and injury prevention: an obscure relationship. *Sports Med*, 34(7), 443-449. <https://doi.org/10.2165/00007256-200434070-00003>
- Yagi, M., Taniguchi, M., Tateuchi, H., Hirono, T., Yamagata, M., Umehara, J., Nojiri, S., Kobayashi, M., & Ichihashi, N. (2022, Jan). Relationship between individual forces of each quadriceps head during low-load knee extension and cartilage thickness and knee pain in women with knee osteoarthritis. *Clin Biomech (Bristol, Avon)*, 91, 105546. <https://doi.org/10.1016/j.clinbiomech.2021.105546>

708 Table 1. Hip and knee angles during static stretching and the results of ANOVAs

Joint	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	F-value	p-value
Hip extension	7 ± 8	9 ± 9 ^a	11 ± 9 ^a	13 ± 9	14 ± 9	15 ± 9	11.1	< .001
angle, deg.				ab	ab	abc		
Knee flexion	87 ± 14	91 ± 13	91 ± 10	92 ± 10	93 ± 10	94 ± 10	10.7	< .001
angle, deg.		a		a	a			

709 Mean ± standard deviation. Neutral position of hip equals 0 degrees. Full extension of knee
710 equals 0 degrees. The results of post-hoc tests with Bonferroni correction are indicated by a, b,
711 and c ($p < 0.05$), representing significant differences compared with Sets 1, 2, and 3, respectively.

712

713 Figure legend

714 Figure 1

715 The experimental design and time-course of measurements. Participants visited the
716 laboratory twice: static stretching or the control condition was performed. After warm up of
717 several knee extension contractions, maximal voluntary contraction (MVC) torque was
718 evaluated twice. They performed submaximal knee extension with ramp-up contraction to
719 35 and 70% of MVC. Because few motor units could be detected during the ramp-up task at
720 70% of MVC, data during the 70% of MVC task were excluded from the present analyses.
721 Following the voluntary knee extension tasks, rectus femoris (RF) and vastus lateralis (VL)
722 stiffness levels were evaluated using shear wave elastography in a relaxed position. Under
723 the static stretching condition, passive stretching comprising hip extension and knee flexion
724 was performed for 1 minute, repeated for 6 sets. Under the control condition, they rested in
725 a supine position for 6 minutes. Immediately after each intervention, shear wave
726 elastography and the voluntary knee extension task were performed again.

727

728

729 Figure 2

730 Representative data on the motor unit (MU) firing rate from the vastus lateralis of one
731 participant. The left panel shows data before intervention (PRE) and the right panel shows
732 data after intervention (POST). The gray line represents torque data. The colored lines

represent firing rates of individual MUs. Between PRE and POST, the same MUs were tracked.

Figure 3

Shear elastic modulus of the rectus femoris (RF) and vastus lateralis (VL) before (PRE) and after (POST) static stretching or control conditions. An interaction was found in RF, and the post-hoc test revealed that the shear elastic modulus of RF significantly decreased after static stretching, but not in the control. There was no interaction in VL. The bars represent mean values, and each plot represents individual data ($n = 14$). If a significant difference is observed, Cohen's d effect sizes was also calculated.

Figure 4

MU recruitment threshold (left panels) and motor unit firing rate (right panels) during the ramp-up contraction task at 35% of MVC. There was an interaction in the recruitment threshold in the vastus lateralis (VL), but not rectus femoris (RF) nor firing rate in either of the muscles. Main effects of time were found in the recruitment threshold and firing rate in both muscles. Post-hoc test of the recruitment threshold of VL revealed significant differences under both conditions (*; $p < 0.01$). Box plots show the quartiles of data, and each gray line shows

753 individual MU data. Cohen's d effect sizes were also calculated.

