Neuromuscular versus Mechanical Stretch-Induced Changes in Contralateral versus Ipsilateral Muscle

AQ1 EMILIANO CÈ[1,2], GIUSEPPE CORATELLA[1], ANGELA VALENTINA BISCONTI[1], MASSIMO VENTURELLI[3], ELOISA LIMONTA[1], CHRISTIAN DORIA[1], SUSANNA RAMPICHINI[1], ~~SUSANNA~~ LONGO[1], and FABIO ESPOSITO[1,2]

[1]Department of Biomedical Sciences for Health (SCIBIS), ~~University of Milan,~~ Milan, ITALY; [2]IRCCS Galeazzi Orthopedic Institute, Milan, ITALY; and [3]Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona, Verona, ITALY

ABSTRACT

C[illegible] G. CORATELLA, A. V. BISCONTI, M. VENTURELLI, E. LIMONTA, C. DORIA, S. RAMPICHINI, S. LONGO, and F. E[illegible]ITO. Neuromuscular versus Mechanical Stretch-Induced Changes in Contralateral versus Ipsilateral Muscle. *Med. Sci. Sports Exerc.*, Vol. 52, No. 6, pp. 00–00, 2020. **Purpose:** Whether or not the homologous contralateral muscle (CM) undergoes stretch-induced force reduction as the stretched muscle (SM) is still unclear. The neuromuscular and mechanical factors underlying the force reduction in CM and SM were investigated. **Methods:** Twenty-one participants underwent unilateral knee extensors passive stretching. In both CM and SM, before, immediately after (POST), 5 ($POST_5$), and 10 min ($POST_{10}$) after passive stretching, maximum voluntary contraction (MVC), peak force (pF), and voluntary activation (VA) were measured. During MVC, the electromyographic and mechanomyographic root mean square (EMG RMS and MMG RMS, respectively) was calculated in *rectus femoris*, *vastus lateralis,* and *vastus medialis*, together with M-wave. The total electromechanical delay (EMD), divided in time delay (Δt) EMG-MMG and Δt MMG-F was calculated. **Results:** In CM at POST, the decrease in MVC (−11%; 95% confidence interval [CI], −13 to −9; effect size [ES], −2.27) was accompanied by a fall in VA (−7%; 95% CI, −9 to −4; ES, −2.29), EMG RMS (range, −22% to −11%; ES, −3.92 to −2.25), MMG RMS (range, −10% to −8%; ES, −0.52 to −0.39) and an increase in Δt EMG-MMG (≈+10%; ES, 0.73 to 0.93). All changes returned to baseline at $POST_5$. In SM, decrease in MVC (−19%; 95%
AQ2 CI, −24 to −18; ES, −3.08), pF (−25%; 95% CI, −28 to −22; ES, −4.90), VA (−10%; 95% CI, −11 to −9; ES, −5.71), EMG RMS (≈−33%; ES, −5.23 to −3.22) and rise in MMG RMS (range, +25% to +32%; ES, 4.21 to 4.98) and EMD (≈+28%; ES, 1.59 to 1.77) were observed at POST and persisted at $POST_{10}$. No change in M-wave occurred. **Conclusions:** The contralateral central motor drive stretch-induced inhibition seems to account for the force reduction in CM. In SM, both central inhibition and mechanical factors concurred. **Key Words:** ELECTROMECHANICAL DELAY, VOLUNTARY ACTIVATION, MAXIMAL VOLUNTARY CONTRACTION, EMG ACTIVITY, MMG ACTIVITY, CONTRALATERAL LIMB

Passive stretching is widely performed in sport and rehabilitation mainly to improve joint range of motion. Concomitantly with the increase in range of motion, a reduction in force-generating capacity during maximal voluntary (1,2) or electrically elicited (3,4) contractions has been often described acutely in the stretched muscle (SM). The increase in range of motion and the reduction in the force-generating capacity were shown to be accounted for both neuromuscular factors, such as an alteration in the afferent feedback given by type I*a*, type II (muscle spindles) (3), type III (mechanoreceptors), and type IV (metabo/nociceptors) fibers (5,6) and mechanical factors, such as alterations in the viscoelastic properties of the muscle-tendon unit (1,7). The neuromuscular factors may have a central, that is, a supraspinal inhibition and a reduction in spinal reflex excitability, and/or a peripheral origin, that is, a possible impairment in the events involved in excitation–contraction coupling process, although its role in reducing the contractile force-generating capability still remains to be elucidated (8). In addition, the mechanical factors further impair the force transmission to the tendon insertion point (1,9). The changes induced by passive stretching were still visible after 2 h from its administration (4,10), so the investigation of the time course of both neuromuscular and mechanical factors should be considered systematically.

Address for correspondence: Fabio Esposito, M.D., Department of Biomedical Sciences for Health (SCIBIS), University of Milan, Via Colombo 71, 20133 Milan, Italy; E-mail: fabio.esposito@unimi.it.
E. C. and G. C. contributed equally to the work.
Submitted for publication June 2019.
Accepted for publication December 2019.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/20/5206-0000/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000002255

Recently, a growing body of literature focused on the possible crossover effects induced by passive stretching on the homologous contralateral muscle (CM), which has not been directly exposed to passive stretching (3,11–16). In CM, an increase in range of motion has been very often reported (11,12,17,18). Conversely, inconsistent results for the force-generating capacity were found. Although some studies reported no change in CM after passive stretching (2,11,12), a reduction in the CM force-generating capacity was also observed (12,15,16,19,20). Because CM is not directly involved in the passive stretching maneuver, it could be argued that neuromuscular (central or peripheral) rather than mechanical factors could be prevalently involved. Four possible mechanisms merging a final common end, that is, the decrease in the contralateral α-motoneuron pool excitability, have been proposed to explain the passive stretching-induced increase in range of motion and reduction in the force-generating capacity in CM: (i) a reduction in the stretch-reflex sensitivity, involving muscle spindles (3,12,16); (ii) an increase in the inhibitory afferent feedback from SM, involving mechanoreceptors and nociceptors (12,16); (iii) an altered spinal-reflex excitability (14); and (iv) an increase in the stretch tolerance, a mechanism associated with the nociceptive nerve endings activity at the muscle/joint level (15).

The neuromuscular factors involved in the force-generating capacity can be further divided into central or peripheral by using the interpolated twitch technique (21). Indeed, through electrical stimulations, it is possible to calculate the voluntary activation and the peak force (pF) recorded after a maximal voluntary contraction as central or peripheral factor, respectively (22). In SM, the central contribution has been identified as a prevalent neuromuscular factor affecting the force-generating capacity (8). Whether or not an impairment in central contribution could occur in CM is still an open question, as well as its possible persistence in both CM and in SM.

For a deeper understanding of the possible crossover effect, the simultaneous recording of the electromyographic (EMG), mechanomyographic (MMG) and force signals could be used (23–25). The MMG is considered the mechanical counterpart of the EMG signal (26,27). Indeed, EMG and MMG provide electrochemical and mechanical information, respectively, on muscle excitation and activation from sarcolemmal depolarization to force transmission to the tendon insertion point (26,28). The continuous dimensional changes of the contracting muscle fibers, indeed, generate pressure waves that can be detected at the skin surface level. Three main physiological mechanisms are included: (i) the gross lateral movement of the contracting muscle at the beginning of muscle contraction, (ii) the subsequent vibrations at the resonance frequency of the muscle, and (iii) the dimensional changes in the active muscle fibers when the force generated during muscle contraction reaches the plateau (26,27). The MMG root mean square is calculated from the oscillation during the force plateauing. Contrarily to the EMG root mean square, MMG root mean square is sensitive to both the number of active motor units (27) and the mechanical characteristics of the contractile and viscoelastic components during muscle contraction (1). This offers the possibility to observe the contribution of both neuromuscular and mechanical factors to muscle excitation and activation. Because mechanical factors are not expected to affect CM, it can be hypothesized that a possible stretching induced reduction in MMG root mean square could derive from neuromuscular factors. Moreover, the frequency content of the MMG signal is strictly related to central drive (26,27). The MMG MF has been proposed to reflect the average firing rate of the active fibers (26,27). Therefore, a stretching-induced reduction in central drive should result in a possible decrease in MMG MF.

Additionally, when combining the EMG and MMG with the force signal, the electromechanical delay (EMD), that is, the latency between the onset of the muscle excitation (EMG) and the following mechanical response at the tendon insertion point (force) can be calculated (23,25). The EMD can be divided into a mainly electrochemical (e.g., the latency between the onset of the EMG and MMG signals) and a mainly mechanical component (e.g., the latency between the onset of the MMG and force signal) (4,23,29). In SM, an EMD elongation was reported, which persisted for at least 15 min after a passive stretching bout and encompassed both the electrochemical and mechanical components (4). However, in CM, no EMD analysis is currently available. In line with a possible reduction in MMG MF previously hypothesized, the EMD in CM should be elongated only in the electrochemical component due to a possible crossed reduction in central drive reflected into a slower sarcolemmal conduction velocity. No changes in the mechanical component of the overall delay should be expected. Lastly, the time course of these possible neuromuscular alterations in CM is, to date, unknown.

