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Is the Interpolated-Twitch Technique-derived Voluntary Activation just Neural? Novel Perspectives from Mechanomyographic Data

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Running Title: VOLUNTARY ACTIVATION AND MECHANICAL FEATURES

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ABSTRACT

Purpose: Voluntary activation determined by interpolation-twitch technique (VA) could be affected by the characteristics of the in-series elastic components. To overcome this possible bias, a novel approach based on the mechanomyographic (MMG) signal to detect voluntary activation (VA_{MMG}) has been proposed. We examined the changes in VA and VA_{MMG} after passive stretching to check the influence of neural and mechanical factors in the force output.

Methods: Twenty-six healthy men underwent VA assessment using the interpolated-twitch technique before and after unilateral passive stretching of the plantar flexors (five 45s-on+15s-off). In addition to the force signal, the MMG signal was detected on *gastrocnemius medialis*, *gastrocnemius lateralis*, and *soleus*. From the force and MMG signal analysis, VA and VA_{MMG} were calculated in the stretched and contralateral non-stretched limb. Joint passive stiffness was also defined.

Results: In the stretched limb, passive stretching increased dorsiflexion range [+18(10)% (P<0.001, ES: 1.54)], whereas reduced joint passive stiffness [-22(8)% (P<0.001; ES: -1.75)], MVC [-15(7)% (P<0.001, ES:-0.87)], VA [-7(3)% (P<0.001; ES:-2.32)], and VA_{MMG} [~-5(2)% (P<0.001; ES: -1.26/-1.14)]. In the contralateral non-stretched limb, passive stretching increased dorsiflexion range [+10(6)% (P<0.001, ES: 0.80)], whereas reduced joint passive stiffness [-3(2)% (P=0.041; ES: -0.27)], MVC [-4(3)% (P=0.035, ES:-0.24)], VA [-4(2)% (P<0.001; ES:-1.77)], and VA_{MMG} [~-2(1)% (P<0.05; ES: -0.54/-0.46)]. The stretch-induced changes in VA correlated with VA_{MMG} (R ranging from 0.447 to 0.583 considering all muscles) and with joint passive stiffness (stretched limb: R=0.503; contralateral non-stretched limb: R=0.530).

Conclusions: VA output is overall influenced by both neural and mechanical factors, not distinguishable using the interpolated-twitch technique. VA_{MMG} is a complementary index to

assess the changes in VA not influenced by mechanical factors and to examine synergistic muscles.

Key Words: STRETCHING, MAXIMUM VOLUNTARY CONTRACTION, POTENTIATED TWITCH, SUPERIMPOSED TWITCH, PLANTAR FLEXORS, STIFFNESS

INTRODUCTION

The voluntary activation (VA) of a skeletal muscle reflects the ability to activate a muscle by increasing the descending drive during a voluntary contraction (1–3). Typically, VA is assessed by the interpolated-twitch technique, which involves an electrically evoked stimulation superimposed during a maximum voluntary contraction (MVC). When stimulating a peripheral nerve during an MVC, a supplementary action potential is added at the motor axon level (4, 5) and, if a given motor unit is not firing fast enough to produce its maximal force, the superimposed action potential generates a twitch-like increase in force from the whole muscle (4, 5). A second twitch evoked with the muscle at rest (i.e., the potentiated twitch) accompanies the superimposed twitch. The ratio between the superimposed and the potentiated twitch defines the level of VA: the more motor units recruited and the faster they fire during an MVC, the greater the VA (4, 5).

Although the interpolated-twitch technique supplies a valid estimation of VA, presents several methodological issues which includes the inability to detect possible differences among the synergistic muscles involved in a given task (6) and the possible influence of the muscle-tendon complex (MTC) stiffness on the overall force output (7–9). Concerning the latter point, previous studies reported that the mechanical properties of the tendon (e.g., less rigidity) causes a substantial overestimation in the VA in a range of 3–12% (7, 8). Comprehensively, determining VA should therefore also take into account the characteristics of the MTC as a whole.

To this purpose, assessing the mechanomyographic signal (MMG) while applying the interpolated-twitch technique may help to minimize the role of the MTC mechanical properties on VA. The MMG is a non-invasive approach that records and quantifies the low-frequency

transverse oscillations propagating from the active muscle fibers to the skin surface during a muscle contraction (10–12). As recently reported, three variables deriving from the MMG signal can be identified during an interpolated-twitch technique procedure (13). In first instance, the gross lateral movement of the contracting fibers at the beginning of the contraction generated by the shortening of the contractile elements, i.e., the MMG peak-to peak during an MVC (MMGp-p_{MVC}) (12, 13), whose amplitude is representative of the whole muscle activation, i.e., the greater the muscle activation, the greater its amplitude (14). Second, the MMG peak-to-peak recorded during the superimposed twitch (MMGp-p_{SUP}) (13), which is positively correlated with the amplitude of the superimposed twitch and the second derivative of the rate of force development, suggesting that MMGp-p_{SUP} may reflect the excitation-contraction coupling of the residual fibers not elicited by the voluntary output (15). Third, the MMG peak-to-peak recorded during the potentiated twitch (MMGp-p_{POT}), which is more affected by peripheral mechanisms such as the excitation-contraction coupling (10–12). Moreover, positive correlations between the MMGp-p_{POT} amplitude and the potentiated twitch were reported (14). In view of the correlations between the amplitude of the MMGp-p_{SUP} and MMGp-p_{POT} with the amplitude of the superimposed and potentiated twitch, a new index has been recently introduced from the ratio between the MMGp-p_{SUP} and the MMGp-p_{POT} to identify the level of VA of the synergistic muscles, i.e., the VA recorded by the MMG signal (VA_{MMG}) (13). The authors indeed observed positive correlations between the VA evoked in leg extensors and the VA_{MMG} recorded individually in *vastus lateralis*, *vastus medialis*, and *rectus femoris* before and after a fatiguing exercise (13). Moreover, VA_{MMG} was able to highlight the differences between *vastus medialis*, *vastus lateralis*, and *rectus femoris* after the fatiguing protocol, with the former presenting larger reduction in VA_{MMG} (13).

Noticeably, both the MMGp-p_{MVC} and MMPp-p_{POT} occur before the slack of the elastic-connective tissue has been fully taken up and the force transmitted to the tendon insertion point, and should be minimally affected by the MTC passive stiffness (16). However, the authors found a negative correlation between the amplitude of the MMG signal during the MVC plateau (expressed as root mean square, RMS) and the MTC passive stiffness (16), and this may raise possible concerns. Indeed, since the MMG RMS during MVC and the MMGp-p_{SUP} are

calculated from the same MMG signal time-window (i.e., the MMG signal corresponding to the force signal plateau), the MMGp-p_{SUP} amplitude could be potentially affected by the MTC passive stiffness level, possibly biasing the $VA_{MMG.}$ However, should no correlation between the MTC passive stiffness and the VA_{MMG} be found, this would imply that VA_{MMG} might be independent of the MTC passive stiffness, as opposed as the traditional interpolated-twitch technique.

To test this hypothesis, passive stretching routine could represent an interesting paradigm. Indeed, after an acute passive stretching bout, a reduction in both VA (17) and joint passive stiffness was reported (16, 18). In this context, passive stretching can therefore help to test the occurrence of possible correlations between VA_{MMG} and joint passive stiffness both before and after its administration, as well as between their stretch-induced changes. Recent literature focusing on the acute effects of passive stretching on the muscle stiffness in synergistic muscles suggested, although with some discrepancy, that biarticular might experience larger decreases in stiffness than monoarticular muscles after passive stretching (19–22). Should a correlation between VA_{MMG} and the joint passive stiffness be found, this could imply lower decrease in VA_{MMG} when separately detected in biarticular compared to monoarticular synergistic muscles.

On these bases, the present study aimed to: (i) determine whether VA_{MMG} is correlated with the joint passive stiffness assessed before and after a passive stretching routine performed on plantar flexors, as well as determine the correlation between their stretch-induced changes, and (ii) check for possible differences in VA_{MMG} between biarticular, i.e., *gastrocnemius medialis* (GM) and *lateralis* (GL), vs monoarticular muscles, i.e., *soleus* (SOL). Should not the

VA_{MMG} and the joint passive stiffness be correlated, the MMG signal could be added to the interpolated-twitch technique to examine the changes in VA not influenced by mechanical factors. To further exclude the role of the mechanical stimulus induced by passive stretching, the dependent variables were also determined in the contralateral non-stretched limb.

METHODS

Study design

Based on a previous investigation (17) the stretch-induced changes in VA were used as main outcome. Considering the study design, i.e., a three-way analysis of variance for repeated measures (*time*: pre vs. post, *limb*: stretched vs. contralateral, *condition*: stretching vs. control), an $\alpha = 0.05$ and a 1-b err = 0.80, the desired sample size computed using statistical software (G-Power 3.1, Dusseldorf, Germany) resulted in 15 participants. Additionally, a preliminary analysis of the sample size calculated on a subsample of 12 participants from the correlation between VA and VA_{MMG} using a correlation bivariate two tails model (correlation r H1 = 0.866, correlation r H0 = 0.600, a = 0.05, 1-b err = 0.80), corrected the required sample size resulted in 23 participants. On these bases, 26 participants were recruited.

Participants

Twenty-six healthy men [age: 24(2) yrs; body mass: 75(3) kg; stature: 1.80(0.03) m; mean (standard deviation)] took part in this study. The inclusion criteria were: (i) no 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 training in the previous 6 months. The local University Ethics Committee approved the study (*CE* 27/17) that was

performed following the principles of the latest version of the Declaration of Helsinki. The participants gave their written informed consent after a full explanation on the purpose of the study and the experimental design. They were informed that they were free to withdraw from the study at any time.

