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## Neuromuscular Correlates of the Contralateral Stretch-induced Strength Loss

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## ABSTRACT

**Purpose.** The current study investigated the effects of unilateral passive stretching on the neuromuscular mechanisms involved in the force-generating capacity of the contralateral muscle.

**Methods.** Twenty-six healthy men underwent unilateral passive stretching of the plantar flexors (5 x 45-s on+15s-off, total stretching time: 225 s). Before and after the stretching protocol, contralateral ankle range of motion (ROM), maximum isometric voluntary contraction (MVC) of the plantar flexors, and electromyographic root mean square (sEMG RMS) of the *soleus* and the *gastrocnemii* muscles were determined. Concurrently, V-wave, maximum and superimposed H-reflex, and M-wave were elicited via nerve stimulation to estimate the supraspinal, spinal, and peripheral mechanisms, respectively. The sEMG RMS, V-wave, and H-reflex were normalized to the M-wave.

**Results.** After passive stretching, contralateral ankle ROM was increased [+8% (1%/15%), effect size (ES) 0.43 (0.02/0.84),  $p<0.001$ ], MVC of the plantar flexors was decreased [-9% (-21%/-2%), ES -0.96 (-1.53/-0.38),  $p<0.001$ ], and the sEMG RMS/M-wave of the *soleus* and the *gastrocnemii* muscles was decreased ( $\approx$  -9%, ES  $\approx$  -0.33,  $p<0.05$ ). Concurrently, the V-wave/M-wave superimposed was decreased in all muscles ( $\approx$  -13%, ES -0.81 to -0.52,  $p<0.05$ ). No change in H-reflex/M-wave and M-wave was observed under both maximum and superimposed condition. The decrease in the MVC and the sEMG RMS of the contralateral muscle was accompanied by a decrease in the V-wave/M-wave but not the H-reflex/M-wave ratios and the M-wave.

**Conclusions.** The present outcomes suggest that only supraspinal mechanisms might be involved in the contralateral decrease in the maximum force-generating capacity.

**Keywords:** H-reflex; M-wave; surface EMG; V-wave; passive stretching, nerve stimulation

## INTRODUCTION

Passive static stretching in sport and rehabilitation can help to improve joint range of motion (ROM), albeit is accompanied by an acute reduction in the force-generating capacity of the stretched muscle (SM) (1, 2). To explain the simultaneous increase in ROM and decrease in force-generating capacity, neuromuscular mechanisms including alteration in the afferent feedback by type-Ia, type-II (muscle spindles) (3), type-III (mechanoreceptors), and type-IV (metabo-/nociceptors) fibers (4, 5), and mechanical mechanisms including a decrease in muscle-tendon unit stiffness (6–8) have been proposed. Specifically, the neuromuscular factors may have their origin in supraspinal inhibition and reduction in spinal reflex excitability, and/or be of peripheral origin, i.e., possible impairment in the events involved in excitation-contraction coupling processes; however, their contribution to reducing contractile force-generating capacity remains to be elucidated (9, 10).

Interestingly, the effect of passive stretching may affect the contralateral homologous muscle (CM) not directly exposed to passive stretching (11). Whereas a number of studies have shown an increase in ROM in the CM (12–15), inconsistent results for the force-generating capacity have been observed with some studies reported no change (13, 14, 16), while others showed a reduction in force-generating capacity in the CM (13, 17–19). Several possible mechanisms merging in a final common end, i.e., decrease in contralateral  $\alpha$ -motoneuron pool excitability, have been proposed to explain the crossover effect in the CM: i) a reduction in stretch-reflex sensitivity involving the muscle spindles via interneurons interposed in the crossed pathway (13, 17, 20); ii) an increase in the inhibitory afferent feedback from the stretched muscle involving mechanoreceptors and nociceptors (13, 17, 19); and consequently iii) an increase in stretch

tolerance (19). Since the CM is not directly involved in the passive stretching maneuver, neuromuscular rather than mechanical factors have been shown to be involved (19). Additionally, analyzing the force signal and concomitantly using an interpolated-twitch technique, central but not peripheral mechanisms were found responsible for the drop in the force-generating capacity in the CM, though the origin of the central neuromuscular mechanisms was not investigated (19).

To examine in depth the origin of the central neuromuscular mechanisms, the analysis of the surface electromyography (sEMG) signal coupled with peripheral nerve stimulation could be used to assess the passive stretching-induced effects on the force-generating capacity in the CM (21). More in detail, the nerve stimulation can evoke the H-reflex, M-wave and V-wave to explore the level of the neuromuscular mechanisms (21–23). Particularly, the H-reflex overall reflects the response of the motoneuron pool to a volley from large-diameter primary muscle spindle afferents (21). The M-wave represents the compound muscle action potential and is usually elicited to estimate the ability of the action potential to propagate across the sarcolemma (22). Remarkably, when evoking the M-wave with a supramaximal stimulation during MVC, the wave is followed by a reflexive response, the V-wave (23, 24). This response results from the motoneuron activation induced by Ia afferents after the pathway has been cleared by the collision of the antidromic and orthodromic waves (24, 25). Such an evoked response is a methodological variant of the H-reflex and its amplitude depends on the number of spinal motoneurons recruited and on their firing frequency during the MVC (25). As such, the V-wave amplitude reflects both spinal processes via reflex excitability and pre- and post-synaptic inhibition, and the level of neural drive in the descending corticospinal pathways (26). Hence, by

assessing the H-reflex during MVC concomitantly with the V-wave, an index of efferent spinal motor output can be retrieved (22). The only study that partially used this approach, examining only spinal responses, found no difference in spinal excitability after a stretching bout in the CM (27).

To date, this systematic approach has never been used to investigate the effects of acute passive stretching on the mechanisms that could contribute to the loss in force-generating capacity in the CM. Therefore, the present study aimed to examine in depth the origin of the possible neuromuscular mechanisms associated with the change in force-generating capacity in the CM, by eliciting the H-reflex, M-wave and V-wave. Since previous studies showed no change in spinal excitability (27) and M-wave (19), we hypothesized that a possible decrease in the force-generating capacity in the CM could be associated with a decrement in the V-wave.

## **MATERIAL AND METHODS**

### **Study design**

For this cross-sectional, within-subject study, the sample size calculation was based on a previous investigation, considering the decreases in force-generating capacity of the CM as the reference parameter (18, 19) and computed using statistical software (G-Power 3.1, Düsseldorf, Germany), using the T-test family. Cohen's *d* effect size (ES) ~ 1.17 was computed using the referenced studies, a two-tail effect,  $\alpha = 0.05$  and a required power  $(1 - \beta) = 0.90$ , the desired sample size resulted in 10 participants. However, given the procedures we used and possible high variability in the signals recorded, 26 participants were recruited to decrease any possible risk of bias.

## Participants

Twenty-six healthy men [age 23(4) years; stature 1.77(0.10) m; body mass 75(11) kg; mean(SD)] volunteered for the present study. The participants were recreationally active. Inclusion criteria were no evident orthopedic and/or neurological pathologies, no lower-limb muscular or joint injuries in the previous 6 months, and no involvement in a systematic passive stretching program in the previous 6 months. The local University Ethics Committee approved the study protocol (CE 27/17); the study was performed in accordance with the principles of the latest version of the Declaration of Helsinki. The participants gave their written, informed consent after receiving an explanation of the purpose of the study and the experimental procedures. The participants were free to withdraw from the study at any time.