Therefore, the present study aimed to investigate whether or not passive stretching may induce an increase in range of motion and a reduction in the force generating capacity in CM. Additionally, the specific contribution of the neuromuscular and/or mechanical factors using both the interpolated-twitch technique and the simultaneous detection of the EMG, MMG, and force signals, was investigated. Lastly, the time course of these possible alterations was also measured.

METHODS

Study Design

The present investigation was designed as a cross-sectional, within-subject study. The sample size calculation was based on a previous investigation, considering the strength loss in CM as the reference parameter (19) and computed using statistical software (G-Power 3.1, Dusseldorf, Germany). Given the present design, the Cohen's *d* effect size (ES) = 0.65 computed using the referenced study, a two-tail effect, $\alpha = 0.05$ and a required power $(1 - \beta) = 0.80$, the desired sample size resulted in 21 participants. AQ3

Participants

Twenty-one healthy male participants (age, 22 yr [3 yr]; stature, 1.75 m [0.08 m]; body mass, 73 kg [9 kg], mean

[SD]) volunteered for the present investigation. The inclusion criteria were: (i) no evident orthopedic and/or neurological pathologies, (ii) no lower-limb muscular or joint injury in the previous 6 months, and (iii) no involvement in a systematic passive stretching routine in the previous 6 months. The local University Ethics Committee approved the study that was performed in accordance with the principles of the latest version of the Declaration of Helsinki. The participants gave their written informed consent after being fully explained on the purpose of the study and the experimental design. The participants were free to withdraw from the study at any time.

Procedures

All measurements were performed in a laboratory with constant room temperature (22°C [1°C]) and humidity (50% [3%]). To minimize the circadian changes in force and joint mobility, the tests were conducted at the same hour between 9:00 AM and 12.00 noon. The participants visited the laboratory three times. In the first session, they familiarized with the experimental set-up and passive stretching technique. Particularly, the participants got accustomed to the maximum voluntary contraction (MVC) and the interpolated twitch technique procedures. On this occasion, a map with some identification points over the skin (moles, scars, angiomas), together with the position of the angle transducers, EMG electrodes, and accelerometers were drawn on transparency sheets, to permit accurate electrodes repositioning consistency within the same area. Because the familiarization could result in a "learning effect," the second visit served as a control, in which the testing procedures were performed without any intervention in both limbs and replicated after the duration of the stretching protocol (POST) and then after 5 min ($POST_5$) and 10 min ($POST_{10}$) from its end. In this session, the limbs were randomly assigned to stretched or CM in the control condition (SM_{CTRL} and CM_{CTRL}, respectively). During the third visit, the participants underwent the unilateral knee extensors passive stretching intervention. The same testing procedures were repeated POST, $POST_5$, and $POST_{10}$. The stretched and contralateral limbs were the same as in the control condition within the second session (i.e., SM for SM_{CTRL} and CM for CM_{CTRL}). The limbs were tested in a randomized order.

To investigate the neuromuscular factors, the voluntary activation, EMG root mean square, mean fiber conduction velocity, M-wave, MMG MF and the delay between the onset of EMG and MMG signal (Δt EMG-MMG) were measured. To investigate the mechanical factors, MMG root mean square and the delay between the onset of the MMG and force signal (Δt MMG-F) were measured.

Measurements

Knee range of motion. The knee joint range of motion was measured by a bi-axial angle transducer (mod. TSD130B; Biopac System Inc., Santa Barbara, CA) previously calibrated, with the participants lying prone. The hip extension = 0° of extension, and hip adduction = 0° in relation to the anatomical position were visually checked by a second biaxial angle transducer positioned at hip level. The same operator performed the knee flexion maneuver until the point of discomfort. Full flexion was reached in 6 s, with a metronome beating time for reproducibility purposes. The angle transducer signals were driven to an A/D converter (mod. UM 150; Biopac System Inc.), sampled at 1000 Hz, directed to an auxiliary input of the electromyography amplifier (mod. EMG-USB; OtBioelettronica, Turin, Italy) and stored on a personal computer. The maneuver was repeated three times. The maximum joint angle reached during the maneuver was considered for the calculation of the maximum range of motion.

Maximum voluntary contraction. The knee extensors MVC was measured on both SM and CM with the participants lying supine, with the tested knee flexed at 90° and firmly secured at ankle level by a Velcro® strap (Velcro Industries Inc., Willemstad, Netherlands Antilles) to a load cell (mod. SM-2000 N operating linearly between 0 and 2000 N; Interface, Crowthorne, UK) for the force signal detection. The hips were maintained extended at 0° and secured by a Velcro® strap to the ergometer. After a standardized warm-up (10 × 2-s contractions at 50% MVC determined during familiarization), three MVC attempts were performed at baseline, whereas one MVC was executed at each posttest to reduce the effects of fatigue. The participants were instructed to push as fast and hard as possible for 3 s. Each MVC attempt was interspersed by at least 2 min of passive recovery. The force signal was driven to an A/D converter (mod. UM 150 Biopac; Biopac System Inc., sampled at 10,240 Hz, directed to an auxiliary input of the electromyography amplifier (mod. EMG-USB; OtBioelettronica) and stored on a personal computer. The maximum force value recorded during the MVC was inserted into the data analysis.

Voluntary activation. The voluntary activation level was calculated using the interpolated twitch technique. At baseline, the interpolated twitch technique was performed eliciting a superimposed doublet with an interpulse duration of 10 ms (100 Hz) (2,22). During this procedure, a stimulating electrode (24 × 24 mm circle electrode; Spes Medica, Battipaglia (SA), Italy) was positioned above the femoral nerve under the iliopubic ligament, whereas the receiving electrode (40 × 90 mm rectangular electrode; Spes Medica) was placed 2 cm under the superior posterior iliac spine. The electrodes were connected to a high-voltage stimulator (Digitimer Stimulator Model DS7AH, Hertfordshire, UK). The amperage (10 mA–1 A) of a 100- to 150-V square-wave pulse [1 ms, with an interpulse duration of 10 ms (22)] was progressively increased until the maximum elicited force was achieved. Thereafter, the doublet was elicited during (superimposed) and 5 s after (potentiated) each MVC. The force signal was also sent to an auxiliary input of the EMG amplifier (mod. EMG-USB; OtBioelettronica). The force signal was exported as .CSV file and converted in AcqKnowledge property file (AcqKnowledge 4.4; Biopac Systems, Goleta, CA). The software allowed calculating

the voluntary activation and the maximum force signal generated by the 100-Hz doublet, which was identified as the pF.

The voluntary activation was calculated as follows (21):

$$\text{voluntary activation} = [1 - (\text{superimposed force}/\text{potentiated force}) \times 100].$$

EMG assessment. The surface EMG signal was detected during the MVC *vastus lateralis, rectus femoris,* and *vastus medialis*. The EMG signal was detected by a linear array of eight electrodes (mod. ELSCH004; OtBioelettronica; probe 45 mm × 20 mm; electrode length 2 mm; interelectrode distance 10 mm) fixed to the skin by dual-adhesive foams (mod. AD004; OtBioelettronica) and filled with conductive gel (Cogel, Comedical, Trento, Italy). The skin area under the EMG electrodes was cleaned with ethyl alcohol, abraded gently with fine sandpaper and prepared with a conductive cream (Nuprep, Weaver and Co., Aurora, CO) to achieve an interelectrode impedance below 2000 Ω. For each muscle, the EMG array was placed over the muscle belly along the direction of the muscle fibers, in accordance with the European recommendations for surface EMG (30). The surface EMG signal was detected during the MVC and acquired by a multichannel amplifier with a sampling rate of 10,240 Hz (mod. EMG-USB; OtBioelettronica; input impedance: >90 MΩ; CMRR: >96 dB), amplified (gain, ×1000) and filtered (filter type: II order Butterworth filter; bandwidth, 10–500 Hz) for further analysis.

MMG assessment. The MMG signal was detected during the MVC and acquired with a sampling rate of 10,240 Hz by a multichannel amplifier (mod. EMG-USB; OtBioelettronica; input impedance, >90 MΩ; CMRR, >96 dB), amplified (gain, ×2), and filtered (filter type: II order Butterworth filter; bandwidth, 4–120 Hz) for further analysis. Three monodirectional accelerometers were placed between the second and the third electrodes of the EMG array on the *vastus lateralis, rectus femoris,* and *vastus medialis* (mod. ADXL103; Analog Devices, Norwood, MA; device weight, <1.0 g; sensitivity, 1000 $mV \cdot g^{-1}$; measure range, ±1.7 g) for MMG detection from the same muscle area as EMG.

EMD assessment. The criteria used to identify the reference points for EMD components have been fully reported in a previous investigation (23). Briefly, during the MVC, the EMD was divided into (i) Δt EMG-MMG, from the EMG to MMG onset; and (ii) Δt MMG-F, from the MMG to force onset. A threshold of three SD above the baseline signal for three consecutive points obtained in a 100-ms interval of the resting condition immediately preceding the contraction was used to the onset of the EMG, MMG, and force signal (23). To this purpose, the EMG, MMG, and force signals during the MVC were exported as .CSV file. Data analysis was performed off-line by a custom-built routine using a commercially available software (Labview 7.1; National Instruments, Austin, TX). The measurements were simultaneously performed in *vastus lateralis, rectus femoris,* and *vastus medialis*.