Procedures

For this cross-sectional study, the participants visited the laboratory four times and were required to come to the laboratory after fasting overnight, abstaining from caffeine and other similar substances for at least 12 h, and not taking part in heavy exercise for at least 48 h before the tests. During the first and the second session, the participants were familiarized with the experimental set-up, getting accustomed to the maximum voluntary contraction (MVC), the interpolated-twitch technique procedures, and the procedures to define the joint passive stiffness On these occasions, a map with some identification points over the skin (moles, scars, angiomas, anatomical landmarks) together with the position of the angle transducers, stimulation, and surface electromyography (sEMG) electrodes, and accelerometers were drawn on transparent sheets, to allow a more accurate and consistent electrodes repositioning within the same area (Fig. 1). More in detail, the distribution of the MMG sensor location was 22±2% of the distance from the tibial plateau to the transmalleolar line for GL, 32±3% for GL and 64±4% for SOL. The participants were also accustomed to the discomfort induced by the passive stretching protocol through a visual analogue scale. Moreover, the participants underwent an experimental procedure to determine any possible between-muscle crosstalk in the MMG signal during stimulation. The outcomes from these two sessions were used to calculate the inter-session reliability. The third and fourth sessions were proposed in a randomized order and consisted in a

unilateral passive stretching routine of the plantar flexors, or the control session. All assessments were performed in a laboratory with constant room temperature [20 (2°C)] and humidity [50% (3%)]. To minimize the circadian changes in force and joint mobility, the tests were conducted at the same time, between 9:00 AM and 12.00 noon.

The ankle range of motion (ROM) and the joint passive stiffness were firstly defined. Second, the interpolated-twitch technique was performed on the plantar flexors during (superimposed twitch) and after the MVC (potentiated twitch) to allow for the VA calculation. During this procedure, besides the force signal, the sEMG and MMG signals were detected on GM, GL and SOL to determine the VA_{MMG} for each muscle. The dependent parameters were assessed before (PRE) and immediately after (POST) the unilateral passive stretching bout involving the plantar flexors. Moreover, the stretch-induced changes were investigated in both limbs (i.e., the stretched and the contralateral non-stretched limb), with the contralateral non-stretched limb assessed as first because the effects of the passive stretching protocol in the contralateral non-stretched limb tend to disappear within 5 min (23), while in the stretched limb were shown to be visible up to two hours after the (24).

Between-muscles crosstalk

By assessing the crosstalk on each single motor point, the intent was to exclude any possible signal noise in the MMG signal deriving from the oscillations of the synergistic muscles during the nerve stimulation. The participants laid prone, with the tested ankle flexed at 90° and firmly secured at the ankle with a Velcro® strap (Velcro Industries Inc., Willemstad, Netherlands Antilles). After cleaning the skin with ethyl alcohol, the main innervation zone of

GM, GL, and SOL was localized by a pen electrode to place the cathode (45 ´ 35 mm rectangular electrode; Spes Medica, Battipaglia, SA, Italy) of the stimulator (Digitimer Stimulator Model DS7AH, Hertfordshire, UK; stimulation characteristics: pulse 1 ms, with an inter-pulse duration of 10 ms]. A common anode (40 ´ 90 mm rectangular electrode; Spes Medica, Battipaglia, SA, Italy) was placed strictly in contact with the other side of the leg. The MMGp-p_{POT} was measured on each muscle by a monodirectional accelerometers [model ADXL103; Analog Devices, Norwood, MA, USA; device weight <1.0 g; sensitivity 1000 mV·g−1; measure range (1.7 g)]. For each muscle, the stimulation amplitude generating the maximum MMGp-p_{POT} was detected with +10 mA steps starting from 30 mA. Such a stimulation current was then increased by +10% during the assessment procedures. After 20 min of passive recovery, the stimulation was evoked individually on each muscle (mean stimulation current: GM = 105±10 mA; GL = 98±8 mA; SOL = 93±7 mA), and the MMGp-p_{POT} generated by the stimulation on each synergistic muscle were recorded. Three stimulations per muscle were elicited, with 3 min of passive recovery between each stimulation. The order of the muscle stimulated was randomized. For each muscle, the average MMGp-p_{POT} of the muscle directly stimulated was first calculated and used to normalize the MMGp-p_{POT} elicited during the stimulations of the other synergistic muscles.

Measurements and data analysis

Dorsiflexion ROM

The ankle was securely fixed to a custom-made ergometer for assessing the dorsiflexion ROM and the joint passive stiffness. A previously calibrated bi-axial angle transducer (mod. TSD 130A, Biopac System, CA, USA) was positioned on the external face of the fibula and on the calcaneum to monitor the changes in ankle ROM. After 10 passive ankle movements

performed by an operator, the dorsiflexion ROM was determined starting with the ankle at its neutral position (~0° of dorsiflexion) (23), and manually slowly dorsiflexed to avoid the activation of any muscle reflex, as monitored by the sEMG signal, until the point of discomfort was reached. The difference between the ankle neutral position and the end of the passive dorsiflexion ROM was considered as the joint ROM.

Joint passive stiffness

To assess the joint passive stiffness, a metal plate was manually fixed at 0°, 10°, 20° of ankle dorsiflexion and at end of the ROM, and the force signal was recorded at each angle (16). As for the passive dorsiflexion ROM, the ankle was moved slowly to avoid the activation of any muscle reflex, as checked by the sEMG signal. The passive resisting torque exerted by the plantar flexors was recorded at each angle as the average of the force values during the first 5 s after the ankle positioning. Such a short time allowed the operator to minimize the influence of the static position on the joint viscoelastic properties (16). The passive torque-angle curve between 0° and 20° of dorsiflexion was fitted with the best polynomial regression model (16, 25), and the slope of this curve at 20° of dorsiflexion (maximum common angle for all participants) represented the joint passive stiffness.

Maximum voluntary contraction

The participants laid prone on a custom-made ergometer (18), with a mobile metal plate connected to a previously calibrated load cell (mod. SM-2000 N, Interface, UK; operating linearly between 0 and 2000 N). The ankle of the dominant limb was firmly secured to the mobile metal plate by a Velcro® strap (Velcro Industries Inc., Willemstad, Netherlands Antilles)

to minimize any heel displacement during the measurement. The hips and the shoulders were also firmly secured to the ergometer. The load cell was constantly kept in line with the axis of force transmission. The force signal was driven to an A/D converter (mod. UM 150, Biopac, Biopac System Inc., Santa Barbara, CA, USA), sampled at 1024 Hz, and stored on a personal computer. The torque was calculated by multiplying the force output by the distance between the apex of the external malleolus and the force application point. After a standardized warm-up (10 ´ 2-s contractions at 50% MVC determined during the familiarization), three MVC attempts were performed at baseline, while one MVC was executed at POST. The participants were instructed to push as fast and hard as possible for 4 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 1000 Hz, and stored on a personal computer. The maximum force value recorded during the MVC was inserted into the data analysis.

Voluntary activation

At baseline, the interpolated-twitch technique was performed eliciting a superimposed and a potentiated twitch consisting of a doublet of 1 ms with an inter-pulse duration of 10 ms (17, 26). The superimposed twitch was elicited during the force signal plateauing visible while exerting the MVC; the potentiated twitch was elicited 5 s after the end of the MVC, at rest. A stimulating electrode (8-mm diameter, Ag-AgCl circle electrode; Medicompex SA,Ecublens, Switzerland) was positioned on the popliteal fossa, while the receiving electrode (50 ´ 100 mm rectangular electrode; Medicompex SA, Ecublens, Switzerland) was placed over the patella. The electrodes were connected to a high-voltage stimulator (Digitimer Stimulator Model DS7AH, Hertfordshire, UK). The amperage of a square wave pulse was increased until the maximum

elicited force was achieved (mean stimulation current = 118±12 mA). The force signal was analyzed with the software AcqKnowledge 4.4 (Biopac Systems, Goleta, CA). The VA was calculated as follows (27):

$$VA = [100 - \left(\frac{superimposed\ twitch}{potentiated\ twitch}\right) \times\ 100]$$

Mechanomyogram signal

The MMG signal was detected by three monodirectional accelerometers (mod. ADXL103; Analog Devices, Norwood, MA; device weight, <1.0 g; sensitivity, 1000 mV·g−1; measure range, ±1.7 g) placed on the point on maximum vertical displacement during a contraction visually inspected on GM, GL, and SOL during both the MVC and the potentiated twitch. The signal was acquired with a sampling rate of 1000 Hz by an A/D converter (mod. UM 150 Biopac; Biopac System Inc.) filtered (filter type: IV order Butterworth filter; bandwidth, 4–120 Hz) and stored on a personal computer, for further analysis, performed with the software AcqKnowledge 4.4 (Biopac Systems, Goleta, CA). The MMG signal was analyzed in time and frequency domain within a 1-s time window detected in the middle of the MVC plateau preceding the superimposed stimulation. From this time window, the MMG RMS and MF (fast Fourier transform method) were calculated in consecutive 250-ms time windows and then averaged. The MMGp-p_{POT} and the delay from the stimulation and the onset of MMGp-p_{POT} were calculated and the latter was used to identify the onset of MMGp-p_{SUP} [PRE: 13.7 (1.2) ms; POST: 14.9 (2.2) ms]. Based on the correlations between the MMGp-p_{SUP} and the superimposed twitch (15, 28), and the MMGp-p_{POT} and the potentiated twitch (14), similar to calculation of VA, we identified the VA_{MMG} as follows (13):

$$VA_{MMG} = [100 - \left(\frac{MMGp - p_{SUP}}{MMGp - p_{POT}}\right) \times 100]$$

sEMG signal

The sEMG signal was detected during the MVC and the potentiated twitch in *GM*, *GL*, and *SOL* by a linear array of eight electrodes (mod. ELSCH008; 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 sEMG 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 sEMG array was placed over the muscle belly along the direction of the muscle fibers, in accordance with the European recommendations for surface EMG (29). The sEMG signal was acquired by a multichannel amplifier with a sampling rate of 2048 Hz (mod. EMG-USB; OtBioelettronica; input impedance: >90 MΩ; CMRR: >96 dB), amplified (gain ´ 1000), and filtered (filter type: IV order Butterworth filter; bandwidth, 10–500 Hz) for further analysis. The sEMG analysis was performed by OtBiolab+ software (OtBioelettronica). The signal was analyzed in time domain within the same 1-s period as for the MMG signal: the sEMG RMS was calculated in consecutive 250-ms time windows and then averaged. The sEMG signal recorded during the potentiated twitch was exported as .csv file and converted in .acq files (AcqKnowledge 4.4; Biopac Systems). The software allowed calculating the maximum peak-to-peak of signal, which was considered as M-wave. The sEMG RMS/M-wave ratio were then calculated for each muscle.