## Experimental procedures

The participants were tested at the same time of the day in a climate-controlled laboratory [temperature 20(1) °C and relative humidity 50(5)%] to minimize confounders due to circadian rhythms. Following previous procedures (19), the participants came to the laboratory three times. During the first session, they were familiarized with the experimental set-up and the passive stretching protocol, i.e., maximum isometric voluntary contraction (MVC) and nerve stimulation technique procedures. Skin landmarks (moles, scars, angiomas) and the position of the angle transducer, sEMG and stimulation electrodes were mapped on a transparency sheet for accurate electrode repositioning consistency within the same area (19). The second and third sessions were randomized. Out of these two, one session served as a control in which the testing procedures were performed without any intervention in both limbs and replicated after a period equal to the duration of the stretching protocol. During the other visit, the participants underwent



unilateral passive stretching of the plantar flexors, and the testing procedures were assessed first in the CM and then in the SM (19), since passive stretching-induced strength loss in the SM is known to last up to 2 hours (28). The order of which leg was stretched first was randomized for the second session and was maintained for the third session. As such, in the control session, the SM and the CM were identified as the SM<sub>CON</sub> and the CM<sub>CON</sub>.

The stretching protocol was performed unilaterally on the plantar flexors by the same experienced operator, and the stretched limb was randomized. Plantar flexors MVC and sEMG root mean square, V-wave, H-reflex, and M-wave were measured in the *gastrocnemius medialis*, the *lateralis*, and the *soleus* muscles of both limbs. Nerve stimulation intensity were determined to evoke H-reflex and M-wave responses at rest in all muscles. The participants performed a standardized warm up (10 × 2-s contractions at 50% MVC determined during the familiarization session) to determine MVC and to evoke superimposed responses.

In a random order, the baseline assessments of MVC in both CM and SM were separated by at least 5 min of passive stretching. Separate nerve stimulations were used to evoke H-reflex, M-wave, and V-wave responses in the *tibialis anterior* only. The participants performed a standardized warm-up prior to MVC and to evoke superimposed responses. Two stimulations were performed for each MVC: the first at rest before (maximal response) and second one during the plateau of MVC (superimposed response) (22). Particularly, this latter was performed because the evoked response at rest may not account for changes Ia afferent pathways during voluntary contraction after stretching (10). Hence, assessing H-reflexes during MVC would be more appropriate, considering the state-dependent changes affecting Ia afferent reflex

excitability and H-reflexes, such as decreases in homosynaptic depression (29) and both homonymous and heteronymous *Ib* inhibition (30), in addition to maintain motoneurons excitability. A rest-period of 1 min was allowed before the beginning of the stretching protocol. Thereafter, a single MVC was performed, and two stimulations were delivered at the same intensity used at baseline to elicit separately each H-reflex and then each M-wave.

## **Measurements**

### *Ankle range of motion*

To monitor the changes in ankle joint ROM, a bi-axial electrogoniometer (TSD 130A, Biopac System, Goleta, CA, USA) was utilized. The electrogoniometer was positioned with one axis on the external face of the fibula and the other on the calcaneum. The electrogoniometer signal was transmitted to an A/D converter (mod. UM 150, Biopac System), sampled at 1000 Hz, and stored on a personal computer. The subject was prone on a medical bed. The starting ankle angle was 90 degrees. To assess the changes in ROM, an operator slowly dorsiflexed the ankle joint manually using a visual feedback that provided her/him with angular displacement by time. The sEMG signal was checked to monitor possible muscle activation during elongation in both stretched muscles and the *tibialis anterior*. Three trials were performed. The maximum angle reached in each set was measured to calculate the maximum ROM. The device showed very high reliability and sensitivity as reported previously (8).

### *Maximum isometric voluntary contraction*

The MVC of the plantar flexors was measured with the participant prone on a medical bed with the ankle on the ergometer and the foot fixed with a Velcro strap to a mobile metal plate

instrumented with a load cell (SM-2000 N, Interface, Scottsdale, AZ, USA). The hips and shoulders were firmly secured to the ergometer. The participants were instructed to push as fast and hard as possible for 4 s and to focus on plantar flexion, avoiding any unnecessary movement (e.g., knee flexion and hip extension/flexion) (22). In case of any unnecessary movement, the trial was discarded, and a further attempt was performed after a 5 min rest. Visual feedback was provided to the participants. The force signal was transmitted to an A/D converter (mod. UM 150, Biopac System, Goleta, CA, USA), sampled at a fixed sampling rate of 1000 Hz, and stored on a personal computer. The maximum force recorded was defined as the MVC and entered in the data analysis. The device showed very high reliability and sensitivity as reported previously (8).

#### *sEMG recordings*

sEMG signal was recorded from the *gastrocnemius medialis*, the *gastrocnemius lateralis*, and the *soleus* muscles. After shaving and cleaning the skin with alcohol to ensure low impedance ( $< 5 \text{ k}\Omega$ ), sEMG signals were obtained with solid hydrogel rounded electrodes (mod. H124SG Kendall ARBO; diameter 10 mm; inter-electrode distance 20 mm; Kendall, Donau, Germany). The electrodes were positioned 2 cm below the insertions of the gastrocnemii over the Achille's tendon for the *soleus*, and over the mid belly of the gastrocnemii muscles for the two gastrocnemii (31). To check for possible coactivation during passive stretching and nerve stimulation, the sEMG signal was also recorded from the *tibialis anterior* muscle (10), with the electrode placed at one-third of the distance on a line between the fibula and the tip of the medial malleolus (31). Following visual inspection of the sEMG signal, the electrodes were eventually replaced should a crosstalk contamination of the sEMG of tibial anterior occur. The sEMG

signal was detected during MVC and nerve stimulation and acquired by a multichannel amplifier at a sampling rate of 2000 Hz (mod. DA100 UM 150, Biopac; input impedance > 90 M $\Omega$ ; CMRR > 96 dB), amplified (gain  $\times$  1000) and filtered (filter type IV order Butterworth filter; bandwidth 10-500 Hz) for further analysis.

### *Nerve stimulation*

Single rectangular pulses (100-150 V pulse of 1 ms in width) were delivered to the tibial nerve to evoke H-reflexes and M-waves of the plantar flexors. An adhesive cathode (8-mm diameter, Ag-AgCl) was placed in the popliteal fossa and an anode (5  $\times$  10 cm, Medicompex SA, Ecublens, Switzerland) was placed over the patella (figure 1). The electrodes were connected to a high-voltage constant-current stimulator (Digitimer Stimulator, Model DS7AH, Hertfordshire, UK). The nerve stimulation and sEMG signal was recorded with a multichannel amplifier at a sampling rate of 2000 Hz (Mod. UM 150, Biopac System, Goleta, CA, USA).