Signal processing and data analysis. The analysis was performed by the software OtBiolab+ (OtBioelettronica). The EMG and the MMG signal epochs were aligned with the same force signal epochs for a simultaneous quantification of the EMG and MMG parameters. The EMG signal was analyzed in time domain within the same 1-s period detected in the middle of the MVC plateau. The MMG signal was analyzed in time and frequency domain using the same time windows for the EMG signal analysis. The EMG and MMG root mean square were calculated in consecutive 250-ms time windows and then averaged. The mean fiber conduction velocity was automatically calculated by the OtBiolab+ software as follows (31):

$$\text{mean fiber conduction velocity} = d/\tau 0$$

where d is the interelectrode distance and τ0 is the delay between the two double differential signals. The MF of the MMG was calculated in consecutive 250-ms time windows applying a Fast Fourier Transform method. The EMG signal recorded during the M-wave elicited by the 100-Hz doublet recorded during the on-phase of the muscle contraction were exported as .CSV file and converted in .ACQ files (AcqKnowledge 4.4; Biopac Systems). The software allowed calculating the maximum peak-to-peak of the M-wave.

Stretching Protocol

During the passive stretching protocol, the participants remained supine with the nonstretched limb supported by a supplemental medical bed and the stretched limb supported by an operator. First, the participants were accustomed to the discomfort induced by the passive stretching through a visual analog scale. Then, an operator flexed the knee of the stretched limb until 80% to 90% of the maximal discomfort point. To minimize a possible muscle reflex activity, the elongation was reached in 6 s and maintained for 45 s (4,10). Five sets interspersed by 15 s of passive recovery were performed, for a total duration of 225 s. The EMG signal was checked during the passive stretching protocol to minimize any possible muscle activation during the elongation. Whether the EMG signal during the passive stretching bout was >5% of that obtained during the MVC, the participant was excluded from the study and replaced with another participant to ensure the warranted statistical power. In the control session, the participants laid supine as relaxed as possible with the knee extended for an equivalent duration.

Statistical Analysis

Statistical analysis was performed using a statistical software package (IBM SPSS Statistics 22, Armonk, NY). To check the normal distribution of the sampling, the Shapiro–Wilk's test was applied. The baseline values of the two experimental sessions were utilized to calculate the interday reliability and sensitivity. To determine the interday reliability, the intraclass correlation coefficient (ICC) and the standard error of the measurement (SEM%) were calculated. The ICC was interpreted as follows: ≥0.90, very high; 0.89 to 0.70, high; 0.69 to 0.50, moderate. The minimal detectable

TABLE 1. Interday reliability (ICC and SEM%) and sensitivity (MDC95%) in the SM and CM.

		SM					CM				
		Day 1, mean (SD)	Day 2. mean (SD)	ICC	SEM%	$MDC_{95\%}$	Day 1, mean (SD)	Day 2, mean (SD)	ICC	SEM%	$MDC_{95\%}$
	ROM (°)	137 (3)	139 (4)	0.93	3	5	139 (3)	138 (4)	0.94	3	5
	MVC (*N*)	665 (41)	665 (42)	0.87	4	8	666 (33)	669 (33)	0.89	4	7
	pF (*N*)	211 (12)	210 (14)	0.95	2	4	212 (13)	214 (11)	0.97	2	4
	VA (%)	97 (2)	96 (3)	0.92	1	2	98 (3)	97 (4)	0.93	1	2
Rectus femoris	EMG RMS (mV)	0.80 (0.04)	0.79 (0.06)	0.83	2	4	0.78 (0.06)	0.80 (0.06)	0.82	2	4
	CV ($m \cdot s^{-1}$)	5.5 (0.7)	5.5 (0.8)	0.90	2	4	5.4 (0.9)	5.5 (0.7)	0.91	2	4
	M-wave (mV)	0.80 (0.06)	0.79 (0.04)	0.93	2	5	0.81 (0.05)	0.80 (0.06)	0.90	2	5
	MMG RMS ($m \cdot s^{-2}$)	0.97 (0.06)	0.97 (0.06)	0.91	3	6	0.96 (0.06)	0.97 (0.06)	0.93	2	5
	MMG MF (Hz)	21 (1)	22 (1)	0.93	2	3	22 (1)	22 (1)	0.94	2	3
	Δt EMG-MMG (ms)	7.7 (1.1)	7.8 (1.1)	0.96	4	8	7.7 (1.3)	7.8 (1.3)	0.98	3	7
	Δt MMG-F (ms)	13.7 (2.2)	13.6 (2.1)	0.97	2	5	13.5 (2.2)	13.6 (2.2)	0.98	2	4
	EMD (ms)	21.4 (3.3)	21.4 (3.1)	0.99	1	3	21.2 (3.5)	21.4 (3.5)	0.98	2	3
Vastus lateralis	EMG RMS (mV)	0.96 (0.05)	0.98 (0.06)	0.94	1	3	0.94 (0.06)	0.96 (0.05)	0.81	3	5
	CV ($m \cdot s^{-1}$)	5.2 (0.4)	5.3 (0.7)	0.91	2	4	5.1 (0.6)	5.3 (0.6)	0.89	2	5
	M-wave (mV)	0.83 (0.05)	0.85 (0.06)	0.94	2	4	0.83 (0.07)	0.84 (0.06)	0.92	2	5
	MMG RMS ($m \cdot s^{-2}$)	0.95 (0.06)	0.95 (0.06)	0.93	2	3	0.96 (0.06)	0.96 (0.06)	0.92	2	4
	MMG MF (Hz)	21 (1)	21 (1)	0.95	1	3	22 (1)	22 (1)	0.96	1	2
	Δt EMG-MMG (ms)	7.7 (1.1)	7.7 (1.4)	0.96	4	8	7.6 (1.2)	7.7 (1.2)	0.97	4	7
	Δt MMG-F (ms)	13.9 (2.2)	13.6 (2.2)	0.97	3	5	13.4 (2.2)	13.7 (2.2)	0.98	2	4
	EMD (ms)	21.6 (3.3)	21.3 (3.5)	0.98	2	4	21.0 (3.4)	21.4 (3.5)	0.97	2	5
Vastus medialis	EMG RMS (mV)	0.90 (0.10)	0.92 (0.06)	0.93	2	3	0.89 (0.05)	0.90 (0.10)	0.82	2	5
	CV ($m \cdot s^{-1}$)	5.2 (0.3)	5.4 (0.2)	0.92	2	3	5.3 (0.2)	5.1 (0.3)	0.94	1	3
	M-wave (mV)	0.80 (0.05)	0.79 (0.04)	0.92	1	3	0.82 (0.05)	0.81 (0.05)	0.93	2	3
	MMG RMS ($m \cdot s^{-2}$)	0.95 (0.06)	0.95 (0.06)	0.94	2	3	0.96 (0.06)	0.96 (0.06)	0.95	1	3
	MMG MF (Hz)	22 (1)	22 (1)	0.94	2	3	23 (1)	22 (1)	0.95	1	2
	Δt EMG-MMG (ms)	7.6 (1.3)	7.3 (1.1)	0.99	2	4	7.4 (1.2)	7.3 (1.2)	0.98	3	6
	Δt MMG-F (ms)	14.2 (2.1)	14.0 (2.1)	0.98	2	4	13.9 (2.2)	14.0 (2.2)	0.98	2	5
	EMD (ms)	21.8 (3.5)	21.3 (3.2)	0.97	2	4	21.3 (3.5)	21.3 (3.5)	0.98	2	4

ROM, range of motion; RMS, root mean square; CV, mean fiber conduction velocity.

change with a 95% confidence interval ($MDC_{95\%}$) was used to detect the sensitivity of the intervention. The differences in range of motion, MVC, pF, and voluntary activation in SM and CM over time were evaluated by a two-way (limb–time) ANOVA for repeated measures. To calculate the between-muscle (*vastus lateralis, rectus femoris,* and *vastus medialis*) differences in the EMG, MMG, and EMD parameters in SM and CM, a three-way (muscle–limb–time) ANOVA for repeated measures was performed. Multiple comparisons were adjusted using the Bonferroni correction. To calculate the differences in the between-limb changes difference in the stretching-induced changes (SM vs CM, SM vs SM_{CTRL}, CM vs CM_{CTRL}), an ANCOVA was performed, assuming the baseline values as covariate. The significance was set at P value <0.05. If not otherwise stated, descriptive statistics are presented as mean (SD). The changes were reported as percentage change with a 95% confidence interval (95% CI). The Cohen's d ES was calculated and interpreted as follows: 0.00 to 0.19, trivial; 0.20 to 0.59, small; 0.60 to 1.19, moderate; 1.20 to 1.99, large; ≥2.00, very large. The 95% CI of the ES was also reported (https://www.cem.org/effect-size-calculator).

RESULTS

The ICC and $MDC_{95\%}$ in CM and SM are reported in Table 1. The ICC ranged from 0.81 to 0.98 and from 0.83 to 0.99 in CM and SM, respectively. The SEM% ranged from 1.1% to 5.7% and from 1.1 to 4.8 in CM and SM, respectively. Lastly, $MDC_{95\%}$ ranged from 2.1% to 10.8% and from 2.1% to 10.2% in CM and SM, respectively. T1

For each parameter, the main findings are shown in the Figures and explained in-text. Each pairwise comparison including ES with 95% CI is reported in the tables as Supplemental Digital Content.

