

Original Article

Passive Stretching and Explosive Jumps Acutely Reduce Spinal Reflex Excitability and Alter Sit-To-Stand Biomechanics in Active Adults

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Abstract

Objectives: To investigate the acute effects of passive stretching and explosive effort countermovement jumps on spinal reflex excitability and sit-to-stand electromyography patterns and vertical stiffness, in active adults. **Methods:** A randomised within-participant crossover design was utilised, with 10 males and 10 females completing two separate sessions, each involving three sets of 60-seconds passive stretching (PS) and five sets of 20 explosive effort (EE) countermovement jumps (100 total); measures taken pre-intervention, post-PS and post-EE. **Results:** Spinal reflex excitability decreased in both post-PS and post-EE conditions ($p < 0.001$) compared to pre-condition measures, with no sex effect ($p = 0.115$). A condition $\times$ sex interaction revealed lower spinal reflex excitability in females post-EE ($p = 0.002$) compared to males. Biomechanically, sit-to-stand vertical stiffness increased in both post-PS and post-EE conditions ($p < 0.001$) compared to pre-condition, and electromyography patterns were influenced by both condition and sex ($p \leq 0.047$). **Conclusions:** Our findings provide insight into the neurophysiological-biomechanical link warranting further investigation as a potential mechanism contributing to the known sex-based differences in lower limb injury risk, informing our understanding of movement control, rehabilitation, and injury prevention strategies.

Keywords: H-reflex, Kinematics, Kinetics, Neuro-mechanics, Motor Adaptation

Introduction

The human motor apparatus, with its many redundant degrees of freedom, is a complex system of interacting components¹. This redundancy enables rapid and acute adaptations to demands for explosive force or increased muscle compliance². Understanding these immediate neuromuscular adjustments can offer insight into movement control and inform the development of

effective interventions to enhance performance and reduce injury risk. Repeated explosive actions, which are common in many physical performance settings (athletic, military, etc.), require rapid neuromuscular activation and generate high force outputs that can induce a transient state of fatigue. This fatigue, in turn, is associated with alterations in biomechanical performance, including variations in muscle recruitment patterns and changes in kinematics and kinetics, which may contribute to the onset of musculoskeletal disorders or injuries³. In this context, passive stretching (PS) interventions, which increase joint range of motion (ROM), have the potential to modulate motor responses. While PS can improve ROM and benefit performance in activities such as dancing⁴, PS results in a transient reduction in muscle power and strength that may be detrimental, in the very short-term, to performance in many other sporting activities^{5,6}.

Previous studies have investigated the influence of PS^{7,8}

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Table 1. Participant descriptive measures. Values are expressed as mean $\pm$ standard deviation.

Variable	Males (n = 10)	Females (n = 10)	Combined (n = 20)
Age (years)	32.6 $\pm$ 13	36.4 $\pm$ 10.3	34.5 $\pm$ 11.6
Height (cm)	178.6 $\pm$ 6.0	168.1 $\pm$ 7.0	173.3 $\pm$ 8.3
Mass (kg)	83.3 $\pm$ 7.8	73.1 $\pm$ 10.0	78.20 $\pm$ 10.16
Body Fat (%)	19.9 $\pm$ 3.8	32.5 $\pm$ 3.1	26.2 $\pm$ 7.3
Lean Mass (kg)	60.7 $\pm$ 12.7	47.6 $\pm$ 7.3	54.2 $\pm$ 12.1
Fat Mass (kg)	15.9 $\pm$ 3.4	20.8 $\pm$ 8.0	18.4 $\pm$ 6.5
Body Mass Index (kg/m ²)	26.0 $\pm$ 2.6	25.6 $\pm$ 2.4	25.8 $\pm$ 2.5
Physical Activity (min/week)	300 $\pm$ 107	335 $\pm$ 129	317 $\pm$ 117

and exercise-induced fatigue^{9,10} on spinal reflex excitability (SRE), by assessing the Hoffmann reflex (H-reflex). The H-reflex is an electrically induced spinal reflex elicited by percutaneous electrical stimulation of the group Ia afferents within a mixed peripheral nerve¹¹. Unlike a tendon tap or stretch reflex, the H-reflex bypasses the muscle spindle, providing useful information on modulation of the monosynaptic reflex activity of the spinal cord and neural pathway functioning^{9,11,12}. Although the effects of both chronic PS programs⁸ and prolonged acute PS⁷ have been studied, there is minimal research on the acute effect of short duration (< five minutes) PS on spinal reflex excitability. Given its ecological validity for physically active individuals, the neurophysiological implications of short duration stretching warrant further investigation. In addition, research has frequently investigated the impact of exercise-induced fatigue, generated by sustained force, on spinal reflex excitability in single-joint muscular contractions¹⁰. However, the limited transfer of single-joint protocols to functional tasks underscores the need for studies examining the neurophysiological impact of repeated bouts of explosive force during a multi-joint movement. Consequently, our understanding of the effects of PS and repeated multi-joint explosive efforts (EE) on motor control during functional movement remains incomplete.