The stimulation intensity started from 5 mA and was progressively increased by 2 mA increments to detect the maximal H-reflex ( $H_{\max}$ ). Thereafter, the stimulation intensity was increased by 5 mA increments to detect the maximal M-wave ( $M_{\max}$ ) until  $M_{\max}$  no longer increased. This last stimulation intensity was then increased by 20% to ensure supramaximal stimulation and used to record the maximal M-wave ( $M_{\max}$ ). At baseline, four stimulations were performed at each intensity to build the whole recruitment curve to determine the optimal stimulation for  $H_{\max}$  and  $M_{\max}$  both in the control and stretching session (22) before starting the passive stretching protocol. More in detail, the procedures to build such a recruitment curve were: an inter-stimulus interval of 10s; the start of the curve was the lowest current which was

sufficient to evoke an H-reflex. The same within-subject stimulation intensities were then used to evoke the superimposed ( $H_{sup}$  and  $M_{sup}$ ) response, both for the baseline and for the post-stretching assessment (22). Because nerve stimulation was optimized for the *soleus* muscle (32), the H-reflex and the M-wave elicited in the *gastrocnemius medialis* and the *gastrocnemius lateralis* muscle were found not to be maximal for each participant: the H-reflex and the M-wave recorded in both gastrocnemii were found to be maximal in 24/26 participants ( $\approx 92\%$ ) and in the ascending part of the recruitment curve in the remaining 2/26 ( $\approx 8\%$ ). The same stimulation intensity was then used to evoke the superimposed responses, both for the baseline and for the post-stretching assessment.

A dedicated software (Mod. Stim 100C, Biopac System Goleta, CA, USA) enabled us to have the same stimulation order (first H-reflex, then M-wave) at a fixed temporal distance (500 ms) for each stimulation. Responses at rest were assessed without prior muscle activity at an interval of 10 s. Superimposed responses were manually triggered after the MVC plateau was attained. The V-wave was recorded during the superimposed M-wave stimulation, at the same time-delay between stimulation onset and the occurrence of the H-reflex (33).

### **Data analysis**

The researchers who analysed the data were blinded to the testing sessions (control or stretching). During the MVCs, the sEMG signal was analyzed in the time domain within a 1-s period of the MVC plateau without any stimulation, and it was assessed by visual inspection. The sEMG root mean square (sEMG RMS) was calculated in consecutive 250-ms time windows, averaged, and then entered in the data analysis. The  $M_{max}$  and  $M_{sup}$  were entered into the data

analysis. The average peak-to-peak amplitudes of the evoked responses were calculated and normalized to the maximal M-wave evoked in the same condition. Thus,  $H_{\max}/M_{\max}$ ,  $H_{\text{sup}}/M_{\text{sup}}$ , and  $V/M_{\text{sup}}$  were defined as dependent variables and were compared pre- and post-stretching. The normalization of the H-reflex to the M-wave was done to provide information about the proportion of the motoneuron pool being activated in the H-reflex (34). As V-wave involves both spinal and supraspinal mechanisms, the comparison of the changes in  $H_{\text{sup}}/M_{\text{sup}}$  and  $V/M_{\text{sup}}$  was proposed as a tool to estimate the relative contribution of both levels to V-wave changes (32). The M-wave accompanying the maximal H-reflex was measured and normalized to the corresponding maximal M-wave ( $\text{Mat}H_{\max}/M_{\max}$  and  $\text{Mat}H_{\text{sup}}/M_{\text{sup}}$ ) (22) to ensure the consistency of the stimulus condition during the whole experiment (35).

### **Stretching protocol**

During the passive stretching protocol, the participants remained prone on the same medical bed and with the same ergometer used for the testing procedures. An operator dorsiflexed the ankle of the stretched limb until 90% of maximal discomfort, according to the subjective response for each participant. Particularly, a 0-10 visual analogue scale was used at this purpose, spanning from no-discomfort to maximal discomfort (5). The stretching intensity was kept constant by means of a constant force output exerted by the operator. The force output between the passively stretched leg and the operator's arms was recorded during the protocol by a load cell (SM-2000 N, Interface, Crowthorne, UK) (5). Specifically, the load cell was positioned 5 cm above the metatarsus of the passively stretched limb and an operator pushed perpendicularly the load cell to stretch plantar flexors. To minimize possible muscle reflex activity, muscle elongation was reached in 6 s and maintained for 45 s (28). In line with previous investigations, five 45 s sets

with 15 s intervals of passive recovery were performed for a total stretching duration of 225 s (7, 19, 36). The sEMG signal was checked during passive stretching to monitor possible muscle activation during elongation. If the sEMG signal during the passive stretching protocol was  $> 5\%$  MVC, the participant was excluded from the study and replaced with another one to ensure statistical power (19). In the current study, no participant was replaced. In the control session, the participants lay prone as relaxed as possible with the ankle at a neutral angle (90 degrees) for an equivalent duration.

### Statistical analysis

Statistical analysis was performed using a statistical software package (IBM SPSS Statistics 22, Armonk, NY, USA). To check the normal distribution of the sampling, the Shapiro-Wilk's test was applied. To determine the inter-day reliability of the dependent parameters, the intraclass correlation coefficient (ICC) and the standard error of measurement (SEM%) were calculated using the baseline values recorded during the second and third session. The ICC was interpreted as:  $\geq 0.90$  *very high*; 0.89-0.70 *high*; 0.69-0.50 *moderate*. The minimal detectable change with a 95% confidence interval (MDC95%) was used to detect the sensitivity of the intervention. The pre-post differences in ROM and MVC in the SM and CM *vs* control were calculated by three-way limb (two levels: SM and CM)  $\times$  session (two levels: stretching and control)  $\times$  time (two levels: pre and post) repeated-measures analysis of variance (ANOVA). The pre-post differences in sEMG RMS, sEMG RMS/ $M_{sup}$ , H-reflex, M-wave, V-wave, H/M ratios, V/ $M_{sup}$ , and MatH/M ratios in the *gastrocnemius medialis*, the *gastrocnemius lateralis*, and the *soleus* muscle *vs.* the control were calculated by four-way (muscle  $\times$  limb  $\times$  session  $\times$  time) repeated-measures ANOVA. To calculate the differences in the between-limb stretch-induced changes (SM *vs* CM,

SM vs SM<sub>CON</sub>, CM vs CM<sub>CON</sub>), analysis of covariance (ANCOVA) was performed, assuming the baseline values as covariate. Partial eta squared ( $\eta_p^2$ ) was calculated and classified as follows: <0.06: *small*; 0.06-0.14: *medium*; and >0.14: *large* (37). Multiple comparisons were performed using Bonferroni's correction. Significance was set at an  $\alpha$  value < 0.05. Unless otherwise stated, descriptive statistics are presented as the mean (standard deviation [SD]). The changes are reported as percentage change with 95% confidence interval (95%CI). Cohen's *d* effect size (ES) was calculated and interpreted as: 0.00-0.19 *trivial*; 0.20-0.59 *small*; 0.60-1.19 *moderate*; 1.20-1.99 *large*;  $\geq 2.00$  *very large* (38). The 95% CI of the ES is also reported

## RESULTS

Table 1 presents the reliability of the dependent parameters: *high* to *very high* reliability was observed for all parameters. No change in Math<sub>max</sub>/M<sub>max</sub> and Math<sub>sup</sub>/M<sub>sup</sub> in any of the groups was noted (Table 2). Homogeneity assumption was met for all parameters.

Figures 2 presents the changes in ROM and MVC. A significant limb  $\times$  session  $\times$  time interaction was found for ROM ( $F_{1,25} = 14.737$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.391$ ). ROM was increased in the CM [8% (1%/15%), ES: 0.43 (0.02/0.84),  $P < 0.001$ ] and SM [31% (15%/46%), ES: 1.18 (0.59/1.77),  $P < 0.001$ ], but not in the CM<sub>CON</sub> ( $P = 1.000$ ) and SM<sub>CON</sub> ( $P = 0.598$ ). The ANCOVA showed that the increase in ROM was greater in the SM than CM [ES: 0.78 (0.20/1.33),  $P < 0.001$ ].