Passive stretching

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 (30). The stretching intensity was kept constant by 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) (30). Specifically, the load cell was positioned 5 cm above the metatarsus of the stretched limb and an operator pushed perpendicularly the load cell to stretch the plantar flexors. To minimize any possible muscle reflex activation, the muscle elongation was reached in 6 s and maintained for 45 s (31). 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 (16, 17, 32). The sEMG signal was checked during passive stretching to monitor any possible muscle activation during the elongation. If the sEMG signal during the passive stretching protocol was > 5% MVC, the participant would be excluded from the study and replaced with another one to ensure statistical power (17). In the current study, no participant was excluded. In the control session, the participants laid prone as relaxed as possible with the ankle at a neutral angle (~0° of dorsiflexion) for an equivalent duration.

Statistical analysis

The statistical analysis was performed using a statistical software package (IBM SPSS Statistics 27, Armonk, NY). The Shapiro–Wilk's and Mauchly tests checked the normal

distribution and the sphericity of the sampling. The Greenhouse-Geisser correction was performed if the sphericity assumption was violated. The outcomes deriving from the first two sessions were used to calculate the intersession reliability and sensitivity. The reliability was calculated using a two-way random, consistency type intraclass correlation coefficient (ICC). Cronbach's was classified as: very high (≥0.90); high (0.89 to 0.70); moderate (0.69 to 0.50) and the percentage standard error of the measurement (SEM%) was calculated. The minimum detectable change with a 95% confidence interval ($MDC_{95\%}$) defined the sensitivity. The PRE-POST differences in dorsiflexion ROM, MVC, pF, VA and joint passive stiffness in the stretched and contralateral non-stretched limb were calculated by a three-way (time ´ stretching ´ limb) analysis of variance (ANOVA) for repeated measures. To calculate the between muscle (GM, GL, and SOL) differences in the MMG and sEMG variables, a four-way (time ´ stretching ´ limb ´ muscle) ANOVA for repeated measures was performed. Further analysis of covariance (ANCOVA) was performed for force, MMG, and sEMG variables taking the baseline values as covariate. Multiple comparisons were adjusted using the Bonferroni correction. The size of the differences was determined by Cohen's *d* or partial eta squared ($\square_p^2$) statistics when appropriated. Cohen's *d value* was classified as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79), and large (≥0.80). h^2_P was classified as small (0.01-0.059), medium (0.06-0.139), and large (≥ 0.14). Pearson's moment product test with bootstrap method correction checked for correlations between VA_{MMG}, VA and the joint passive stiffness. The correlations' magnitude was classified as trivial for a coefficient (*R*) <0.1, low for *R* between 0.11 and 0.30, moderate for *R* between 0.31 and 0.50, high for *R* between 0.51 and 0.70, very high for *R* between 0.71 and 0.90, nearly perfect for *R* between 0.91 and 0.99, and perfect for *R* = 1 (33). The determination

coefficient (R^2) was also calculated. Statistical significance was set at P value <0.05. Unless otherwise stated, descriptive statistics are presented as mean (SD).

Results

The reliability (ICC and SEM%) and sensitivity variables ($MDC_{95\%}$) are reported in Table 1. The ICC ranged from 0.966 to 0.995, and the SEM% ranged from 2.33% to 4.43%. $MDC_{95\%}$ ranged from 4.57% to 8.69%.

Between-muscle crosstalk

Table 2 reports the MMGp-p_{POT} elicited during the direct stimulation of one muscle and those from the muscle not-directly stimulated. Crosstalk signals ranged from 6(1)% to 10(3)% were found from the muscles not directly stimulated.

Dorsiflexion ROM

ANOVA showed a time ´ stretching ´ limb interaction in dorsiflexion ROM ($F_{1,99}$= 19.79, P<0.001, h^2_P = 0.287). After stretching, the dorsiflexion ROM (Fig. 2 panel A) increased after stretching in the stretched limb by 24(13)% [P<0.001, ES = 1.54 (1.23 to 1.84)] and in the contralateral non-stretched limb by 10(6)% [P<0.001, ES = 0.80 (0.52 to 1.07)], with larger increases in the stretched compared to the contralateral non-stretched limb [P<0.001, ES = 0.89 (0.29 to 1.46)]. No change was found in the control session (P = 0.559 and 0.617 in the two limbs).

Joint passive stiffness

ANOVA showed a time ´ stretching ´ limb interaction in the joint passive stiffness ($F_{1,99}$= 16.66, P<0.001, h^2_P = 0.212). The joint passive stiffness (Fig. 2 panel B) decreased after stretching by -22(8)% [P<0.001, ES = -1.75 (-2.07 to -1.44)] and -3(2)% [P=0.041, ES = -0.27 (-0.54 to 0.00)] in the stretched and in the contralateral non-stretched limb, respectively. Decrements were larger in the stretched compared to the contralateral non-stretched limb [P<0.001, ES = 1.47 (0.86 to 2.08)]. No change was found in the control session (P = 0.661 and 0.436 in the two limbs).

MVC

ANOVA showed a time ´ stretching ´ limb interaction in MVC ($F_{1,99}$ = 16.66, P<0.001, h^2_P = 0.212). After stretching the plantar flexor muscles MVC (Fig. 3, panel A) was reduced in the stretched [-13(7)%, P<0.001, ES = -0.74 (-1.01 to -0.46)] and in the contralateral non-stretched limb [-4(3)%, P=0.035, ES = -0.24 (-0.50 to -0.03)], with a wider decrease in the stretched compared to the contralateral non-stretched limb [P<0.001, ES = 0.48 (0.07 to 1.28)]. No change was found in the control session (P = 0.568 and 0.686 in the two limbs).

Potentiated twitch

ANOVA showed a time ´ stretching ´ limb interaction in potentiated twitch ($F_{1,99}$ = 12.67, P<0.001, h^2_P = 0.159). The potentiated twitch (Fig 3. panel B) decreased after stretching only in the stretched limb by -7(5)% [P<0.001, ES = -0.36 (-0.63 to -0.09)]. No change occurred in the contralateral non-stretched limb and in both limbs in the control session (P range: 0.298 to 0.874).

VA

ANOVA showed a time ´ stretching ´ limb interaction in VA ($F_{1,99}$= 7.63, P<0.001, h^2_P = 0.145).VA (Fig 3, panel C) was reduced after stretching in the stretched limb by -7(3)%, [P<0.001, ES = -2.36 (-2.71 to -2.01) and in the contralateral non-stretched limb by -4 (2)% [P<0.001, ES = -1.77 (-2.09 to -1.46)], with larger reductions in the stretched compared to the contralateral non-stretched limb [P<0.001, ES = -1.06 (-1.35 to -0.78)]. No difference occurred in the control session (P = 0.690 and 0.197 in both limbs].

VA_{MMG}

ANOVA showed no time ´ stretching ´ limb ´ muscle interaction ($F_{1,299}$ = 1.14, P = 0.330, h^2_P = 0.007), but retrieved a time ´ stretching ´ limb in VA_{MMG} ($F_{1,99}$= 10.19, P<0.001, h^2_P = 0.118). As shown in Fig. 4, after stretching the VA_{MMG} decreased by a similar extent in the *two gastrocnemii* and in the SOL of the stretched limb [~ -5 (2)%, P<0.001 in all muscles, ES range = -1.26 to -1.13] and of the contralateral non-stretched limb [~ -2 (1)%, P<0.05 in all muscles, ES range = -0.54 to -0.46]. with larger decreases in the stretched than in the contralateral non-stretched limb [P<0.001 in all the muscles, ES range = 0.57 to 0.89]. No change occurred in the control session (P range = 0.100 - 0.860).

MMG RMS

ANOVA showed no time ´ stretching ´ limb ´ muscle interaction in MMG RMS ($F_{1,299}$= 1.69, P = 0.187, h^2_P = 0.011). A time ´ stretching ´ limb was retrieved in MMG RMS ($F_{1,99}$= 28.52, P<0.001, h^2_P = 0.748). After stretching the MMG RMS increased in the stretched limb by about 28(3)% (P<0.001 in all the muscles), while it decreased in the contralateral non-stretched

limb by about -5(2)%, (P<0.001 in all the muscles). No change occurred in the control session (P range = 0.217 to 0.960).

MMG MF

ANOVA showed no time ´ stretching ´ limb ´ muscle interaction in MMG MF ($F_{1,299}$= 1.39, P = 0.250, h^2_P = 0.009). A time ´ stretching ´ limb was retrieved in MMG MF ($F_{1,99}$=3.26, P = 0.04, h^2_P = 0.021). MMG MF decreased after stretching in the stretched limb by about -16(5)% (P <0.001 in all the muscles) and in the contralateral non-stretched limb by about -5(2)% (P <0.001 in GM, GL and SOL), with greater reductions in the stretched compared to the contralateral non-stretched limb (P range. 0.001 to <0.001, *ES* range: 0.46 to 0.95]. No change was observed in the control session (P range: 0.209 to 0.570).