The individual data for the range of motion are reported in Figure 1. Time–limb interaction was found for range of motion ($F_{3,240} = 12.14$, $P < 0.001$). A main effect for factor time F1

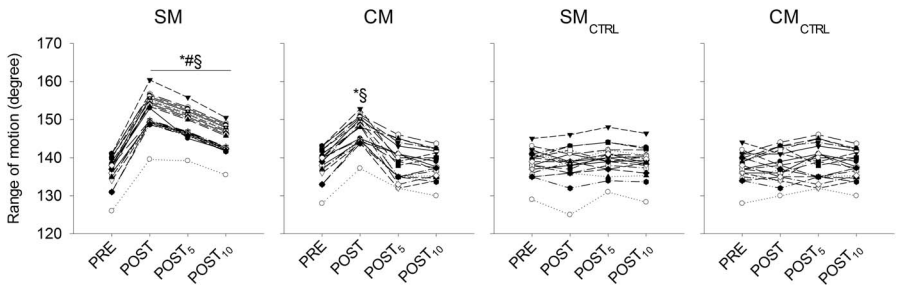


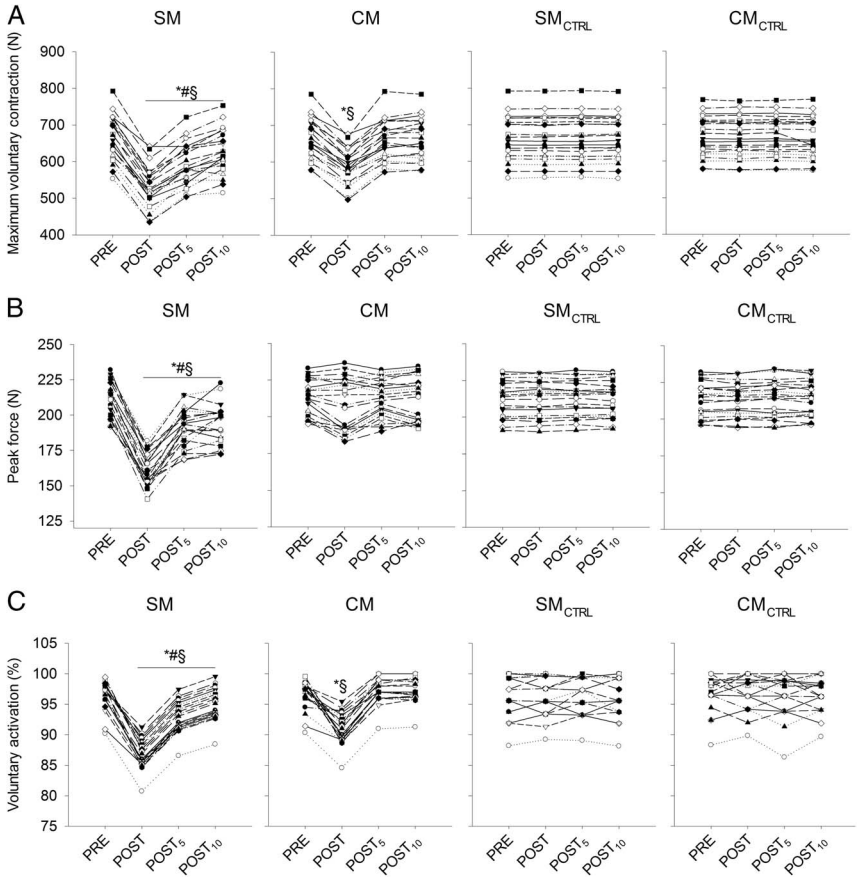
FIGURE 1—The individual data for the knee range of motion in the SM, CM, SM_{CTRL}, and CM_{CTRL} are shown for baseline (PRE), immediately after (POST), after 5 ($POST_5$) and 10 min of recovery ($POST_{10}$) from the passive stretching bout. *$P < 0.05$ vs PRE, #$P < 0.05$ SM vs CM, §SM vs SM_{CTRL} or CM vs CM_{CTRL}.

was observed ($F_{3,\ 20}$ = 17.57, P < 0.001). In CM, compared with PRE, range of motion increased at POST (6%; 95% CI, 1 to 11; P < 0.001), returning to baseline within 5 min. In SM, range of motion increased at POST (11%; 95% CI, 10 to 13; P < 0.001) and remained higher at $POST_5$ (9%; 95% CI, 8 to 10; P < 0.001) and $POST_{10}$ (6%; 95% CI, 5 to 7; P < 0.001). No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb ($F_{3,20}$ = 14.91, P < 0.001). The changes in range of motion at any time were greater in SM versus CM or versus SM_{CTRL}, and in CM versus CM_{CTRL} only at POST (see Table, Supplemental Digital Content 1, where Cohen's d ES with 95% CI of the changes in range of motion is shown, http://links.lww.com/MSS/B891).

F2 Figure 2 shows the individual data for the MVC (A), pF (B), and voluntary activation (C). Time–limb interaction was found for maximal voluntary contraction ($F_{3,240}$ = 10.72, P < 0.001). A main effect for factor time was observed ($F_{3,20}$ = 12.98, P < 0.001). In CM, the MVC decreased at POST (−11%; 95% CI, −13 to −9; P < 0.001), returning to baseline within 5 min. In SM, the MVC decreased at POST (−19%; 95% CI, −20 to −18; P < 0.001) and remained lower at $POST_5$ [−9% (−11 to −8), P < 0.001] and $POST_{10}$ (−6%; 95% CI, −7 to −4; P = 0.006). No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb ($F_{3,20}$ = 13.58 P < 0.001). The changes in the MVC at any time were greater in SM versus CM or versus SM_{CTRL}, and in CM versus CM_{CTRL} only at POST (see Table, Supplemental Digital Content 2, where Cohen's d ES with 95% CI of the changes in MVC is shown, http://links.lww.com/MSS/B892).

Time–limb interaction was found for the pF ($F_{3,240}$ = 9.09, P < 0.001). A main effect for factor time was observed ($F_{3,20}$ = 8.69, P < 0.001). In CM, the pF did not show any change. In SM, the pF decreased at POST (−25%; 95% CI, −28 to −22; P < 0.001) and remained lower at $POST_5$ (−10%; 95% CI, −13 to −7; P < 0.001) and $POST_{10}$ (−9; 95% CI, −11 to −6; P < 0.001). No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb ($F_{3,20}$ = 9.16, P < 0.001). The changes in pF at any time were greater in SM versus CM or versus SM_{CTRL}, whereas no difference occurred in CM versus CM_{CTRL} (see Table, Supplemental Digital Content 2, where Cohen's d ES with 95% CI of the changes in pF is shown, http://links.lww.com/MSS/B892).

Time–limb interaction was found for the voluntary activation ($F_{3,240}$ = 10.54, P < 0.001). A main effect for factor time was observed ($F_{3,20}$ = 15.39, P < 0.001). In CM, the voluntary activation decreased at POST (−7; 95% CI, −9 to −4; P < 0.001),



AQ4 FIGURE 2—The individual data for the MVC (A), VA, and pF (C) in the SM, CM, SM_{CTRL}, and CM_{CTRL} are shown for baseline (PRE), immediately after (POST), after 5 ($POST_5$) and 10 min of recovery ($POST_{10}$) from the passive stretching bout. *P < 0.05 vs PRE, #P < 0.05 SM vs CM, §SM vs SM_{CTRL} or CM vs CM_{CTRL}.

returning to baseline at $POST_{10}$ In SM, compared with PRE, the voluntary activation decreased at POST (−10%; 95% CI, −11 to −9; $P < 0.001$) and remained lower at $POST_5$ (4%; 95% CI, −5 to −3; $P < 0.001$), returning to baseline at $POST_{10}$. No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb ($F_{3,20} = 13.88$, $P < 0.001$). The changes in voluntary activation at POST and $POST_5$ were greater in SM versus CM or versus SM_{CTRL}, whereas no difference occurred in CM versus CM_{CTRL} only at POST (see Table, Supplemental Digital Content 2, where Cohen's *d* ES with 95% CI of the changes in voluntary activation is shown, http://links.lww.com/MSS/B892).