754

755

756 Figure 5

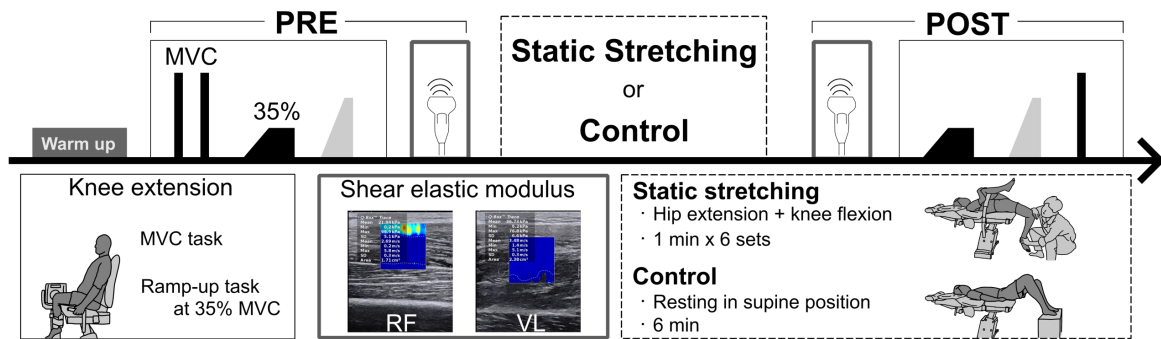
757 Maximal voluntary contraction knee extension torque before (PRE) and after (POST) static

758 stretching or control conditions. There was a main effect of time, but no interaction. The

759 bars represent mean values, and each plot represents individual data (n = 14). Cohen's d

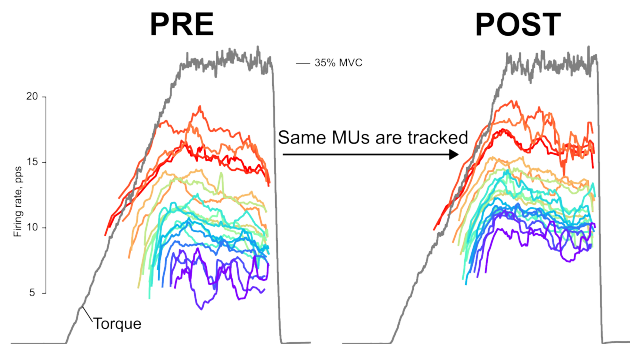
760 effect sizes were also calculated.

761



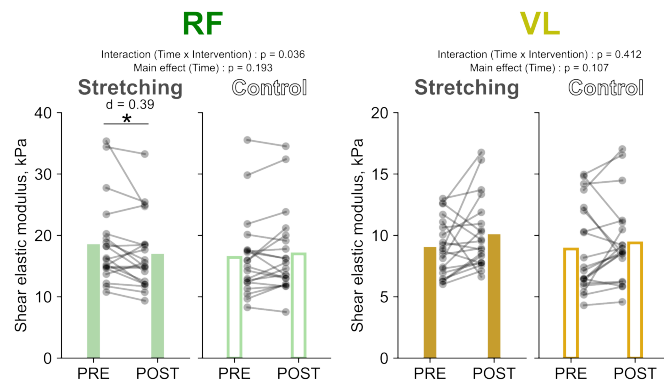
762

763



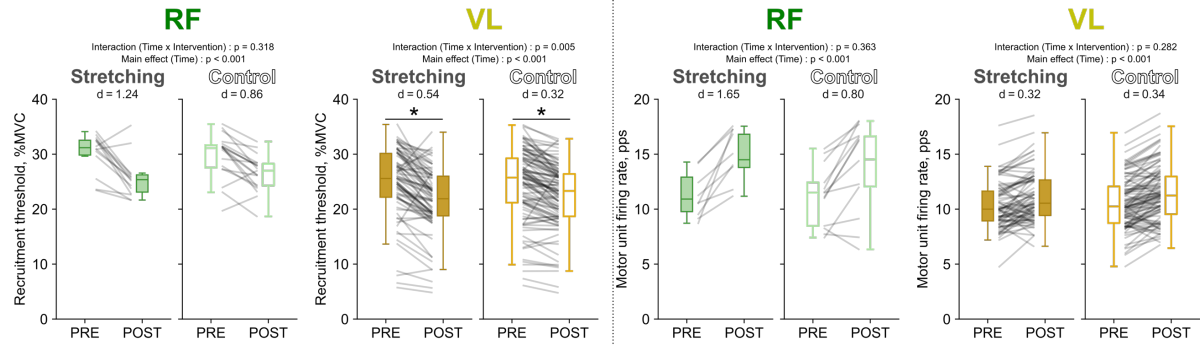
764

765



766

767



768

769