A significant limb  $\times$  session  $\times$  time interaction was found for MVC ( $F_{1,25} = 12.777$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.338$ ). MVC was decreased in the CM [-9% (-21%/-2%), ES: -0.96 (-1.53/-0.38),  $P <$



0.001] and SM [-17% (-22%/-12%), ES: -2.39 (-3.10/-1.68),  $P < 0.001$ ], but not in the CM<sub>CON</sub> ( $P = 0.603$ ) and SM<sub>CON</sub> ( $P = 0.192$ ). The ANCOVA showed that the decrease in the MVC was greater in the SM than CM [ES: 0.71 (0.15/1.27),  $P < 0.001$ ].

Figure 3 presents the changes in sEMG RMS/M<sub>sup</sub>. No muscle  $\times$  limb  $\times$  session  $\times$  time interaction was found for the sEMG RMS/M<sub>sup</sub> ( $F_{2,24} = 1.618$ ,  $P = 0.218$ ,  $\eta_p^2 = 0.119$ ), although significant limb  $\times$  session  $\times$  time interaction was observed ( $F_{1,25} = 9.015$ ,  $P = 0.006$ ,  $\eta_p^2 = 0.349$ ), indicating similar trend in all muscles. Taking all muscles together, the sEMG RMS/M<sub>sup</sub> was decreased in the CM by  $\approx$ -9% (ES: -0.33,  $P < 0.05$ ) and in SM by -17% (ES: -0.52,  $P < 0.05$ ). No change was observed during the control session in the CM<sub>CON</sub> and SM<sub>CON</sub> ( $P > 0.05$ ). The ANCOVA showed that the decrease in the sEMG RMS/M<sub>sup</sub> was greater in the SM than CM (ES: 0.80,  $P < 0.05$ ).

Figure 4 shows the reduction in V/M<sub>sup</sub> in the three muscles. No muscle  $\times$  limb  $\times$  session  $\times$  time was found for V/M<sub>sup</sub> ( $F_{2,24} = 0.774$ ,  $P = 0.472$ ,  $\eta_p^2 = 0.021$ ), although significant limb  $\times$  session  $\times$  time interaction was observed ( $F_{1,25} = 12.463$ ,  $P = 0.002$ ,  $\eta_p^2 = 0.333$ ), indicating similar trends for all muscles. Taking all muscles together, the V/M<sub>sup</sub> was decreased by -13% (ES: -0.81,  $P < 0.05$ ) in the CM, while in the SM it was decreased by -23% (ES: -1.35,  $P < 0.05$ ). No change was observed during the control session in the CM<sub>CON</sub> and SM<sub>CON</sub> ( $P > 0.05$ ). The ANCOVA showed that the decrease in the V/M<sub>sup</sub> was greater in the SM compared to CM (ES: 0.65,  $P < 0.05$ ).

The ANOVA showed no muscle  $\times$  limb  $\times$  session  $\times$  time for  $H_{\max}$  ( $F_{2,24} = 0.553$ ,  $P = 0.582$ ,  $\eta_p^2 = 0.016$ ),  $H_{\sup}$  ( $F_{2,24} = 0.717$ ,  $P = 0.498$ ,  $\eta_p^2 = 0.019$ ),  $M_{\max}$  ( $F_{2,24} = 1.181$ ,  $P = 0.324$ ,  $\eta_p^2 = 0.094$ ) and  $M_{\sup}$  ( $F_{2,24} = 1.404$ ,  $P = 0.265$ ,  $\eta_p^2 = 0.106$ ). Moreover, no muscle  $\times$  limb  $\times$  time interaction in  $H_{\max}$  ( $F_{1,25} = 1.051$ ,  $P = 0.365$ ,  $\eta_p^2 = 0.078$ ),  $H_{\sup}$  ( $F_{1,25} = 0.823$ ,  $P = 0.451$ ,  $\eta_p^2 = 0.028$ ),  $M_{\max}$  ( $F_{1,25} = 0.847$ ,  $P = 0.441$ ,  $\eta_p^2 = 0.035$ ) and  $M_{\sup}$  ( $F_{1,25} = 0.467$ ,  $P = 0.632$ ,  $\eta_p^2 = 0.013$ ). No change was noted in the H-reflex or the M-wave in any condition in any muscle ( $P > 0.05$ ) (Table 2). Consequently, no change in H/M ratios was observed in any condition ( $P > 0.05$ ).

## DISCUSSION

While the effects of passive stretching have been extensively investigated in the SM, the current study sought to examine the effects of passive stretching on the neuromuscular mechanisms influencing the force-generating capacity of the CM. After a passive stretching protocol, the ROM was increased and the MVC, the sEMG RMS, and the  $V/M_{\sup}$  were decreased in the CM, while no change in the H/M ratios or the M-wave was observed. The decrease in  $V/M_{\sup}$  and the concomitant lack of change in H/M ratios and M-wave suggest that supraspinal rather than spinal and peripheral mechanisms could underlie the drop in the force-generating capacity of the CM.

### *Preliminary considerations*

No change in any parameter was observed in the  $SM_{CON}$  and  $CM_{CON}$ . Additionally, the *high* to *very high* reliability recorded here indicates that the observed findings are attributable mainly to the intervention and not to the low reproducibility of the protocols. The MatH accompanying the H-reflex ensured consistency in nerve stimulation (35). The similar pre-post MatH/M ratios

indicated that the H-reflex was recorded within the same portion of the recruitment curve, with stable nerve stimulation (22).

To verify the effectiveness of the stretching protocol, we assessed the ROM, MVC, sEMG RMS, and nerve stimulation in the stretched muscle as well. In line with previous studies, the ankle ROM was increased, while the MVC and the sEMG RMS of the plantar flexors were decreased in the SM (8, 19, 28). The decrease in the sEMG RMS/ $M_{sup}$  observed here (39, 40), together with the decrease in the  $V/M_{sup}$  without any simultaneous change in H/M ratios and M-wave, may indicate that the supraspinal activity was affected, in line with what was previously shown (23, 39). Although, the supraspinal level cannot be clearly identified with the present design. For instance, recent studies using transcranial magnetic stimulation to investigate modifications in cortical activity following passive stretching found no change (10, 41–43). Therefore, excluding the changes at the cortical level, it is likely that the efferent drive generated at the sub-cortical level (i.e., basal ganglia, cerebellum, and ventral-anterior and ventral-lateral thalamus nuclei) could have been affected by passive stretching (44). The lack of change in the H/M ratios indicates that passive stretching does not impair the spinal contribution to the force-generating capacity (9). Similarly, a recent study observed no change at the spinal level after passive stretching (10). Although the role of the muscle spindles and of the free nerve endings was hypothesized to affect the force-generating capacity at the spinal level (9), it is possible that the present procedures were not adequate to capture these changes, as recently acknowledged (10). Indeed, the H-reflex bypasses the muscle spindles, so that it is not influenced by their sensitivity (10). Nonetheless, it could still be hypothesized that stretching might reduce the intrafusal muscle spindle discharge through a muscle spindle desensitization, albeit without any change in

the H-reflex (3, 10, 42). In line with the literature, no change in the M-wave was observed, indicating that the decrease in the force-generating capacity cannot be ascribed to any peripheral mechanism (e.g., impaired propagation of the neuromuscular action potential or excitation-contraction coupling) (9, 10, 19, 45).