Whilst differences in spinal reflex excitability between sexes have been demonstrated, the physiological mechanisms underlying this variation remain unclear^{13,14}. It has been postulated that decreased spinal reflex excitability alters joint stiffness and stability¹⁵, potentially increasing lower-limb ligamentous injury rates, which are higher among females¹⁶. Similarly, although variations in movement kinematics and kinetics between sexes are well documented¹⁷, there is limited information on sex-based lower limb electromyography (EMG) pattern variations. Given the implications for movement control and injury risk, the primary aim of this study was to examine the acute effects of PS and EE on a spinal reflex excitability

measure derived from the H-reflex in healthy, physically active adults. Secondary and exploratory aims include investigating associated changes in sit-to-stand lower-limb muscle activation patterns and vertical stiffness as complementary indicators of neuromuscular function, as well as examining potential sex-based differences in these responses.

Materials and Methods

Participants

Twenty participants (10 males and 10 females), who were all right leg dominant, volunteered for the study (Table 1). All participants were over 18 years of age, were physically active through engagement in at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic activity each week (or an equivalent combination of both) for a minimum of 3 months before the study, and were free of any diseases, injuries or illnesses that may influence the results. No participants were excluded from the study after enrolment.

Study Procedures

This study used a randomised within-participant crossover design. Participants attended the laboratory at the same time of day on three occasions, each separated by a 6-14 day washout period (median seven days). The first session served as a familiarisation, during which descriptive data were collected and participants were exposed to all testing measures and protocols. Sessions two and three were the experimental sessions. A counterbalanced design was used across the two experimental testing sessions; all participants completed both the PS and EE protocols in each session, but in opposite orders (PS then EE or EE then PS), with the order randomly assigned across the two experimental sessions. Thus, half of the participants performed PS first in Session two and EE first in Session three, while the other half completed EE first in Session two and PS first in Session three.

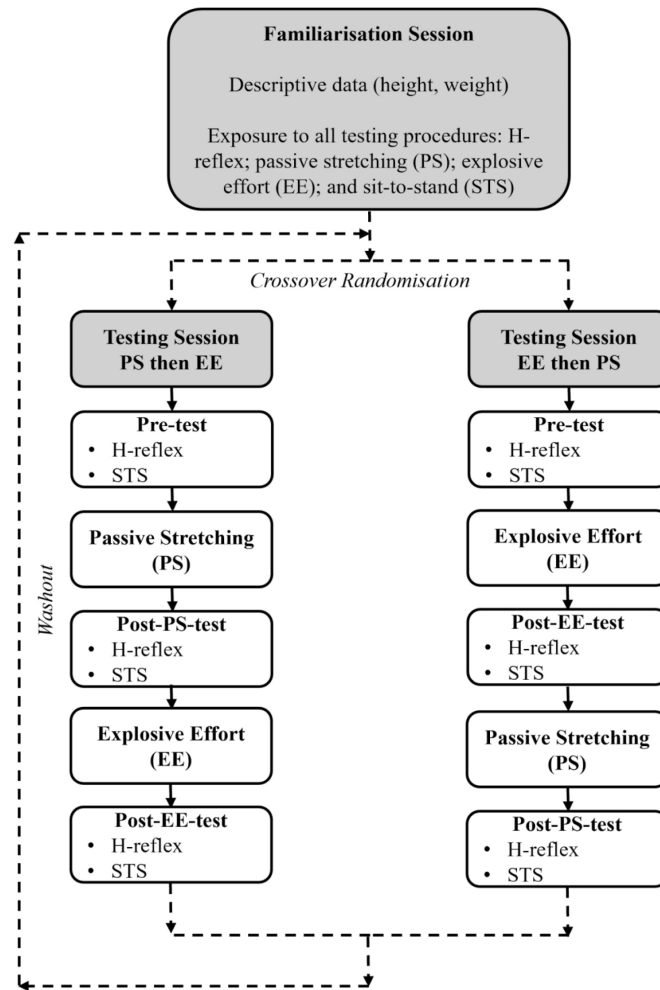


Figure 1. Schematic overview of the experimental protocol. All participants completed both testing sessions in a randomised counterbalanced order (PS then EE; and EE then PS), separated by a 6–14 day washout. Following a familiarisation session, each testing session included pre-, post-first intervention, and post-second intervention assessments of the H-reflex and sit-to-stand (STS). Solid lines = within a session. Dashed lines = between sessions.