Cohen's d ES with 95% CI of the changes in MMG RMS and MF are shown in Supplemental Table 1 (see Supplemental Digital Content, http://links.lww.com/MSS/C736).

MMGp-p_{MVC}

No time ´ stretching ´ limb ´ muscle interaction was observed for MMGp-p_{MVC} ($F_{1,299}$= 0.99, P = 0.373, h^2_P = 0.007). A time ´ stretching ´ limb interaction was observed in MMGp-p_{MVC} ($F_{1,99}$= 11.64, P<0.001, h^2_P = 0.171). Stretching reduced MMGp-p_{MVC} in the stretched and contralateral non-stretched limb by about -13(4)% and -6(1)%, respectively (P<0.001 in all the muscles), with larger decrease in the stretched than in the contralateral non-stretched limb (P<0.001, *ES* range: 0.30 to 0.46). No change occurred in the control session (P range: 0.176 to 0.942).

MMGp-p_{SUP}.

No time ´ stretching ´ limb ´ muscle interaction was observed for MMGp-p_{SUP} ($F_{1,299}$= 0.15, P = 0.864, h^2_P = 0.001). A time ´ stretching ´ limb interaction was observed in MMGp-p_{SUP} ($F_{1,99}$= 6.48, P<0.001, h^2_P = 0.115). MMGp-p_{SUP} increased after stretching in the stretched limb by about 26(2)% and contralateral non-stretched limb by about 16(3)% (P<0.001 in all the muscles), with larger increase in the stretched than in the contralateral non-stretched limb (P<0.001 in all the muscles, *ES* range: 0.531– 0.701). No change occurred in the control session (P range: 0.305 to 0.760).

MMGp-p_{POT}.

No time ´ stretching ´ limb ´ muscle interaction was retrieved for MMGp-p_{POT} ($F_{1,299}$= 1.85, P = 0.159, h^2_P = 0.012). A time ´ stretching ´ limb interaction was retrieved in MMGp-p_{POT} ($F_{1,99}$= 17.06, P<0.001, h^2_P = 0.183). The MMGp-p_{POT} was reduced after stretching only in the stretched limb by ~ -13 (6)%, (P <0.001 in all muscles), while it was unchanged in the contralateral non-stretched limb and in both limbs in the control session [~0 (3)%, P range 0.164 - 0.982].

Cohen's d ES with 95% CI of the changes in MMGp-p_{MVC}, MMGp-p_{SUP}, and MMGp-p_{POT} are shown in Supplemental Table 2 (see Supplemental Digital Content, http://links.lww.com/MSS/C736).

sEMG RMS.

No time ´ stretching ´ limb ´ muscle interaction for sEMG RMS was retrieved ($F_{1,299}$=1.03, P=0.358, h^2_P=0.007), whereas it was found a time ´ stretching ´ limb interaction ($F_{1,99}$=30.61, P<0.001, h^2_P=0.093). After stretching sEMG RMS was decreased in both the stretched [~ -17 (13)%, P<0.001 for all muscles] and the contralateral non-stretched limb [~ -6 (7)%, P range 0.001 - <0.001], with larger decrease in the stretched than in the contralateral non-stretched limb (P<0.001 in all the muscles, *ES* range: 0.707 – 1.148). No change was observed in the control session (P>0.05).

M-wave.

ANOVA did not show a time ´ exercise ´ limb ´ muscle interaction for M-wave. No changes occurred in the M-wave in both limbs and in both conditions (P>0.05).

sEMG RMS/M-wave.

ANOVA did not show a time ´ stretching ´ limb ´ muscle interaction for sEMG RMS/M-wave. ($F_{1,299}$=0.29, P=0.745, h^2_P=0.002), whereas it was found a time ´ stretching ´ limb interaction ($F_{1,99}$=15.28, P<0.001, h^2_P=0.049). After stretching sEMG RMS/M-wave was decreased in both the stretched and the contralateral non-stretched limb (P<0.001 in all muscles), with larger decreases in the stretched limb wave than in the contralateral non-stretched limb (P range 0.01 - <0.001, *ES* range: 0.38 – 0.51). No change was observed in the control session (P>0.05).

Cohen's d ES with 95% CI of the changes in sEMG RMS, M-wave, and sEMG RMS/M-wave are shown in Supplemental Table 3 (see Supplemental Digital Content, http://links.lww.com/MSS/C736).

Correlations.

The correlations among VA_{MMG}, VA, and the joint passive stiffness are shown in Table 3 (PRE or POST values) and Figure 5 (D% PRE-POST changes), respectively. *Moderate* to *very high* positive correlations were observed for between VA_{MMG} and VA in PRE and POST values and between the stretch-induced D% PRE-POST changes. *Moderate* to *high* positive correlations were found between the joint stiffness and VA in PRE, POST, and D% PRE-POST changes, but not with VA_{MMG}. No correlation was found between VA_{MMG} and EMG RMS/M-wave.

The correlations between the joint passive stiffness and MMG RMS, MMGp-p_{MVC}, MMGp-p_{SUP}, and MMGp-p_{POT} in the three muscles of the stretched and in the contralateral non-stretched limb are shown in Supplemental Table 4 (see Supplemental Digital Content, http://links.lww.com/MSS/C736).

DISCUSSION

The aims of the current study were to: (i) determine whether VA_{MMG} is correlated with the ankle joint passive stiffness before and after a passive stretching routine involving the plantar flexors, as well as determine the correlation between the stretch-induced changes, and (ii) check for possible differences in VA_{MMG} between biarticular, i.e., GM and GL, vs monoarticular muscles, i.e., SOL. The present findings highlighted that: (i) no correlation was found between VA_{MMG} and the joint passive stiffness, neither when considering the raw values before and after passive stretching nor when considering the percentage stretch-induced changes, and (ii) VA_{MMG} decreased after stretching in GM, GL, and SOL by a similar extent, thus showing no difference between biarticular and monoarticular muscles. Importantly, VA_{MMG} presented a *very high* inter-

session reliability and a sufficient sensitivity to detect the changes in the voluntary activation variations even in the contralateral non-stretched limb not involved in the stretching routine. VA_{MMG} appears able to monitor the synergic muscles voluntary activation and is minimally affected by the joint passive mechanical properties.

Preliminary considerations: effects of stretching on ROM, MVC, and MMG signal.

We observed *very high* inter-session reliability, *moderate* SEM% for VA_{MMG}, and an adequate sensitivity, as reported in a previous study (13). In addition, the MMG signal crosstalk values spanned from 6% to 10%, in agreement with previous literature (13, 34–36). These results suggest that VA_{MMG} was minimally influenced by possible methodological biases. It should be reminded that the activation of the muscle is different when the stimulation occurs at the motor point or at the nerve (37). For example, the former elicits the contraction of all fibers in the three plantar flexors while the latter implies the contraction of a group of fibers of a single muscle, beginning with those nearest the skin. Therefore, the present results should be interpreted aware of this difference.

In line with previous investigations, after passive stretching dorsiflexion ROM increased whereas the MVC and the joint passive stiffness decreased in both the stretched and the contralateral non-stretched limb, with greater changes occurring in the former (17, 23). The current increase in dorsiflexion ROM and the simultaneous reduction in MVC and joint passive stiffness are due to both neuromuscular (38) and mechanical factors (16). The former include an alteration in the motor pathway potentially acting at central level that could depress the force-generating capacity in both limbs, as suggested here by the reduction in sEMG RMS/M-wave, in

line with previous studies (23, 39). Moreover, the decrease in joint passive stiffness could be also caused by a reduction in muscle tone associated with a decrease in nociceptive activity, increasing the stretch-tolerance both in the stretched and in the contralateral non-stretched limb (40). In contrast, the mechanical factors act only on the stretched limb and reflects the changes in the passive stiffness that, while on the one hand permitted a wider dorsiflexion ROM, on the other hand reduced the force transmission effectiveness during the contraction (17, 23). Concomitantly, the MMG RMS of the stretched limb increased by a similar extent in the three muscles, while it decreased in the contralateral non-stretched limb. Moreover, the MMG RMS in the stretched limb is also influenced by the in-parallel viscoelastic elements (41), while in the contralateral non-stretched limb could reflect a reduced number of active motor units recruited during the MVC, since not mechanically involved in the stretching maneuver (17). The decrease in the MMG MF in both the stretched and the contralateral non-stretched limb could reflect the fall in firing rate of the high-threshold motor units during the MVC (41). Also in this case, the similar decrease in MMG RMS and MF found in the three muscles suggest an analogous reduction in muscle excitation occurring after stretching.

VA, VA_{MMG} and joint passive stiffness correlations.

Passive stretching reduced the VA, the VA_{MMG}, and the joint passive stiffness in both the stretched and in the contralateral non-stretched limb. The decrease in VA in both limbs is in agreement with a previous investigation (17). It was suggested that such a reduction could have been the result of a passive stretch-induced inhibitory effect occurring at supraspinal level, leading to a simultaneous reduction in the motor drive toward both limbs (42–44). Such an inhibitory effect could have derived from afferent feedback originating from the peripheral

mechanoreceptors, proprioceptors and nociceptors located in the stretched limb possibly transferred to the contralateral hemisphere, resulting in a contralateral reduction in VA (42–44). 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 pathways allowing the afferent stimuli to be crossed over.

Reductions in VA_{MMG} were observed in GM, GL and SOL both in the stretched and in the contralateral non-stretched limb. Previous studies have shown that synergistic biarticular muscles underwent greater increases in passive stiffness than monoarticular muscles while passively lengthened (19–22). This would derive from the hypothesized greater variations in passive stiffness in biarticular muscles because of the need to stabilize more joints simultaneously (19–22). However this was not the case here, where the reductions in VA_{MMG} were similar between GM, GL and SOL in both limbs. This would imply that the differences in the joint passive stiffness between biarticular and monoarticular muscles may be transitory and more visible during the passive stretching protocol, while after stretching such differences tend to disappear. In line, VA_{MMG} decreased similarly in the contralateral non-stretched limb, that did not undergo any mechanical stress. As a whole, VA_{MMG} does not seem to convey the possible between-muscle difference in mechanical properties.