The changes in EMG root mean square, conduction velocity and M-wave are shown in Figure 3. There was no muscle–time–limb interaction for EMG root mean square ($F_{6,720} = 0.93$, $P = 0.470$), mean fiber conduction velocity ($F_{6,720} = 1.18$, $P = 0.311$) and M-wave ($F_{6,720} = 0.65$, $P = 0.689$), indicating that rectus femoris, vastus lateralis and vastus medialis experienced similar trends for each parameter. In CM, considering all muscles, EMG root mean square decreased by ≈−16% ($P < 0.05$) at POST, mean fiber conduction velocity by ≈−16% ($P < 0.05$) and returned to baseline within 5 min. No change in M-wave occurred. In SM, EMG root mean square decreased at POST (≈−33%, $P < 0.001$) and remained lower at $POST_5$ (≈−29%, $P < 0.05$) and $POST_{10}$ (≈−20%, $P < 0.05$). Mean fiber conduction velocity decreased at POST (≈−22%, $P < 0.001$) and remained lower at $POST_5$ (≈−22%, $P < 0.05$) and $POST_{10}$ (≈−12%, $P < 0.05$). No change in M-wave occurred. No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb in EMG root mean square ($F_{3,20} = 8.54$, $P < 0.001$), mean fiber conduction velocity ($F_{3,20} = 8.67$, $P < 0.001$), but not for M-wave ($F_{3,20} = 0.36$, $P = 0.969$). The changes in EMG root mean square and mean fiber conduction velocity at POST and $POST_5$ and $POST_{10}$ were greater in SM versus CM or versus SM_{CTRL}, whereas these changes in CM versus CM_{CTRL} were greater only at POST. No between-limb difference in M-wave was retrieved (see Table, Supplemental Digital Content 3, Supplemental Digital Content 4 and Supplemental Digital Content 5, where Cohen's *d* ES with 95% CI of the changes in EMG root mean square, conduction velocity and M-wave, respectively is shown, http://links.lww.com/MSS/B893,

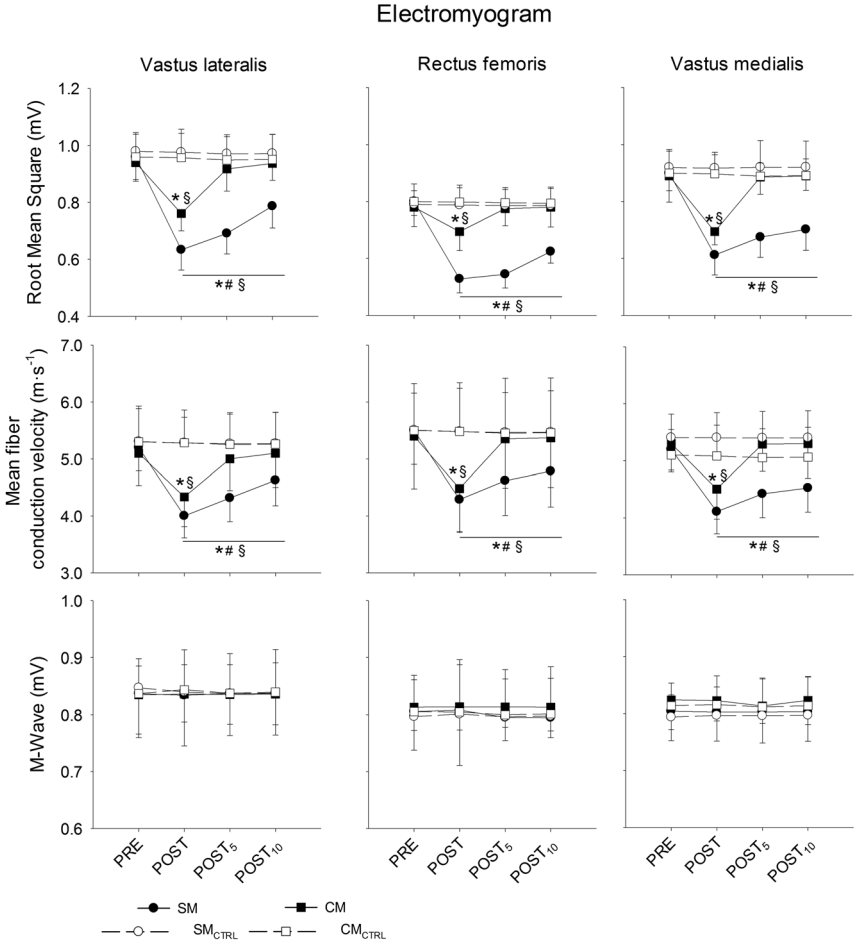


FIGURE 3—The changes in EMG root mean square, mean fiber conduction velocity, and M-wave in vastus lateralis (left side column), rectus femoris (central column), and vastus medialis (right side column) are shown for the SM (*closed circle*), CM (*closed square*), SM_{CTRL} (*open circle*), and CM_{CTRL} (*open square*) at baseline (PRE), immediately after (POST), after 5 ($POST_5$) and 10 min of recovery ($POST_{10}$) from the passive stretching bout. *$P < 0.05$ vs PRE, #$P < 0.05$ SM vs CM, §SM vs SM_{CTRL} or CM vs CM_{CTRL}.

http://links.lww.com/MSS/B894, and http://links.lww.com/MSS/B895).

The changes in MMG root mean square and MF are shown in Figure 4. There was no muscle–time–limb interaction for MMG root mean square ($F_{6,720}$ = 0.71, P = 0.639) and MMG MF ($F_{6,720}$ = 0.60, P = 0.724), indicating that rectus femoris, vastus lateralis and vastus medialis experienced similar trends for each parameter. In CM, considering all muscles, MMG root mean square decreased by ≈−9% (P < 0.05), MMG MF by ≈−18% (P < 0.05) and returned to baseline within 5 min. In SM, MMG root mean square increased at POST (≈+29%, P < 0.001) and remained higher at $POST_5$ (≈+16%, P < 0.05) and $POST_{10}$ (≈+7%, P < 0.05). The MMG MF decreased at POST (≈−24%, P < 0.001) and remained lower at $POST_5$ (≈−14%, P < 0.05) and $POST_{10}$ (≈−10%, P < 0.05). No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb in MMG root mean square ($F_{3,20}$ = 13.12, P < 0.001) and MMG MF ($F_{3,20}$ = 12.74, P < 0.001). The changes in MMG root mean square and MMG MF at POST, $POST_5$, and $POST_{10}$ were greater in SM versus CM or versus SM_{CTRL}, whereas these changes in CM versus CM_{CTRL} were greater only at POST (see Table, Supplemental Digital Content 6 and Supplemental Digital Content 7, where Cohen's d ES with 95% CI of the changes in MMG root mean square and MF, respectively, is shown, http://links.lww.com/MSS/B896 and http://links.lww.com/MSS/B897).

The changes in the total EMD, Δt EMG-MMG and Δt MMG-F are shown in Figure 5. There was no muscle–time–limb interaction for the total EMD ($F_{6,720}$ = 0.91, P = 0.493), Δt EMG-MMG ($F_{6,720}$ = 0.60, P = 0.728) and Δt MMG-F ($F_{6,720}$ = 0.67, P = 0.671), indicating that rectus femoris, vastus lateralis and vastus medialis experienced similar trends for each parameter. In CM, considering all muscles, the total EMD and Δt EMG-F did not change, whereas EMG-MMG increased by ≈+10% (P < 0.05) at POST and returned to baseline within 5 min. In SM, the total EMD increased at POST (≈+28%, P < 0.001) and remained lower at $POST_5$ (≈+19%, P < 0.05) and $POST_{10}$ (≈+12%, P < 0.05). Δt EMG-MMG increased at POST (≈+26%, P < 0.001) and remained higher at $POST_5$ (≈+15%, P < 0.05) and $POST_{10}$ (≈+8%, P < 0.05). Δt MMG-F increased at POST (≈+29%, P < 0.001) and remained higher at $POST_5$ (≈+21%, P < 0.05) and $POST_{10}$ (≈+14%, P < 0.05). No change in SM_{CTRL} and CM_{CTRL} was observed. ANCOVA retrieved a main effect for factor limb in the total EMD ($F_{3,20}$ = 8.82, P < 0.001), Δt EMG-MMG ($F_{3,20}$ = 9.33, P < 0.001) and Δt MMG-F ($F_{3,20}$ = 9.13, P < 0.001). The changes in the total EMD, Δt EMG-MMG, and Δt MMG-F at POST, $POST_5$, and $POST_{10}$ were greater in SM versus CM or versus SM_{CTRL}, whereas these changes in CM versus CM_{CTRL} were greater only at POST (see Table, Supplemental Digital Content 8, Supplemental Digital Content 9 and Supplemental Digital Content 10, where Cohen's d ES with 95% CI of the

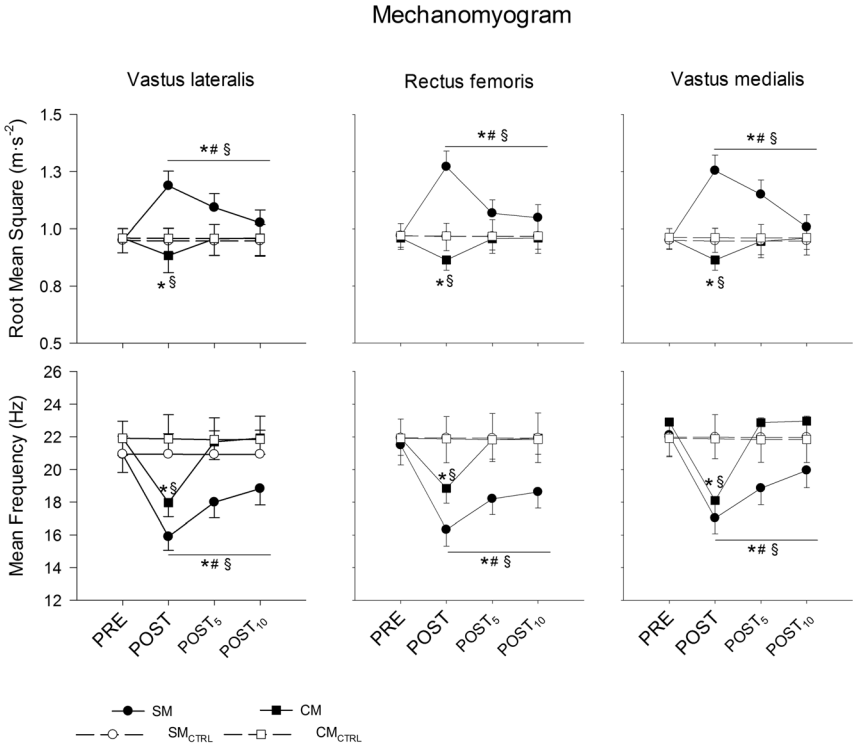


FIGURE 4—The changes in MMG root mean square and MF in vastus lateralis (left side column), rectus femoris (central column), and vastus medialis (right side column) are shown for the SM (*closed circle*), CM (*closed square*), SM_{CTRL} (*open circle*), and CM_{CTRL} (*open square*) at baseline (PRE), immediately after (POST), after 5 ($POST_5$) and 10 min of recovery ($POST_{10}$) from the passive stretching bout. *P < 0.05 vs PRE, #P < 0.05 SM vs CM, §SM vs SM_{CTRL} or CM vs CM_{CTRL}.