#### *Stretch-induced changes in the contralateral muscle*

The observation of a *small* increase in ROM in the CM is shared by previous reports (11–14, 19). An increase in ROM is attributable to a decrease in muscle-tendon unit stiffness, which depends on: (i) the cross-link between actin and myosin filaments (46); (ii) the non-contractile proteins of the endo-sarcomeric (47) and the exo-sarcomeric cytoskeleton (48); (iii) the changes in the viscoelastic properties of the connective tissue located within and surrounding the muscle (47); and (iv) an augmented stretch-tolerance during passive stretching, involving a reduction in muscle tone possibly associated with a reduction in nociceptive activity (49). The first three factors hark back to mechanical stimuli, in which the CM was not involved. Consequently, the augmented stretch-tolerance is the only factor that could be hypothesized, since it is the only factor involving neuromuscular pathways (19).

The passive stretching protocol led to a *moderate* decrease in MVC in the CM. Inconsistent results for this phenomenon have been reported, since some studies showed a decline in maximal force (17–19), while others did not (13, 14, 16). Such a discrepancy may derive from the muscle stretched, the stretching modality (e.g., passive or dynamic stretching), the overall duration of the passive stretching protocol, and the stretching intensity (9). Particularly, it should be acknowledged that the duration of the present stretching protocol might be longer than what

actually happen in the practice (50, 51), so that the effects might be greater. The force-generating capacity depends on both neuromuscular and mechanical factors. A recent study showed a decrease in sEMG RMS as a gross neuromuscular process and excluded the mechanical factors as possible mechanisms in the CM (19). Since the sEMG is the gross sum of the central and peripheral contribution to the skeletal muscle activation, the present procedures enabled us to better detect the origin of the neuromuscular mechanisms and possibly identify whether supraspinal, spinal and/or peripheral mechanisms were involved.

Given the normalization of all parameters to the M-wave amplitude, it is important to note that no change in the M-waves was observed in the *gastrocnemius medialis*, the *gastrocnemius lateralis* or the *soleus* muscles, while the sEMG/M<sub>sup</sub> was decreased in all muscles. This means that the peripheral neuromuscular mechanisms, associated with the propagation properties of the nerve and sarcolemmal action potential and with the excitation-contraction coupling, were not affected by the passive stretching protocol (19). As such, the changes in the sEMG/M<sub>sup</sub> may be ascribed to central neuromuscular factors (10, 39). Since the V-wave and the H-reflex were normalized to the M-waves, the possible changes in the V/M<sub>sup</sub> and the H/M ratios could be explained by supraspinal and/or spinal mechanisms.

As hypothesized, we observed no change in the H/M ratios in the CM. The lack of change in H/M<sub>max</sub> suggests that no change in homosynaptic depression (29) or homonymous and heteronymous *Ib* inhibition (30) have occurred. Moreover, the unchanged H/M<sub>sup</sub> points out that there is no alteration in the *Ia* afferent pathways during the voluntary contraction after passive stretching did not occur (10). This is in line with the only previous study that assessed the spinal

contribution to the CM after passive stretching and reported no change in the spinal reflex excitability (27). Such a lack of change in spinal reflex was observed after a shorter and less intense passive stretching protocol and technique (transcutaneous spinal cord stimulation) (27). Hypothetically, a change in the spinal contribution might be ascribed to a change in a motoneuron facilitatory mechanism from the neuromuscular spindles in the SM towards the motoneurons in the CM via the interneurons interposed in the crossed pathway (20, 40, 52, 53). Such a change in motoneurons facilitatory mechanism may be mediated by two possible factors: i) an alteration in the excitability of the Ia afferent-motoneuron reflex pathway to transmit muscle spindle activity; ii) a decrease in the intrafusal muscle spindle discharge through desensitization of the muscle spindle, thus decreasing motoneuron activity (10, 40, 52). Since no mechanical stimulus and response occurred in the CM (19), the muscle spindle desensitization is not expected to be involved. However, the present results also indicate that the former mechanism was not even involved.

*Moderate* decreases in the  $V/M_{sup}$  in all contralateral muscles were found. A decrease in V-wave amplitude without any concomitant change in  $H/M_{sup}$  would point out a reduced motoneuron recruitment or firing rate, which may indicate lower supraspinal contribution to the motoneuron pool (54). Notwithstanding, the motoneuron firing rate is not only an index of the supraspinal input to the motoneuron, but also of the response to all inputs to the motoneuron, thereby making a clear identification of the origin of a change in V-wave amplitude difficult to achieve (25). Since these procedures were used here for the first time in the CM, a direct comparison with the literature cannot be made. Using a different approach (i.e., interpolated-twitch technique), voluntary activation was reduced after passive stretching in the CM, indicating a reduction in the

neural central drive (19). However, voluntary activation does not permit distinguishing the supraspinal from the spinal neuromuscular mechanisms (21). Additionally, neither the V-wave nor the voluntary activation can indicate accurately whether the changes occur at the cortical and/or the subcortical level (55). Nevertheless, previous studies reported no change in motor-evoked potential in the motor cortex activating the SM, indicating that passive stretching does not impair the cortical activity (41, 42), making it unlikely that a change in the motor cortex activates the CM. Excluding any change at the cortical level, it is likely that subcortical mechanisms might be responsible for the drop in the V-wave. The subcortical neural patterns associated with the cortical-subcortical circuits between the basal ganglia and the cerebellum have been suggested as possible pathways that allow the crossover of the afferent stimuli from the muscle spindles, mechanoreceptors, and metabo-nociceptors (44). Lastly, it was shown that passive stretching could affect the monoaminergic (e.g., norepinephrine and serotonin) drive in a non-localized way via an increase in parasympathetic activity (56), possibly reducing the force-generating capacity of the CM (9, 40). It should be noted that the changes in the CM had lesser extent than in the SM, as recently shown (19). The inhibitory interneurons within the subcortical circuits may have mediated such a lower crossover efferent response towards CM (57).

The present study has a number of limitations. First, the cortical mechanisms were not investigated. We acknowledge that different methods (e.g., transcranial magnetic stimulation or electrical stimulation of the cervico-medullary junction) could have deepened the supraspinal mechanisms. Future studies are needed to explore these possible mechanisms in the CM. Second, the H-reflex is a product of small proportion of motoneurons, so that undetectable changes are still possible (25). Third, no information about the role played by the peripheral receptors is

provided. Lastly, the current procedures were conducted at a neutral ankle angle position (i.e., 90 degrees), and assessing the procedures at shorter or longer muscle lengths could result in different outcomes.

## CONCLUSION

The present study was conceived to investigate the effects of unilateral passive stretching on the neuromuscular mechanisms behind the reduction in the force-generating capacity of the CM. The decrease in the MVC and the sEMG RMS of the CM was accompanied by a decrease in the  $V/M_{sup}$  but not the H/M ratios and the M-wave, suggesting that only supraspinal mechanisms might be involved in the contralateral decrease in the maximum force-generating capacity.



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## **CONFLICT OF INTEREST**

The authors have no conflict of interest. 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. The results of the present study do not constitute endorsement by ACSM.

## **AVAILABILITY OF DATA AND MATERIAL**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## **ADDITIONAL INFORMATION SECTION**

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

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

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## FIGURE CAPTIONS

**Figure 1.** The electrode placement is shown.

**Figure 2.** Individual data of the ankle range of motion (ROM) and maximum voluntary contraction (MVC) of the plantar flexor in the stretched (SM) and the contralateral muscle (CM) and in the control condition (SM<sub>CON</sub> and CM<sub>CON</sub>).