Moderate correlations were found between the stretch-induced reductions in VA and VA_{MMG}. Intriguingly, VA presented a *moderate* correlation with the joint passive stiffness in both the stretched and the contralateral non-stretched limb. Both the superimposed and the

potentiated twitch (i.e., the two variables constituting the VA) are generated after the tensioning of the in-series elastic elements, so that their mechanical behavior could possibly affect both amplitudes (7–9). Nevertheless, when examining the positive correlation between VA_{MMG} and VA in the contralateral non-stretched limb as in the stretched limb, it appears clear that such a correlation may derive mainly from neural mechanisms. In contrast, if VA_{MMG} would have been influenced mainly by mechanical factors, the correlation between VA_{MMG} and VA in the contralateral non-stretched limb would have not occurred. Interestingly, the joint passive stiffness appeared to explain approximatively 25% to 34% of the variance of the VA. While indubitably neural factors play a role in the determination of the VA, still the influence of the mechanical properties may lead to question the appropriateness of defining VA as something uniquely related to the changes in supraspinal factors.

In contrast, $VA_{MMG,}$ as well as MMGp-p_{POT} or MMGp-p_{SUP} (i.e., the two variables constituting the VA_{MMG}) were not correlated with the joint passive stiffness. Individually, MMGp-p_{POT} derives from the in-series elastic elements located at the muscle level before the tensioning, so that its amplitude should not be affected by their mechanical characteristics (16). As such, the lack of correlation with the joint passive stiffness could have been supposed. On the contrary, the MMGp-p_{SUP} is expected to be sensitive to the changes in mechanical properties of the in-parallel viscoelastic elements (16, 41), that could have affected the amplitude of MMGp-p_{SUP} particularly in the stretched limb. However, no correlation with the joint passive stiffness was found, as well as for VA_{MMG}. Interestingly, MMG RMS during the MVC correlated inversely with the joint passive stiffness as seen in previous studies, so that the reduced joint passive stiffness permitted greater oscillations of the parallel elastic elements (16). When super-

imposing a twitch, the consequent muscle oscillations derive primarily from the greater activation evoked electrically, and secondarily from the mechanical characteristics of the joint passive stiffness (41, 45, 46). This may account for the lack of correlation between the VA_{MMG} and MMGp-p_{SUP} with the joint passive stiffness, being the former more associated with the muscle activation evoked electrically rather than the joint passive stiffness.

The present study comes with some acknowledged limitations. First, it was not possible to obtain MMG information concerning the changes in voluntary activation in the antagonist muscles, which is one of the limitations intrinsic to the VA with the present MMG-based procedures. Second, assessing passive stiffness directly from each muscle as done in previous investigation (19–22) instead of from the joint would have provided more detailed information about each muscle. Third, the MMG signal is affected by the amount of subcutaneous fat. Similarly, the specific sex-dependent muscle architecture motor unit recruitment characteristics may affect the VA_{MMG} estimates (47, 48). Consequently, the present data assessed in healthy men should be extended to other populations (e.g., women) with caution. Fourth, a single MVC was performed after the stretching protocol and it may not be fully representative of the force-generating capacity, so that a second MVC trial should be considered. Future studies addressing these three limitations are needed.

CONCLUSIONS

Stretching plantar flexors induced concomitant reductions in VA during the plantar flexion, VA_{MMG} of GM, GL and SOL and in the joint passive stiffness. The reductions in VA_{MMG} were similar between the muscles. These stretch-induced changes were also visible in the

contralateral non-stretched limb, albeit with lower extents. Additionally, while VA correlated with the joint passive stiffness, VA_{MMG} did not. Lastly, the VA_{MMG} presents a high intersession reliability and an adequate sensitivity in detecting the variations in voluntary activation induced by passive stretching.

The current results open for some methodological novelties. The interpolated-twitch technique is traditionally used for determining VA. However, i) interpreting the force signal does not allow distinguishing the synergistic individual muscles that are involved in the task and ii) the force signal appears influenced by mechanical factors. We propose to include the interpretation MMG with the force signal, with the intent to distinguish the behavior of each synergistic muscle and to account for the mechanical properties of the joint that affect the extent of the VA. In the light of these outcomes, the VA_{MMG} can be used as an alternative/complementary index to VA to quantify the voluntary activation, especially in all those circumstances in which more synergistic muscles are involved in muscle contraction and could present different levels of voluntary activation.

Author Contributions

Conception or design of the study: G.C., E.C., F.E. Acquisition, analysis, or interpretation of data for the study: E.C., C.D., M.B., N.T., S.R., E.L., S.L. Drafting the manuscript or revising it critically for important intellectual content: G.C., E.C., S.L., F.E. All the authors have approved the final version of the manuscript and agree to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

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Ethics approval

The local University Ethics Committee approved the study (*CE* 27/17), which was performed following the principles of the latest version of the Declaration of Helsinki. All experiments were conducted at the Physiology Labs of the School of Sport Science, Università degli Studi di Milano.

Participant Consent

All participants gave their written, informed consent after receiving an explanation of the study purpose and design. The participants were free to withdraw from the study at any time. All the participants gave their consent for data and study publication.

Availability of data and material

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

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REFERENCES

1. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*. 2001;81(4):1725–89.
2. Rekling JC, Funk GD, Bayliss DA, Dong X-W, Feldman JL. Synaptic control of motoneuronal excitability. *Physiol Rev*. 2000;80(2):767–852.
3. de Haan A, Gerrits KHL, de Ruiter CJ. Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol (1985)*. 2009;107(1):355-7; discussion 357-8.
4. Belanger AY, McComas AJ. Extent of motor unit activation during effort. *J Appl Physiol Respir Environ Exerc Physiol*. 1981;51(5):1131–5.
5. Merton PA. Voluntary strength and fatigue. *J Physiol*. 1954;123(3):553–64.
6. Behm DG, Whittle J, Button D, Power K. Intermuscle differences in activation. *Muscle Nerve*. 2002;25(2):236-43.
7. Arampatzis A, Mademli L, De Monte G, Walsh M. Changes in fascicle length from rest to maximal voluntary contraction affect the assessment of voluntary activation. *J Biomech*. 2007;40(14):3193–200.
8. Suter E, Herzog W. Effect of number of stimuli and timing of twitch application on variability in interpolated twitch torque. *J Appl Physiol (1985)*. 2001;90(3):1036–40.
9. Bulow P, Nørregaard J, Danneskiold-Samsøe B, Mehlsen J. Twitch interpolation technique in testing of maximal muscle strength: influence of potentiation, force level, stimulus intensity and preload. *Eur J Appl Physiol Occup Physiol*. 1993;67(5):462–6.
10. 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.

11. Cè E, Longo S, Limonta E, Coratella G, Rampichini S, Esposito F. Peripheral fatigue : new mechanistic insights from recent technologies. *Eur J Appl Physiol*. 2020;120(1):17–39.
12. Orizio C. Muscle sound: bases for the introduction of a mechanomyographic signal in muscle studies. *Crit Rev Biomed Eng*. 1993;21(3):201–43.
13. Cè E, Coratella G, Doria C, et al. Determining voluntary activation in synergistic muscles: a novel mechanomyographic approach. *Eur J Appl Physiol*. 2022;122(8):1897-913.
14. Gobbo M, Cè E, Diemont B, Esposito F, Orizio C. Torque and surface mechanomyogram parallel reduction during fatiguing stimulation in human muscles. *Eur J Appl Physiol*. 2006;97(1):9–15.
15. Ohta Y, Shima N, Yabe K. Superimposed mechanomyographic response at different contraction intensity in medical gastrocnemius and soleus muscles. *Int J Sport Health Sci*. 2007;5:63–70.
16. 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.
17. Cè E, Coratella G, Bisconti AV, et al. Neuromuscular versus mechanical stretch-induced changes in contra- versus ipsilateral muscle. *Med Sci Sports Exerc*. 2020;52(6):1294–306.
18. Longo S, Cè E, Rampichini S, et al. Correlation between stiffness and electromechanical delay components during muscle contraction and relaxation before and after static stretching. *J Electromyogr Kinesiol*. 2017;33:83–93.

19. Akagi R, Takahashi H. Acute effect of static stretching on hardness of the gastrocnemius muscle. *Med Sci Sports Exerc*. 2013;45(7):1348–54.

20. Akagi R, Takahashi H. Effect of a 5-week static stretching program on hardness of the gastrocnemius muscle. *Scand J Med Sci Sports*. 2014;24(6):950–7.

21. Akagi R, Fukui T, Kubota M, Nakamura M, Ema R. Muscle shear moduli changes and frequency of alternate muscle activity of plantar flexor synergists induced by prolonged low-level contraction. *Front Physiol*. 2017;8:708.

22. Andrade RJ, Freitas SR, Hug F, et al. Chronic effects of muscle and nerve-directed stretching on tissue mechanics. *J Appl Physiol (1985)*. 2020;129(5):1011–23.

23. Coratella G, Cè E, Doria C, Borrelli M, Longo S, Esposito F. Neuromuscular correlates of the contralateral stretch-induced strength loss. *Med Sci Sports Exerc*. 2021;53(10):2066–75.

24. 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.

25. Mizuno T, Matsumoto M, Umemura Y. Viscoelasticity of the muscle-tendon unit is returned more rapidly than range of motion after stretching. *Scand J Med Sci Sports*. 2013;23(1):23–30.

26. Coratella G, Grosprêtre S, Gimenez P, et al. Greater fatigability in knee-flexors vs . knee-extensors after a standardized fatiguing protocol. *Eur J Sport Sci*. 2018;18(8):1110-8.