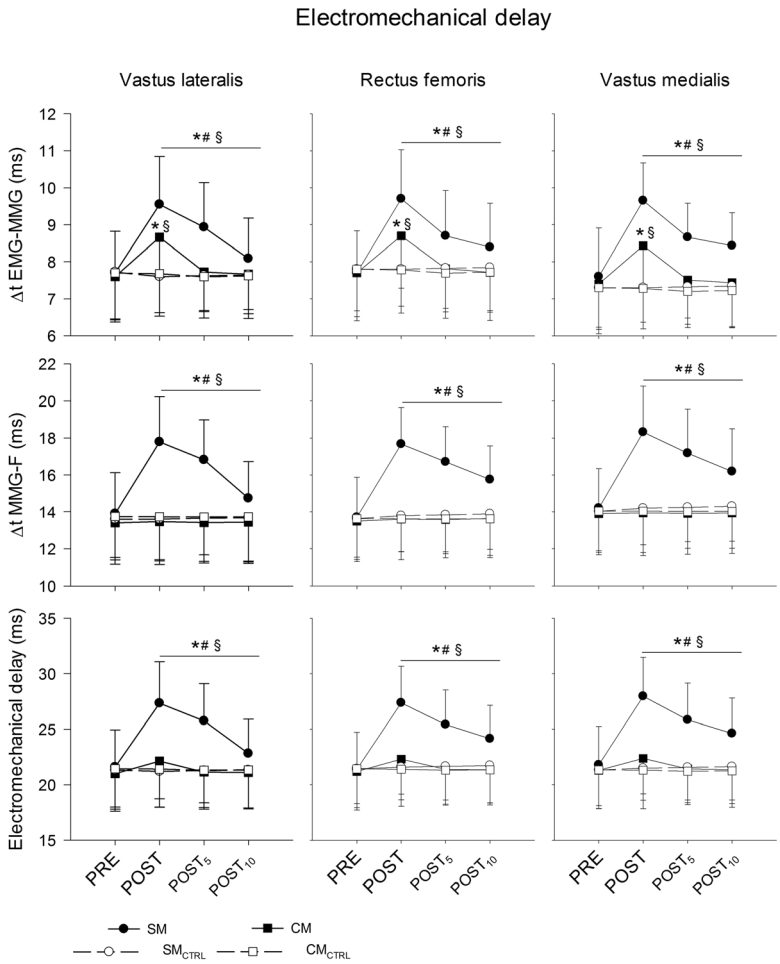


FIGURE 5—The changes in the delay between the EMG and MMG signals onset (Δt EMG-MMG, upper panels), the delay between the MMG and force signals onset (Δt MMG-F, middle panels), and in the total EMD (lower panels) in vastus lateralis (left side column), rectus femoris (central column), and vastus medialis (right side column) are shown for the SM(*closed circle*), CM (*closed square*), SM_{CTRL} (*open circle*), and CM_{CTRL} (*open square*) at baseline (PRE), immediately after (POST), after 5 ($POST_5$) and 10 min of recovery ($POST_{10}$) from the passive stretching bout. *$P < 0.05$ vs PRE, #$P < 0.05$ SM vs CM, §SM vs SM_{CTRL} or CM vs CM_{CTRL}.

changes in the total EMD, Δt EMG-MMG and Δt MMG-F respectively is shown, http://links.lww.com/MSS/B898, http://links.lww.com/MSS/B899, http://links.lww.com/MSS/B900).

DISCUSSION

The current study investigated whether or not passive stretching could induce changes in the range of motion and muscle force-generating capacity in CM, similarly to what usually reported in SM. The contribution of the neuromuscular and mechanical factors underlying the passive stretching-induced changes in CM was also assessed using the interpolated-twitch technique and the simultaneous detection of the EMG, MMG and force signals. In CM, the range of motion increased and the MVC decreased immediately after the passive stretching bout but both recovered within 5 min. Additionally, the changes in range of motion and MVC in CM were accompanied by a reduction in voluntary activation, EMG and MMG activity and by a rise in Δt EMG-MMG. A return to baseline within 5 min with no further change was observed also in these parameters. Altogether, the present findings support the hypothesis that the changes in range of motion and force-generating capacity in CM seem to be mainly affected by crossed neuromuscular factors occurring at the central level. All these stretching-induced changes were greater and longer-lasting in SM. Lastly, as expected, no change in any parameter occurred in in SM_{CTRL} and CM_{CTRL} and the *high* reliability recorded here indicates that the observed findings are attributable to the intervention and not to the possible bias introduced by the study design and not even to low reproducibility of the protocols used.

Range of motion. Increases in range of motion were already observed in both CM and SM (11,12,15,17). However, the changes in CM observed here returned to baseline sooner (within 5 min) than those in SM (>10 min). An increase in range of motion is usually attributable to a decrease in the muscle-tendon unit stiffness, which depends on: (i) the cross-link between the actin and myosin filaments (32); (ii) the noncontractile proteins of the endosarcomeric (33) and exosarcomeric cytoskeletons (34); (iii) the changes in the viscoelastic properties of the connective tissue located within and surrounding the muscle (33); and (iv) an augmented stretch-tolerance

occurring during passive stretching, involving a reduction in muscle tone possibly associated with a reduction in the nociceptive activity (35). The first three factors mainly hark back to mechanical stimuli, which CM was not involved in. Consequently, the augmented stretch tolerance is the only factor that could have occurred in CM, being the only one involving neuromuscular pathways. In contrast, all the aforementioned mechanisms could account for the increment in range of motion observed in SM immediately after passive stretching, thus involving both neuromuscular and mechanical factors (4,10). The protracting of these mechanisms over time was shown to be different because the mechanical factors can last over 2 h (4,10), whereas the neuromuscular factors are no longer impaired after 5 to 10 min (35). Taking it together, the rise in stretch-tolerance might be pointed as the most likely mechanism to explain the increase in range of motion in CM.

Force. The force-generating capacity depends on both neuromuscular (8) and mechanical factors (1). The procedures adopted in the present study allowed to partition the contribution of each factor, as discussed in the following sections. In CM, a reduction in the MVC occurred, returning to baseline within 5 min. The literature reported inconsistent results on this phenomenon, because some studies showed falls in maximal force (15,16,19,20), whereas other studies did not observe any change (2,11,12). Such an inconsistency may derive from the type of muscle stretched, the stretching modality (e.g., passive or dynamic stretching), the overall duration of the passive stretching protocol and the stretching intensity (8). In SM, as previously reported in the literature, the MVC and the pF were reduced more than 10 min after passive stretching administration, as in previous investigations (1,10) reporting long-lasting passive stretching-induced reductions in force (2) that persisted up to 2 h (4,10). (4,10)

AQ5

Voluntary activation. The loss in voluntary activation in CM and SM observed here are suggestive of a passive stretching-induced inhibitory effect occurring at supraspinal level, leading to a simultaneous reduction in the motor drive toward both CM and SM (11,12,16). This inhibitory effect may derive from an afferent feedback originating from the peripheral mechanoreceptors, proprioceptors and nociceptors located in SM (3,12,15,16). It seems that such an inhibitory afferent feedback might have been transferred to the contralateral hemisphere, resulting in a reduction in the voluntary activation shown here in CM. The interhemispheric connection between the cortical motor areas via *the corpus callosum* (36) and the subcortical neural pathways associated with the cortical-subcortical loops between the basal ganglia and cerebellum (37) have been proposed as possible pathways permitting the afferent stimuli to be crossed over. It should be noted that the cortical activity may not be affected by a passive stretching bout, given the unchanged motor evoked potential reported in previous studies (38–40). Additionally, a passive stretching bout could alter the monoaminergic (e.g., norepinephrine and serotonin) drive to the motoneurons' excitability in a nonlocalized way via an increase in parasympathetic activity (41), possibly decreasing muscle force in CM and SM (8,42). In CM, the voluntary activation returned to baseline after 5 min, in line with the decrement in MVC. In SM, the drop in voluntary activation disappeared after 10 min. In line with the present findings, voluntary activation returned to baseline after 15 min (8) in plantar flexors but was still reduced after passive stretching bout in quadriceps and hamstrings (43), leading to hypothesize a muscle-specific effect.