\*  $p < 0.05$  post vs. pre

#  $p < 0.05$  vs control

§  $p < 0.05$  SM vs. CM

**Figure 3.** Mean(SD) of the surface electromyographic signal root mean square normalized to the superimposed M-wave (sEMG RMS/M<sub>sup</sub>) in the two gastrocnemii and the soleus of the stretched (SM) and the contralateral muscle (CM) and in the control condition (SM<sub>CON</sub> and CM<sub>CON</sub>).

\*  $p < 0.05$  post vs. pre

#  $p < 0.05$  vs. control

§  $p < 0.05$  vs. CM.

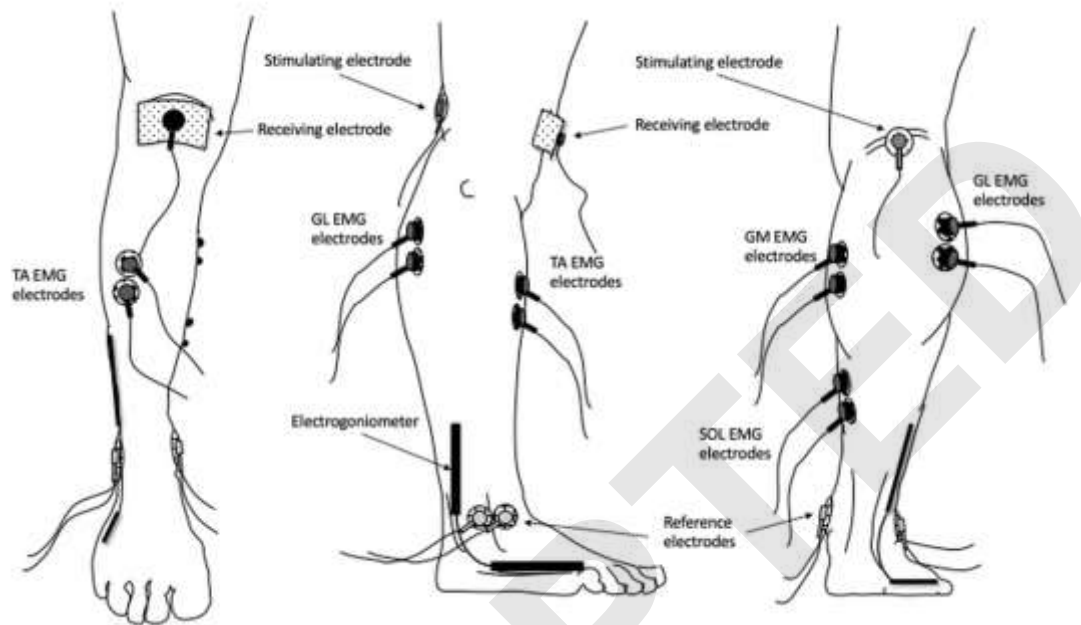
**Figure 4.** Mean(SD) of the V-wave normalized to the superimposed M-wave (V/M<sub>sup</sub>) in the two gastrocnemii and the soleus of the stretched (SM) and the contralateral muscle (CM) and in the control condition (SM<sub>CON</sub> and CM<sub>CON</sub>).

\*  $p < 0.05$  post vs. pre

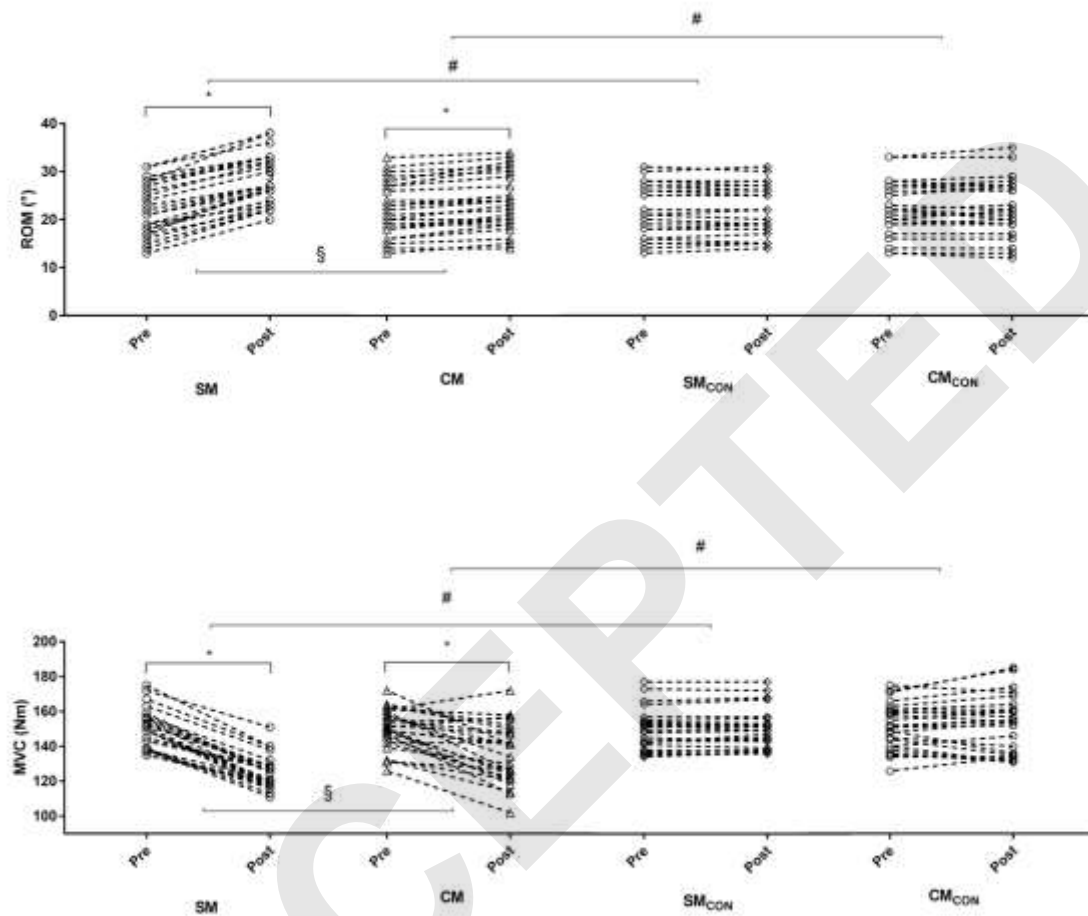
#  $p < 0.05$  vs. control

§  $p < 0.05$  vs. CM.