27. Rozand V, Grosprêtre S, Stapley PJ, Lepers R. Assessment of neuromuscular function using percutaneous electrical nerve stimulation. *J Vis Exp*. 2015;(103):52974.

28. Ohta Y, Shima N, Yabe K. In vivo behaviour of human muscle architecture and

mechanomyographic response using the interpolated twitch technique. *J Electromyogr Kinesiol*. 2009;19(3):e154–61.

29. Hermens HJ, Freriks B, Merletti R, et al. European recommendations for surface electromyography: results of the SENIAM project. *Roessingh Res Dev*. 2000;10(5):361–74.

30. Venturelli M, Rampichini S, Coratella G, et al. Heart and musculoskeletal hemodynamic response to repetitive bouts of quadricepes static stretching. *J Appl Physiol (1985)*. 2019;127(2):376–84.

31. 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.

32. Venturelli M, Rampichini S, Coratella G, et al. Heart and musculoskeletal hemodynamic responses to repetitive bouts of quadriceps static stretching. *J Appl Physiol (1985)*. 2019;127(2) :376-84.

33. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3–13.

34. Ismail MRM, Lam CK, Sundaraj K, Rahiman MHF. Fatigue effect on cross-talk in mechanomyography signals of extensor and flexor forearm muscles during maximal voluntary isometric contractions. *J Musculoskelet Neuronal Interact*. 2021;21(4):481–94.

35. Islam MA, Sundaraj K, Ahmad RB, Sundaraj S, Ahamed NU, Ali MA. Cross-talk in mechanomyographic signals from the forearm muscles during sub-maximal to maximal isometric grip force. *PLoS One*. 2014;9(5):e96628.

36. Beck TW, DeFreitas JM, Stock MS. An examination of cross-talk among surface

mechanomyographic signals from the superficial quadriceps femoris muscles during isometric muscle actions. *Hum Mov Sci*. 2010;29(2):165–71.

37. Bergquist AJ, Clair JM, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae. *J Appl Physiol (1985)*. 2011;110(3):627–37.

38. Trajano GS, Nosaka K, Blazevich AJ. Neurophysiological mechanisms underpinning stretch-induced force loss. *Sports Med*. 2017;47(8):1531-41.

39. Cè E, Coratella G, Doria C, et al. No effect of passive stretching on neuromuscular function and maximum force-generating capacity in the antagonist muscle. *Eur J Appl Physiol*. 2021;121(7):1955–65.

40. Magnusson SP, Simonsen EB, Aagaard P, Soørensen H, Kjær M. A mechanism for altered flexibility in human skeletal muscle. *J Physiol*. 1996;497(1):291–8.

41. 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.

42. 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.

43. Marchetti PH, De Oliveira Silva FHD, Soares EG, et al. Upper limb static-stretching protocol decreases maximal concentric jump performance. *J Sports Sci Med*. 2014;13(4):945–50.

44. 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.

45. 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.

46. Orizio C, Perini R, Veicsteinas A. Muscular sound and force relationship during isometric contraction in man. *Eur J Appl Physiol Occup Physiol*. 1989;58(5):528–33.

47. Inglis JG, Gabriel DA. Sex differences in motor unit discharge rates at maximal and submaximal levels of force output. *Appl Physiol Nutr Metab*. 2020;45(11):1197–207.

48. Kubo K, Kanehisa H, Azuma K, et al. Muscle architectural characteristics in young and elderly men and women. *Int J Sports Med*. 2003;24(2):125–30.

FIGURE LEGENDS

Figure 1. Experimental setup for the between-muscle crosstalk determination (panel A), for the mechanomyographic, surface electromyographic, and force signals detection (panel B), and the MMG sensors location with respect to the leg anatomical landmarks (panel C).

Figure 2. Individual data and percentage changes in the dorsiflexion range of motion (ROM, panel A) and the joint passive stiffness (panel B) after passive stretching and control in the stretched and contralateral non-stretched limb.

$^{*}P<0.05$ post *vs*. pre;

$^{c}P<0.05$ *vs.* control;

$^{\#}P<0.05$ contralateral non-stretched *vs*. stretched limb.

Figure 3. Individual data and percentage changes in the maximum voluntary contraction (MVC, panel A), potentiated twitch (panel B), and voluntary activation from the force signal analysis (VA, panel C) of the plantar flexors after passive stretching and control in the stretched and contralateral non-stretched limb.

$^{*}P<0.05$ post *vs*. pre;

$^{c}P<0.05$ *vs.* control;

$^{\#}P<0.05$ contralateral non-stretched *vs*. stretched limb

Figure 4. Individual data and percentage changes in the voluntary activation from mechanomyographic signal analysis (VA_{MMG}) after passive stretching (panel A) and control

(panel B) in the stretched and contralateral non-stretched limb.

GM: gastrocnemius medialis

GL: gastrocnemius lateralis

SOL: soleus

$^{*}P<0.05$ post *vs*. pre;

$^{c}P<0.05$ *vs.* control;

$^{\#}P<0.05$ contralateral non-stretched *vs*. stretched limb.

Figure 5. Correlations between the percentage changes between post and pre-stretching values (D) between the voluntary activation from mechanomyographic signal analysis (VA_{MMG}, panel A), and the voluntary activation from force and MMG signal analysis (VA), and the ankle joint passive stiffness (panel B).

GM: gastrocnemius medialis

GL: gastrocnemius lateralis

SOL: soleus

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplemental Digital Content.docx

Table S1 - Root mean square and mean frequency of the mechanomyographic signal of the three muscles for the stretched and contralateral non-stretched limb, before and after stretching and control

Table S2 - Peak-to-peak of the mechanomyographic signal during maximum voluntary contraction, superimposed, or potentiated from the three muscles on the intervention and contralateral non-stretched limb, before and after stretching or control

Table S3 - Root mean square of the surface electromyographic signal, M-wave, and sEMG RMS/M-wave ratio of the three muscles in the exercising and contralateral non-stretched limb, before and after exercise and control

Table S4 - Correlations between the joint passive stiffness, and the root mean square of the mechanomyographic signal, the peak-to-peak of the mechanomyographic signal calculated during the maximum voluntary contraction, the superimposed, and the potentiated stimulation in the *gastrocnemius medialis*, *gastrocnemius lateralis*, and *soleus* in the stretched and the contralateral non-stretched limb, before, after stretching, and between the POST-PRE percentage differences