EMG. In both CM and SM, EMG root mean square, and mean fiber conduction velocity were reduced immediately after the passive stretching bout. The drop in EMG root mean square is suggestive of a reduction in the gross motor drive occurring concurrently at the supraspinal, spinal, and/or peripheral level (8). The reduction in mean fiber conduction velocity suggests that the velocity at which the activated motor units are recruited during a muscle contraction decreased (44). Particularly, mean fiber conduction velocity depends mainly on the type of recruited motor units, because the higher the number of high-threshold motor units activated, the higher the mean fiber conduction velocity (44), suggesting that the passive stretching bout could have mainly inhibited the high-threshold motor units. The present findings agree with a previous study reporting a decrease in EMG root mean square in the contralateral homologous quadriceps (19). However, no previous studies recorded the mean fiber conduction velocity in the CM. Based on the current outcomes, it seems that a specific crossed inhibition may have occurred in CM in the high-threshold motor units. In CM, EMG root mean square and mean fiber conduction velocity returned to baseline within 5 min, whereas in SM they remained depressed longer than 10 min (14). In contrast, no change occurred in M-wave, both in CM and in SM. As reported previously, an unchanged electrically-evoked EMG response after passive stretching indicates that the neuromuscular synapsis efficiency and the sarcolemma action-potential propagation properties have been preserved (31). Thus, the reduction in EMG root mean square and mean fiber conduction velocity but not in M-wave suggests that the reduction in motor drive could be ascribed to central but not peripheral neuromuscular factors.

MMG. The MMG root mean square adds mechanical information to the neuromuscular data provided by the EMG root mean square (26). In CM, the decrease in MMG root mean square immediately after the passive stretching bout is coupled with the aforementioned reduction in EMG root mean square, and could reflect a reduced number of active motor units recruited during the MVC (26). On the contrary, in SM MMG root mean square increased and remained higher compared with baseline up to 10 min. It is to bear in mind that during the plateau phase of contraction, both the in-series elastic components and the contractile elements reach their isometric condition, during which the MMG signal amplitude is mainly influenced by the contractile and in-parallel viscoelastic elements (26). A rise in MMG root mean square was reported in the *gastrocnemius medialis* muscle after a similar passive stretching protocol (10). Additionally, MMG root mean

square was reported to be inversely correlated with the muscle, tendon and muscle-tendon unit stiffness, measured as the passive force/angle relationship (1). The authors showed that such a correlation persisted after passive stretching and addressed it to an augmented mechanical compliance (1). In line, the increased MMG root mean square shown here in SM leads to argue that the mechanical factors might have exceeded the neuromuscular factors. Therefore, this corroborates the hypothesis that the changes in CM are mainly due to neuromuscular rather than mechanical factors. The different behavior of the MMG root mean square in CM versus SM offers an interesting point of view to understand the contribution of the neuromuscular and mechanical factors in decreasing the force-generating capacity. In this scenario, although CM appears to be sensitive to the neuromuscular factors only, SM is likely affected by both neuromuscular and mechanical factors.

The MMG MF decreased in both CM and SM but remained lower than baseline after 10 min only in SM. The reduction in MMG MF reflects the fall in firing rate of the high-threshold motor units (26). Taking it together, the different behavior of the MMG signal in CM versus SM offers an interesting point of view to understand the contribution of the neuromuscular and mechanical factors in decreasing the force-generating capacity. In this scenario, although CM appears to be sensitive to the neuromuscular factors only, SM is likely affected by both neuromuscular and mechanical factors.

Delays. The total EMD is the sum of Δt EMG-MMG and Δt MMG-F. These represent the delay from the onset of the EMG to MMG signal and from the onset of the MMG to the beginning of the force generation, respectively (23). Specifically, the former denotes the time delay between the action-potential propagation along the sarcolemma underneath the EMG electrodes and the cross-bridge activation, that is, the first mechanical event occurring at the muscle-belly level before the tensioning of the in-series elastic components (4). Instead, the latter monitors the overall duration of the mechanical events after the cross-bridge formation, that is, the elongation of the in-series elastic components, the transmission of the force exerted to the tendon insertion point (4). The raise in Δt EMG-MMG may depend on: (i) the amount of force generated during the maximum voluntary isometric contraction (23); (ii) the sarcolemmal excitability (45); (iii) a possible reduction in Ca^{2+} release and sensitivity (45); (iv) a desensitization of the link between the dihydropyridine and ryanodine receptors (45); (v) a possible impairment of the t-tubules depolarization process; (vi) alterations in the contractile cross-bridge efficiency and elastic properties of the semi-active proteins (e.g., titin) (46). Particularly, the first two mechanisms include eventual changes in supraspinal motor drive (23). In CM, only Δt EMG-MMG increased immediately after the passive stretching bout, recovering within 5 min, whereas in SM, the elongation of the total EMD was due to the simultaneous rises in both Δt EMG-MMG and Δt MMG-F. The slower and longer-lasting Δt EMG-MMG delay observed in SM versus CM can be accounted for the simultaneous contribution of both peripheral and supraspinal mechanisms, whereas only the supraspinal mechanisms can be pointed out in CM, that is, the first aforementioned mechanism. The elongation in Δt EMG-MMG in CM possibly due to a different motor drive (slower sarcolemmal conduction velocity) is in line with the decrease in MMG MF, which reflects a reduction of the average firing rate of the active fibers. Lastly, the increase in Δt MMG-F in SM, but not in CM, reflects the decrement in muscle and tendon stiffness only after a mechanical stress directly in the stretched limb (23).

Limitations. The present study comes with some acknowledged limitations. First, the spinal mechanisms possibly implied in the passive stretching-induced effect on CM and SM have not been investigated. It is acknowledged that the present design could have benefited from this further assessment and future studies should address this point. Second, no information about the role played by the peripheral receptors has been provided. Future studies are required to clarify their role. Thirdly, the entire procedures were conducted at a "neutral" ankle angle position (i.e., 90°). Because it was shown that muscle length affected the stretching-induced neuromuscular response (47,48), assessing the procedures at short or long muscle length could result in different outcomes. Future studies should check this. Lastly, the present results may depend specifically on the stretching modality, its overall duration and the muscles involved. Different procedures may likely incur in different outcomes.

CONCLUSIONS

In conclusion, a passive stretching bout increased the range of motion in both CM and SM, although it returned to baseline within 5 min in CM and persisted in SM up to 10 min. Additionally, as shown, the passive stretching-induced strength loss in CM seems to be mainly affected by crossed neuromuscular factors occurring at the central level that disappeared within 5 min. Conversely, the strength loss seen in SM depends on both neuromuscular and mechanical factors that overall persist up to 10 min.

The authors are grateful to the participants that volunteered for the present procedures.

The present investigation was funded by a dedicated grant (PSR2017_CE) provided by the Department of Biomedical Sciences for Health, University of Milan. The authors declared no professional relationship with companies or manufacturers that might benefit from the results of the present study. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. Results of the present study do not constitute endorsement by ACSM.

The authors approved the final version of the article.

The authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

The present study was conducted in the Exercise Physiology Laboratory of the Department of Biomedical Sciences for Health, University of Milan.

E. C. and G. C. contributed equally to the work. E. C., G. C., F. E. conceived the experimental design of the work. E. C., G. C., A. V. B., M. V., E. L., C. D., S. D., S. L. collected, analyzed and interpreted the data. E. C., G. C., A. V. B., M. V., E. L., C. D., S. D., S. L., F. E. drafted the work and revised it critically.