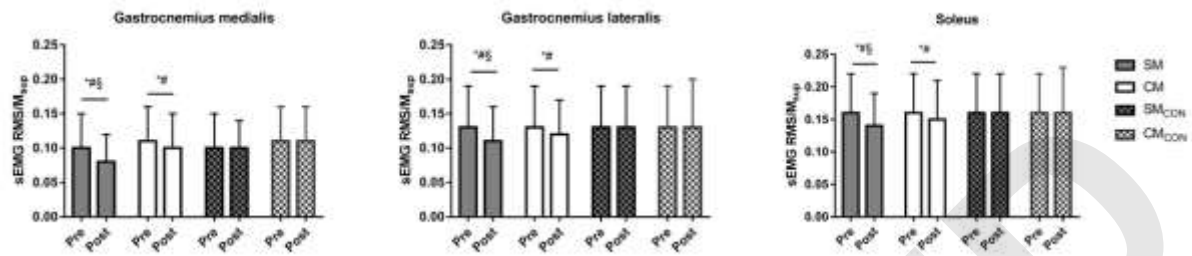
**Figure 1**



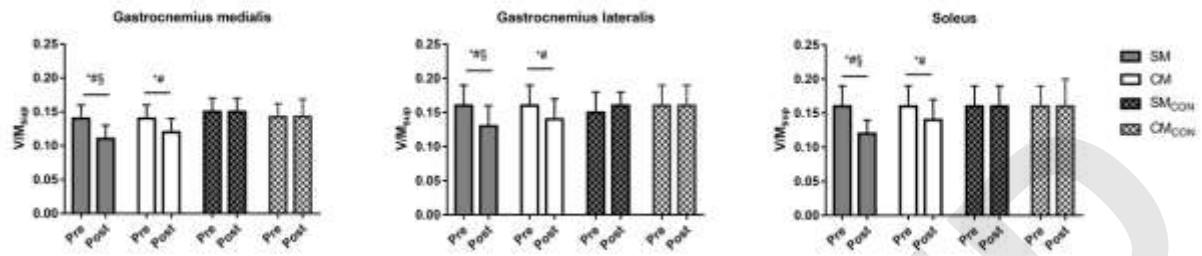
**Figure 2**



**Figure 3**



**Figure 4**



**Table 1.** Interday reliability [intraclass correlation coefficient (ICC) and standard error of measurement as percentage (SEM%)] and sensitivity (minimum detectable change as percentage (MDC<sub>95%</sub>) in the stretched and the contralateral non-stretched muscle.

|                                    |                     | <i>Stretched muscle</i> |       |       |       |       |      |                    | <i>Contralateral muscle</i> |       |       |       |       |      |                    |
|------------------------------------|---------------------|-------------------------|-------|-------|-------|-------|------|--------------------|-----------------------------|-------|-------|-------|-------|------|--------------------|
|                                    | Variable<br>(mV)    | Stretch                 | SD    | Con   | SD    | ICC   | SEM% | MDC <sub>95%</sub> | Stretch                     | SD    | Con   | SD    | ICC   | SEM% | MDC <sub>95%</sub> |
| <i>Gastrocnemius<br/>medialis</i>  | H <sub>max</sub>    | 0.721                   | 0.230 | 0.721 | 0.230 | 0.874 | 11.3 | 22.2               | 0.710                       | 0.222 | 0.711 | 0.222 | 0.895 | 10.1 | 19.8               |
|                                    | H <sub>sup</sub>    | 0.704                   | 0.229 | 0.723 | 0.235 | 0.910 | 9.8  | 19.1               | 0.710                       | 0.223 | 0.713 | 0.227 | 0.904 | 9.8  | 19.2               |
|                                    | M <sub>max</sub>    | 4.114                   | 1.578 | 4.082 | 1.525 | 0.891 | 12.5 | 24.5               | 4.182                       | 1.867 | 4.207 | 1.879 | 0.913 | 13.2 | 25.8               |
|                                    | M <sub>sup</sub>    | 4.080                   | 1.528 | 4.092 | 1.527 | 0.896 | 12.1 | 23.6               | 4.100                       | 1.527 | 4.156 | 1.687 | 0.895 | 12.6 | 24.7               |
|                                    | V-wave              | 0.581                   | 0.241 | 0.625 | 0.255 | 0.926 | 11.2 | 21.9               | 0.580                       | 0.234 | 0.585 | 0.241 | 0.876 | 3.4  | 6.6                |
|                                    | MatH <sub>max</sub> | 1.180                   | 0.454 | 1.182 | 0.455 | 0.911 | 11.5 | 22.5               | 1.186                       | 0.455 | 1.192 | 0.453 | 0.900 | 15.0 | 29.5               |
|                                    | MatH <sub>sup</sub> | 1.190                   | 0.467 | 1.187 | 0.460 | 0.888 | 13.1 | 25.6               | 1.197                       | 0.476 | 1.205 | 0.469 | 0.896 | 12.6 | 24.6               |
| <i>Gastrocnemius<br/>lateralis</i> | H <sub>max</sub>    | 0.706                   | 0.226 | 0.708 | 0.222 | 0.895 | 10.3 | 20.2               | 0.703                       | 0.223 | 0.703 | 0.224 | 0.887 | 10.7 | 21.0               |
|                                    | H <sub>sup</sub>    | 0.708                   | 0.228 | 0.707 | 0.222 | 0.910 | 9.6  | 18.7               | 0.704                       | 0.228 | 0.707 | 0.222 | 0.895 | 10.3 | 20.2               |
|                                    | M <sub>max</sub>    | 3.264                   | 1.215 | 3.228 | 1.123 | 0.904 | 11.2 | 21.9               | 3.264                       | 1.202 | 3.293 | 1.222 | 0.911 | 11.0 | 21.6               |
|                                    | M <sub>sup</sub>    | 3.279                   | 1.229 | 3.254 | 1.228 | 0.911 | 11.2 | 22.0               | 3.264                       | 1.229 | 3.294 | 1.142 | 0.881 | 12.5 | 24.5               |
|                                    | V-wave              | 0.533                   | 0.214 | 0.498 | 0.221 | 0.934 | 10.8 | 21.3               | 0.534                       | 0.202 | 0.537 | 0.209 | 0.899 | 6.2  | 12.1               |
|                                    | MatH <sub>max</sub> | 0.950                   | 0.363 | 0.932 | 0.335 | 0.895 | 12.0 | 23.6               | 0.963                       | 0.368 | 0.970 | 0.399 | 0.903 | 20.4 | 39.9               |
|                                    | MatH <sub>sup</sub> | 1.095                   | 0.368 | 1.084 | 0.356 | 0.902 | 10.4 | 20.4               | 1.101                       | 0.376 | 1.108 | 0.387 | 0.918 | 14.6 | 28.5               |
| <i>Soleus</i>                      | H <sub>max</sub>    | 0.718                   | 0.186 | 0.719 | 0.185 | 0.889 | 8.6  | 16.8               | 0.718                       | 0.185 | 0.710 | 0.185 | 0.893 | 8.5  | 16.6               |
|                                    | H <sub>sup</sub>    | 0.716                   | 0.184 | 0.718 | 0.186 | 0.921 | 7.3  | 14.2               | 0.716                       | 0.182 | 0.718 | 0.186 | 0.905 | 7.9  | 15.5               |
|                                    | M <sub>max</sub>    | 2.164                   | 0.699 | 2.162 | 0.696 | 0.915 | 9.4  | 18.4               | 2.159                       | 0.675 | 2.170 | 0.715 | 0.929 | 8.6  | 16.8               |
|                                    | M <sub>sup</sub>    | 2.192                   | 0.691 | 2.204 | 0.700 | 0.904 | 9.8  | 19.2               | 2.205                       | 0.699 | 2.236 | 0.688 | 0.913 | 9.2  | 18.1               |
|                                    | V-wave              | 0.351                   | 0.141 | 0.347 | 0.136 | 0.909 | 12.0 | 23.4               | 0.356                       | 0.141 | 0.359 | 0.149 | 0.904 | 12.6 | 24.7               |
|                                    | MatH <sub>max</sub> | 0.622                   | 0.213 | 0.626 | 0.212 | 0.909 | 10.3 | 20.1               | 0.625                       | 0.205 | 0.632 | 0.222 | 0.875 | 12.0 | 24                 |
|                                    | MatH <sub>sup</sub> | 0.644                   | 0.215 | 0.639 | 0.211 | 0.913 | 9.8  | 19.2               | 0.645                       | 0.209 | 0.649 | 0.218 | 0.898 | 10.5 | 21                 |

H<sub>max</sub>: H-reflex at rest. H<sub>sup</sub>: H-reflex superimposed. M<sub>max</sub>: M-wave at rest. M<sub>sup</sub>: M-wave superimposed. MatH<sub>max</sub>: M-wave at H-reflex intensity at rest. MatH<sub>sup</sub>: M-wave at H-reflex intensity superimposed.