Figure 1

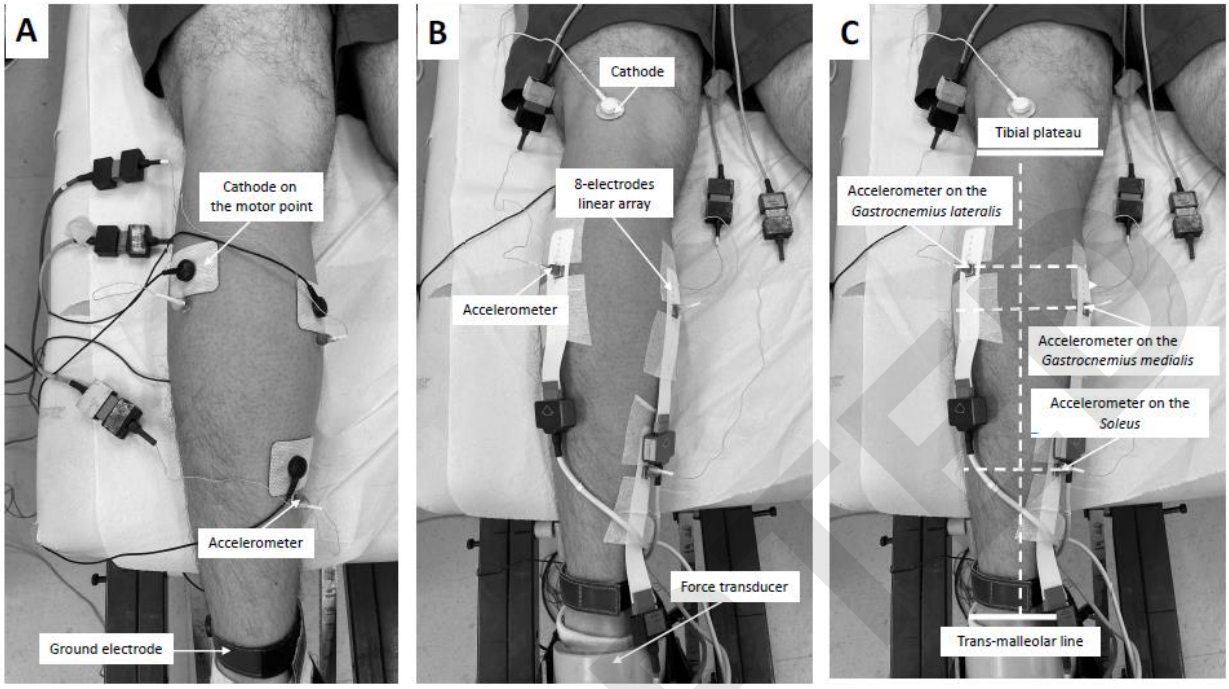

Figure 2

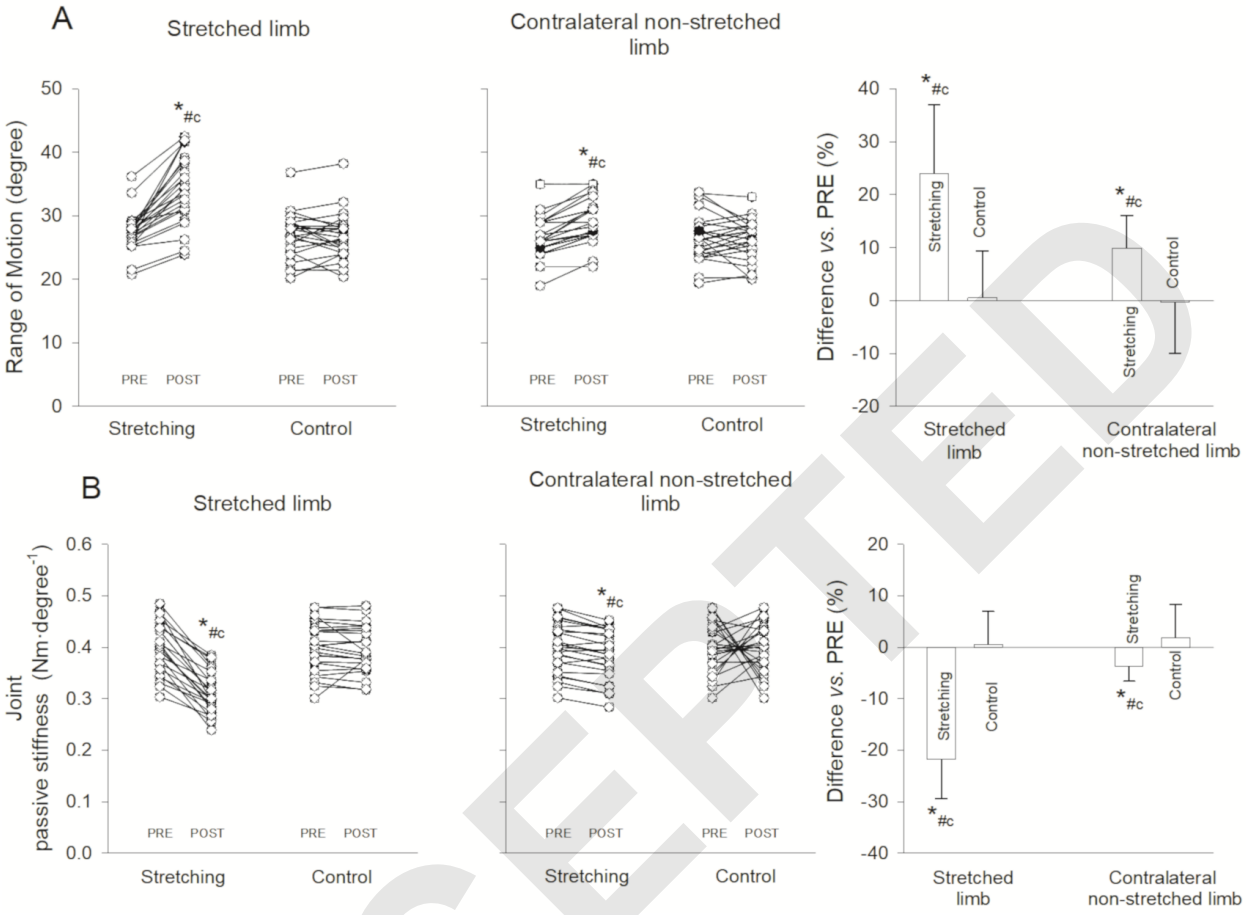

Figure 3

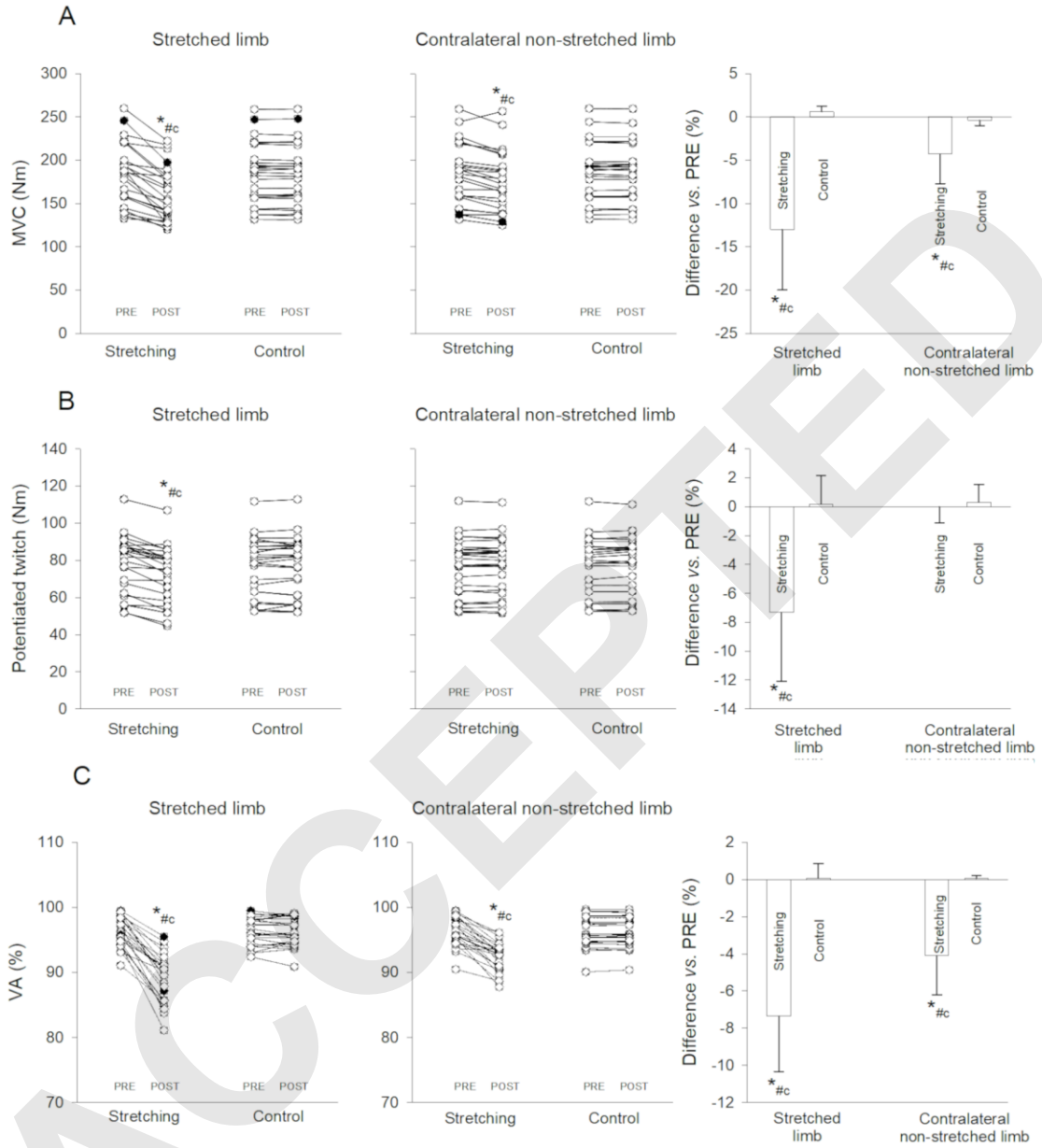

Figure 4

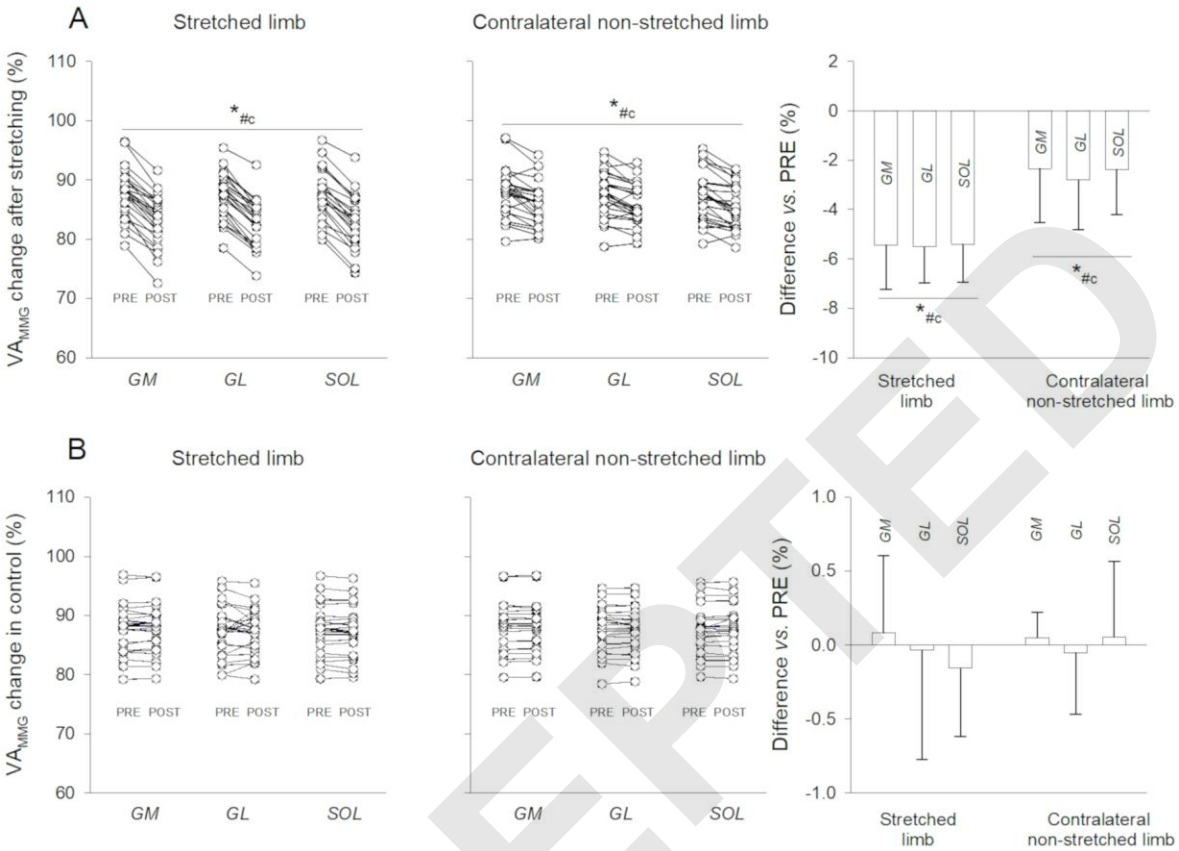

Figure 5

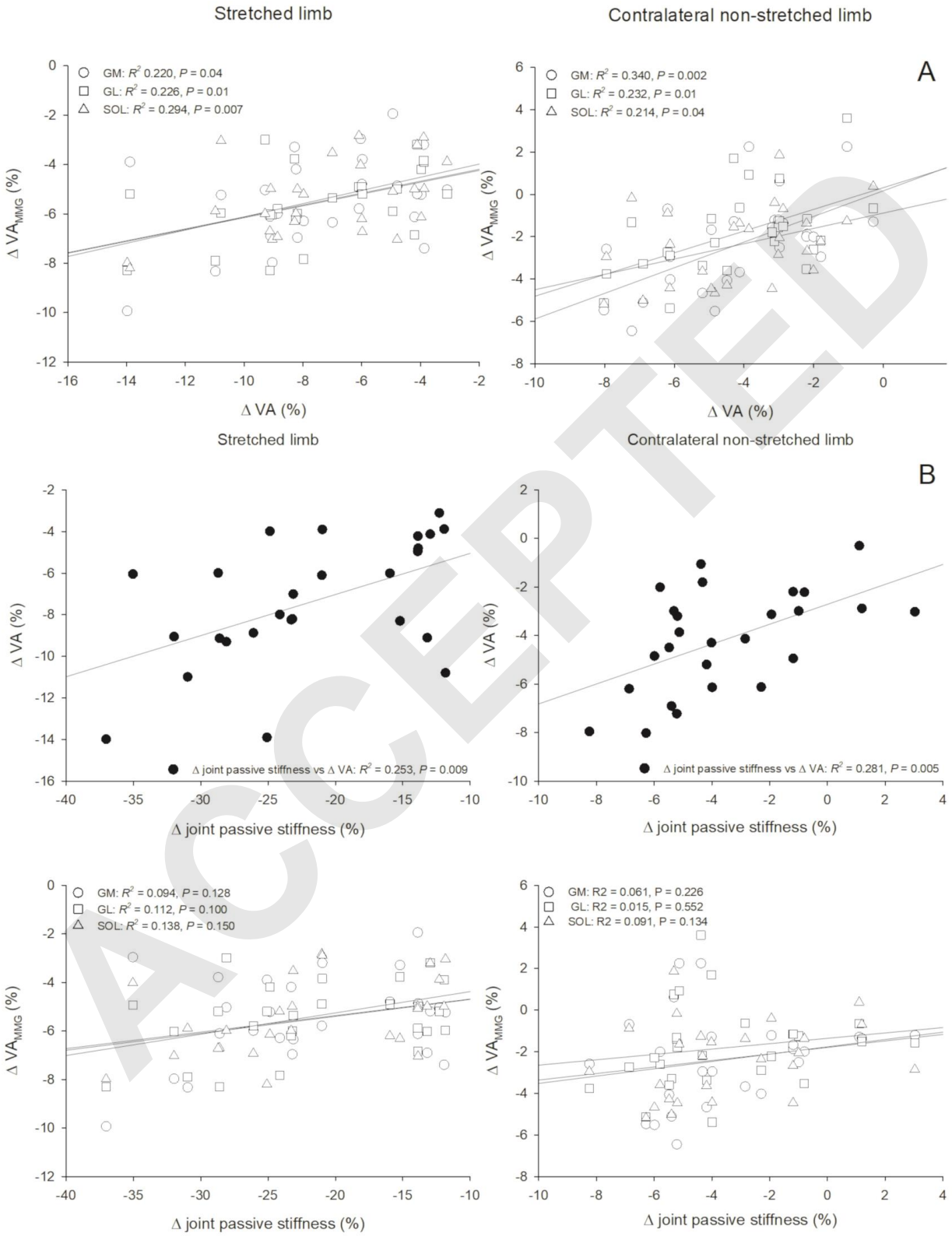

Table 1. The inter-session reliability (ICC and SEM%) and sensitivity (MDC95%) are shown for each MMG dependent parameter (n = 26) in the *gastrocnemius medialis* (GM), *gastrocnemius lateralis* (GL) and *soleus* (SOL).

		Trial 1 [m(SD)]	Trial 2 [m(SD)]	ICC	SEM%	$MDC_{95\%}$
MMGp-p_{MVC} ($mm \cdot s^{-2}$)	*GM*	16.58 (3.76)	16.58 (3.93)	0.966	4.278	8.385
	GL	16.73 (4.11)	16.76 (4.00)	0.986	2.866	5.618
	SOL	16.82 (4.06)	16.72 (4.05)	0.989	2.537	4.972
MMGp-p_{SUP} ($mm \cdot s^{-2}$)	*GM*	1.86 (0.68)	1.84 (0.68)	0.993	3.073	6.024
	GL	1.93 (0.65)	1.93 (0.66)	0.983	4.433	8.689
	SOL	1.89 (0.65)	1.89 (0.67)	0.986	4.123	8.081
MMGp-p_{POT} ($mm \cdot s^{-2}$)	*GM*	15.36 (3.55)	15.27 (3.59)	0.995	2.332	4.570
	GL	15.52 (3.62)	15.43 (3.64)	0.990	2.348	4.601
	SOL	15.44 (3.66)	15.38 (3.66)	0.988	2.604	5.104

MMG, mechanomyogram; p-p_{MVC}, peak-to-peak during maximum isometric voluntary contraction; p-p_{SUP}, peak-to-peak elicited during superimposed stimulation; p-p_{POT}, peak-to-peak elicited during the potentiated stimulation.

Table 2. Between-muscle crosstalk. MMG, mechanomyogram; p-p_{POT}, peak-to-peak elicited during potentiated stimulation; *GM*, *gastrocnemius medialis*; *GL*, *gastrocnemius medialis*; *SOL*, *soleus*.

	Stimulated muscle	**Crosstalk**					
	MMGp-p_{POT} ($m \cdot s^{-2}$)	*GM* MMGp-p_{POT} ($m \cdot s^{-2}$)	Δ%	*GL* MMGp-p_{POT} ($m \cdot s^{-2}$)	Δ%	*SOL* MMGp-p_{POT} ($m \cdot s^{-2}$)	Δ%
GM	14.8 (1.2)	---	---	0.8 (0.1)	6 (1)	0.9 (0.2)	6 (2)
GL	14.9 (3.9)	1.3 (0.3)	9 (4)	---	---	1.4 (0.3)	10 (3)
SOL	15.0 (3.9)	1.4 (0.3)	10 (3)	1.3 (0.4)	9 (3)	---	---