REFERENCES

1. Longo S, Cè E, Rampichini S, Devoto M, Limonta E, Esposito F. Mechanomyogram amplitude correlates with human gastrocnemius medialis muscle and tendon stiffness both before and after acute passive stretching. *Exp Physiol*. 2014;99(10):1359–69.
2. Power K, Behm D, Cahill F, Carroll M, Young W. An acute bout of static stretching: effects on force and jumping performance. *Med Sci Sports Exerc*. 2004;36(8):1389–96.
3. Avela J, Kyröläinen H, Komi PV. Altered reflex sensitivity after repeated and prolonged passive muscle stretching. *J Appl Physiol*. 1999;86(4):1283–91.
4. Esposito F, Limonta E, Cè E. Passive stretching effects on electromechanical delay and time course of recovery in human skeletal muscle: new insights from an electromyographic and mechanomyographic combined approach. *Eur J Appl Physiol*. 2011;111(3):485–95.
5. Venturelli M, Cè E, Limonta E, et al. Central and peripheral responses to static and dynamic stretch of skeletal muscle: mechano- and metaboreflex implications. *J Appl Physiol*. 2017;122(1):112–20.
6. Venturelli M, Rampichini S, Coratella G, et al. Heart and musculoskeletal hemodynamic response to repetitive bouts of quadricepes static stretching. *J Appl Physiol*. 2019;127(2):376–84.
7. Magnusson SP, Simonsen EB, Aagaard P, Sørensen H, Kjaer M. A mechanism for altered flexibility in human skeletal muscle. *J Physiol*. 1996;497(Pt 1):291–8.
8. Trajano GS, Nosaka K, Blazevich AJ. Neurophysiological mechanisms underpinning stretch-induced force loss. *Sports Med*. 2017; 47(8):1531–41.
9. Cè E, Longo S, Rampichini S, et al. Stretch-induced changes in tension generation process and stiffness are not accompanied by alterations in muscle architecture of the middle and distal portions of the two gastrocnemii. *J Electromyogr Kinesiol*. 2015;25(3): 469–78.
10. Esposito F, Limonta E, Cè E. Time course of stretching-induced changes in mechanomyogram and force characteristics. *J Electromyogr Kinesiol*. 2011;21(5):795–802.
11. da Silva JJ, Behm DG, Gomes WA, et al. Unilateral plantar flexors static-stretching effects on ipsilateral and contralateral jump measures. *J Sports Sci Med*. 2015;14(2):315–21.
12. Marchetti PH, Silva FH, Soares EG, et al. Upper limb static-stretching protocol decreases maximal concentric jump performance. *J Sports Sci Med*. 2014;13(4):945–50.
13. Behm DG, Cavanaugh T, Quigley P, Reid JC, Nardi PSM, Marchetti PH. Acute bouts of upper and lower body static and dynamic stretching increase non-local joint range of motion. *Eur J Appl Physiol*. 2016;116(1):241–9.
14. Masugi Y, Obata H, Inoue D, Kawashima N, Nakazawa K. Neural effects of muscle stretching on the spinal reflexes in multiple lower-limb muscles. *PLoS One*. 2017;12(6):e0180275.
15. Chaouachi A, Padulo J, Kasmi S, Othmen AB, Chatra M, Behm DG. Unilateral static and dynamic hamstrings stretching increases contralateral hip flexion range of motion. *Clin Physiol Funct Imaging*. 2017;37:23–9.
16. Cramer JT, Housh TJ, Johnson GO, Miller JM, Coburn JW, Beck TW. Acute effects of static stretching on peak torque in women. *J Strength Cond Res*. 2004;18(2):236–41.
17. Lima BN, Lucareli PR, Gomes WA, et al. The acute effects of unilateral ankle plantar flexors static- stretching on postural sway and gastrocnemius muscle activity during single-leg balance tasks. *J Sports Sci Med*. 2014;13(3):564–70.
18. Rouissi M, Chtara M, Owen A, et al. Effect of leg dominance on change of direction ability amongst young elite soccer players. *J Sports Sci*. 2016;34(6):542–8.
19. Cramer JT, Housh TJ, Weir JP, Johnson GO, Coburn JW, Beck TW. The acute effects of static stretching on peak torque, mean power output, electromyography, and mechanomyography. *Eur J Appl Physiol*. 2005;93(5–6):530–9.
20. Cramer JT, Housh TJ, Coburn JW, Beck TW, Johnson GO. Acute effects of static stretching on maximal eccentric torque production in women. *J Strength Cond Res*. 2006;20(2):354–8.
21. Rozand V, Grosprêtre S, Stapley PJ, Lepers R. Assessment of neuromuscular function using percutaneous electrical nerve stimulation. *J Vis Exp*. 2015;(103).
22. Coratella G, Grosprêtre S, Gimenez P, Mourot L. Greater fatigability in knee-flexors vs. knee- extensors after a standardized fatiguing protocol. *Eur J Sport Sci*. 2018;18(8):1110–8.
23. Cè E, Rampichini S, Agnello L, Limonta E, Veicsteinas A, Esposito F. Effects of temperature and fatigue on the electromechanical delay components. *Muscle Nerve*. 2013;47(4):566–76.
24. Cè E, Rampichini S, Monti E, Venturelli M, Limonta E, Esposito F. Changes in the electromechanical delay components during a fatiguing stimulation in human skeletal muscle: an EMG, MMG and force combined approach. *Eur J Appl Physiol*. 2017;117(1):95–107.
25. Rampichini S, Cè E, Limonta E, Esposito F. Effects of fatigue on the electromechanical delay components in gastrocnemius medialis muscle. *Eur J Appl Physiol*. 2014;114(3):639–51.
26. Cè E, Rampichini S, Esposito F. Novel insights into skeletal muscle function by mechanomyography: from the laboratory to the field. *Sport Sci Health*. 2015;11(1):1–28.
27. Orizio C, Gobbo M, Diemont B, Esposito F, Veicsteinas A. The surface mechanomyogram as a tool to describe the influence of fatigue on biceps brachii motor unit activation strategy. Historical basis and novel evidence. *Eur J Appl Physiol*. 2003;90(3–4):326–36.
28. Vigotsky AD, Halperin I, Lehman GJ, Trajano GS, Vieira TM. Interpreting signal amplitudes in surface electromyography studies in sport and rehabilitation sciences. *Front Physiol*. 2018;8:985.
29. Sasaki K, Sasaki T, Ishii N. Acceleration and force reveal different mechanisms of electromechanical delay. *Med Sci Sports Exerc*. 2011;43(7):1200–6.
30. Hermens HJ, Freriks B, Merletti R, et al. European recommendations for surface ElectroMyoGraphy. *Roessingh Res Dev*. 1999;8–11.
31. Cè E, Paracchino E, Esposito F. Electrical and mechanical response of skeletal muscle to electrical stimulation after acute passive stretching in humans: a combined electromyographic and mechanomyographic approach. *J Sports Sci*. 2008;26(14): 1567–77.
32. Proske U, Morgan DL. Do cross-bridges contribute to the tension during stretch of passive muscle? *Review*. 1999;20(5–6):433–42.
33. Borg TK, Caulfield JB. Morphology of connective tissue in skeletal muscle. *Tissue Cell*. 1980;12(1):197–207.
34. Magid A, Law DJ. Myofibrils bear most of the resting tension in frog skeletal muscle. *Science*. 1985;230(4731):1280–2.
35. Magnusson SP, Simonsen EB, Aagaard P, Dyhre-Poulsen P, McHugh MP, Kjaer M. Mechanical and physiological responses to stretching with and without preisometric contraction in human skeletal muscle. *Arch Phys Med Rehabil*. 1996;77(4):373–8.
36. Carson RG. Neural pathways mediating bilateral interactions between the upper limbs. *Brain Res Rev*. 2005;49(3):641–62.
37. Wiesendanger R, Wiesendanger M. Cerebello-cortical linkage in the monkey as revealed by transcellular labeling with the lectin wheat germ agglutinin conjugated to the marker horseradish peroxidase. *Exp Brain Res*. 1985;59(1):105–17.
38. Pulverenti TS, Trajano GS, Kirk BJC, Blazevich AJ. The loss of muscle force production after muscle stretching is not accompanied by altered corticospinal excitability. *Eur J Appl Physiol*. 2019;119(10): 2287–99.
39. Budini F, Gallasch E, Christova M, Rafolt D, Rauscher AB, Tilp M. One minute static stretch of plantar flexors transiently increases H reflex excitability and exerts no effect on corticospinal pathways. *Exp Physiol*. 2017;102(8):901–10.
40. Budini F, Kemper D, Christova M, Gallasch E, Rafolt D, Tilp M. Five minutes static stretching influences neural responses at spinal

level in the background of unchanged corticospinal excitability. *J Musculoskelet Neuronal Interact*. 2019;19(1):30–7.
41. Kruse NT, Scheuermann BW. Cardiovascular responses to skeletal muscle stretching: "stretching" the truth or a new exercise paradigm for cardiovascular medicine? *Sports Med*. 2017;47(12):2507–20.
42. Trajano GS, Seitz LB, Nosaka K, Blazevich AJ. Can passive stretch inhibit motoneuron facilitation in the human plantar flexors? *J Appl Physiol*. 2014;117(12):1486–92.
43. Reid JC, Greene R, Young JD, Hodgson DD, Blazevich AJ, Behm DG. The effects of different durations of static stretching within a comprehensive warm-up on voluntary and evoked contractile properties. *Eur J Appl Physiol*. 2018;118(7):1427–45.
44. Merletti R, Farina D, Gazzoni M. The linear electrode array: a useful tool with many applications. *J Electromyogr Kinesiol*. 2003;13(1):37–47.
45. Bruton JD, Lännergren J, Westerblad H. Mechano-sensitive linkage in excitationcontractioncontraction coupling in frog skeletal muscle. *J Physiol*. 1995;484(3):737–42.
46. Reisman S, Allen TJ, Proske U. Changes in passive tension after stretch of unexercised and eccentrically exercised human plantarflexor muscles. *Exp Brain Res*. 2009;193(4):545–54.
47. Balle SS, Magnusson SP, McHugh MP. Effects of contract-relax vs static stretching on stretch-induced strength loss and length-tension relationship. *Scand J Med Sci Sports*. 2015;25(6):764–9.
48. Herda TJ, Cramer JT, Ryan ED, McHugh MP, Stout JR. Acute effects of static versus dynamic stretching on isometric peak torque, electromyography, and mechanomyography of the biceps femoris muscle. *J Strength Cond Res*. 2008;22(3):809–17.

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES

AQ1 = Please check if authors name are correctly captured for given names (in red) and surnames (in blue) for indexing after publication.

AQ2 = Some data were assumed to 95% CI values. Please check.

AQ3 = Is this a reference citation? Please check.

AQ4 = Please discuss part B in the caption.

AQ5 = Please check citations here if appropriate.

END OF AUTHOR QUERIES