**Table 2:** Maximum (max) and superimposed (sup) H-reflex and M-wave and M-wave at H-reflex intensity over M wave ratios (MatH/M) in the *gastrocnemius medialis* (GM), the *lateralis* (GL), and the soleus (Sol) of the stretched (SM) and the contralateral (CM) limb before (pre) and after (post) a passive stretching bout or an equivalent period of rest ( $SM_{CON}$  and  $CM_{CON}$ ).

|   |     | SM          |             |          | CM          |             |          | SM <sub>CON</sub> |             |          | CM <sub>CON</sub> |             |          |
|---|-----|-------------|-------------|----------|-------------|-------------|----------|-------------------|-------------|----------|-------------------|-------------|----------|
|   |     | pre         | post        | <i>p</i> | pre         | post        | <i>p</i> | pre               | post        | <i>p</i> | pre               | post        | <i>p</i> |
| <b>H<sub>max</sub> (mV)</b>               | GM  | 0.72 (0.23) | 0.72 (0.24) | 0.147    | 0.71 (0.22) | 0.70 (0.23) | 0.621    | 0.72 (0.23)       | 0.73 (0.24) | 0.502    | 0.71 (0.22)       | 0.71 (0.21) | 0.833    |
|   | GL  | 0.71 (0.23) | 0.70 (0.22) | 0.217    | 0.70 (0.22) | 0.70 (0.23) | 0.348    | 0.71 (0.22)       | 0.71 (0.22) | 0.701    | 0.70 (0.22)       | 0.71 (0.23) | 0.251    |
|   | Sol | 0.72 (0.19) | 0.73 (0.19) | 0.288    | 0.72 (0.18) | 0.72 (0.19) | 0.372    | 0.72 (0.18)       | 0.72 (0.18) | 0.216    | 0.72 (0.18)       | 0.72 (0.19) | 0.311    |
| <b>H<sub>sup</sub> (mV)</b>               | GM  | 0.70 (0.23) | 0.71 (0.23) | 0.373    | 0.71 (0.22) | 0.71 (0.23) | 0.930    | 0.71 (0.23)       | 0.72 (0.24) | 0.269    | 0.71 (0.22)       | 0.71 (0.22) | 0.850    |
|   | GL  | 0.71 (0.23) | 0.70 (0.22) | 0.110    | 0.70 (0.23) | 0.70 (0.22) | 0.898    | 0.71 (0.22)       | 0.71 (0.21) | 0.950    | 0.70 (0.22)       | 0.71 (0.23) | 0.415    |
|   | Sol | 0.72 (0.18) | 0.72 (0.19) | 0.834    | 0.72 (0.18) | 0.72 (0.18) | 0.728    | 0.72 (0.19)       | 0.72 (0.18) | 0.486    | 0.72 (0.18)       | 0.72 (0.19) | 0.591    |
| <b>M<sub>max</sub> (mV)</b>               | GM  | 4.03 (1.49) | 4.07 (1.47) | 0.431    | 4.02 (1.51) | 4.06 (1.55) | 0.258    | 4.01 (1.47)       | 4.03 (1.49) | 0.523    | 4.05 (1.56)       | 4.04 (1.58) | 0.571    |
|   | GL  | 3.96 (1.22) | 3.95 (1.21) | 0.215    | 3.96 (1.20) | 3.97 (1.23) | 0.703    | 3.96 (1.12)       | 3.94 (1.16) | 0.602    | 3.90 (1.22)       | 3.91 (1.22) | 0.660    |
|   | Sol | 3.56 (1.10) | 3.56 (1.11) | 0.163    | 3.55 (1.11) | 3.56 (1.11) | 0.759    | 3.56 (1.10)       | 3.56 (1.11) | 0.690    | 3.54 (1.11)       | 3.58 (1.10) | 0.632    |
| <b>M<sub>sup</sub> (mV)</b>               | GM  | 4.08 (1.53) | 4.13 (1.50) | 0.496    | 4.10 (1.63) | 4.18 (1.64) | 0.219    | 4.09 (1.53)       | 4.12 (1.51) | 0.611    | 4.16 (1.69)       | 4.20 (1.76) | 0.244    |
|   | GL  | 3.98 (1.23) | 3.94 (1.20) | 0.155    | 3.96 (1.23) | 3.93 (1.27) | 0.163    | 3.95 (1.23)       | 3.93 (1.15) | 0.467    | 3.99 (1.14)       | 3.93 (1.22) | 0.228    |
|   | Sol | 3.58 (1.09) | 3.60 (1.06) | 0.402    | 3.56 (1.10) | 3.55 (1.11) | 0.173    | 3.56 (1.10)       | 3.52 (1.08) | 0.905    | 3.57 (1.09)       | 3.56 (1.07) | 0.193    |
| <b>MatH<sub>max</sub>/M<sub>max</sub></b> | GM  | 0.09 (0.02) | 0.10 (0.02) | 0.524    | 0.09 (0.03) | 0.08 (0.01) | 0.232    | 0.10 (0.02)       | 0.09 (0.01) | 0.274    | 0.10 (0.02)       | 0.10 (0.02) | 0.732    |
|   | GL  | 0.11 (0.01) | 0.10 (0.04) | 0.863    | 0.11 (0.02) | 0.11 (0.02) | 0.412    | 0.09 (0.02)       | 0.10 (0.02) | 0.618    | 0.10 (0.02)       | 0.09 (0.01) | 0.406    |
|   | Sol | 0.10 (0.01) | 0.11 (0.01) | 0.286    | 0.11 (0.01) | 0.11 (0.01) | 0.445    | 0.10 (0.02)       | 0.11 (0.01) | 0.528    | 0.11 (0.01)       | 0.10 (0.01) | 0.486    |
| <b>MatH<sub>sup</sub>/M<sub>sup</sub></b> | GM  | 0.10 (0.03) | 0.10 (0.04) | 0.934    | 0.10 (0.04) | 0.10 (0.05) | 0.496    | 0.11 (0.03)       | 0.09 (0.03) | 0.532    | 0.10 (0.04)       | 0.10 (0.04) | 0.789    |
|   | GL  | 0.11 (0.03) | 0.11 (0.02) | 0.957    | 0.11 (0.02) | 0.11 (0.03) | 0.924    | 0.11 (0.02)       | 0.11 (0.03) | 0.492    | 0.11 (0.01)       | 0.11 (0.02) | 0.866    |
|   | Sol | 0.11 (0.01) | 0.11 (0.01) | 0.715    | 0.11 (0.01) | 0.10 (0.01) | 0.830    | 0.10 (0.01)       | 0.11 (0.01) | 0.285    | 0.10 (0.01)       | 0.10 (0.01) | 0.976    |