Table 3. Correlations (Pearson's correlation coefficient, R; determination coefficient, R^2; and P value) between the joint passive stiffness, voluntary activation (VA), and voluntary activation from the mechanomyographic signal analysis (VA_{MMG}) in the *gastrocnemius medialis* (*GM*), *gastrocnemius lateralis* (*GL*), and *soleus* (*SOL*) in the stretched and the contralateral non-stretched limb, before (PRE) and after (POST) stretching.

				VA (N = 26, *R*, R^2, *P*)			VA_{MMG} *GM* (N = 26, *R*, R^2, *P*)			VA_{MMG} *GL* (N = 26, *R*, R^2, *P*)			VA_{MMG} *SOL* (N = 26, *R*, R^2, *P*)		
Stretching	Stretched limb	PRE	Joint passive stiffness	0.521	0.271	0.006	0.318	0.101	0.113	0.235	0.055	0.248	0.256	0.066	0.207
			VA				0.900	0.810	0.000	0.848	0.719	0.000	0.866	0.750	0.000
		POST	Joint passive stiffness	0.527	0.278	0.006	0.198	0.039	0.333	0.287	0.082	0.155	0.266	0.071	0.189
			VA				0.601	0.361	0.001	0.632	0.399	0.001	0.580	0.336	0.002
	Contralateral limb	PRE	Joint passive stiffness	0.473	0.224	0.015	0.324	0.105	0.106	0.308	0.095	0.126	0.254	0.065	0.210
			VA				0.772	0.596	0.000	0.767	0.588	0.000	0.673	0.453	0.000
		POST	Joint passive stiffness	0.478	0.228	0.013	0.329	0.108	0.101	0.265	0.070	0.19	0.283	0.080	0.161
			VA				0.447	0.200	0.022	0.501	0.251	0.009	0.448	0.202	0.021
Control	Stretched limb	PRE	Joint passive stiffness	0.520	0.270	0.007	0.363	0.132	0.068	0.335	0.112	0.095	0.278	0.077	0.169
			VA				0.895	0.801	0.000	0.869	0.755	0.000	0.855	0.731	0.000
		POST	Joint passive stiffness	0.547	0.299	0.008	0.147	0.021	0.473	0.100	0.010	0.627	0.090	0.008	0.663
			VA				0.8	0.7	0.0	0.7	0.6	0.0	0.8	0.7	0.0

						46	16	00	91	26	00	66	50	00
	PRE	Joint passive stiffness	0.590	0.348	0.002	0.283	0.080	0.171	0.323	0.104	0.110	0.312	0.097	0.101
Contralateral limb		VA				0.857	0.734	0.000	0.810	0.656	0.000	0.851	0.724	0.000
	POST	Joint passive stiffness	0.512	0.262	0.007	0.343	0.118	0.086	0.333	0.111	0.097	0.255	0.065	0.208
		VA				0.853	0.728	0.000	0.827	0.684	0.000	0.845	0.714	0.000