Each experimental session included three measurement time points: 1) Pre, 2) immediately after the first intervention (Post-PS or Post-EE, depending on whether PS or EE was performed first), and 3) immediately after the second intervention (Post-PS or Post-EE, depending on whether PS or EE was performed second). The pre-testing involved H-reflex assessment followed by a biomechanical assessment of the sit-to-stand (STS) task. Following pre-testing, participants were provided with 2 minutes of rest prior to completing either the EE protocol or the PS protocol. Immediately following EE or PS protocol completion, participants underwent re-assessment of their H-reflex and re-completed the STS task. Participants were then provided with 2 minutes of rest before completing the EE protocol (if they had initially completed the PS protocol)

or the PS protocol (if they had initially completed the EE protocol). Immediately following completion of the EE or PS protocol, participants underwent a final re-assessment of their H-reflex and re-completed the STS task. The order in which each participant completed the EE and PS protocols was reversed during their second experimental session (Figure 1). Given that both interventions were completed within each session, the second post-intervention measurement reflects the sequential application of both protocols within a counterbalanced design.

Spinal reflex excitability was assessed before the biomechanical assessment of the sit-to-stand (STS) task to minimise the potential for H-reflex recovery and a return to baseline values. To control and standardise factors that could influence the study's outcomes, prior to

experimental testing sessions, participants were assessed for total mood disturbance (TMD) via the abbreviated 'Profile of Mood States' (POMS) questionnaire¹⁸, and measures of muscle soreness were obtained from the vastus lateralis and soleus of the dominant leg, with the participant seated with their hips, knees, and ankles flexed to 90° (0° being full extension). A handheld algometer (Force One™ FDIX, Wagner Instruments, Greenwich, USA) with a 10 mm diameter flat rubber contact surface was used to apply a 50 N load to the muscle for 3-seconds, with participants providing a rating of muscle soreness from 0 to 10, where 0 indicated no soreness and 10 indicated extreme soreness¹⁹.

Participants completed a 24-hour nutrition diary leading into session two and were advised to replicate the same diet in the 24-hours before session three²⁰. They were asked to continue their regular physical activities and abstain from caffeine, alcohol and strenuous activity for 48 hours before testing. Participant height was also measured to the nearest 0.1 cm using a stadiometer (Sentry Medical) and body mass was measured to the nearest 0.01 kg using calibrated electronic scales (A&D Digital Platform Scale, model HW-150K).

Experimental Conditions

The Post-PS condition targeted the triceps surae muscles. Participants stood facing a wall, with feet hip-width apart, and both hands on the wall for support. They slowly and progressively flexed both knees, lowering their body while keeping both feet flat on the floor, until they reported a stretch intensity of 'maximal stretch without pain' in the calf of the dominant leg²¹. At this point, they held the static stretch for a period of 60-seconds. After 60 seconds, they slowly released the stretch and returned to the starting position. Three repetitions of 60-second stretches were completed with a 30-second rest between each repetition²¹.

The Post-EE condition consisted of five sets of 20 (100 in total) maximal effort vertical countermovement jumps (CMJs) with a 15 seconds rest between each set. Each set began with participants stepping off a 40 cm box, leading with their non-dominant leg, and landing on both feet. Immediately after landing, they performed 20 consecutive maximal-effort CMJs, using self-selected arm swing, and were instructed to jump as high and fast as possible for all repetitions in each set. Verbal encouragement was provided to the participants throughout each set. Borg's Rating of Perceived Exertion (RPE) was assessed immediately after completing the protocol using the 6-20 scale, with participants reporting a mean RPE of 16.25 (SD = ± 1.89).

Electromyography (EMG)

Surface electromyography (EMG) electrodes (Trigno, Delsys Inc., Boston, MA, USA) were placed on the dominant

limb and positioned over the vastus lateralis, rectus femoris, vastus medialis, lateral head of gastrocnemius, medial head of gastrocnemius, tibialis anterior, and long head of biceps femoris²². A further electrode was positioned over the soleus muscle in accordance with the SENIAM guidelines²³. All electrodes were wirelessly connected to an amplifier where signals were obtained with a gain of 909 mV/mV and a common mode rejection ratio < -80 dB (Trigno, Delsys Inc., Boston, MA, USA). Each electrode site was first prepared by shaving, lightly abrading, and cleansing the skin with isopropyl alcohol.

Spinal Reflex Excitability Assessment

The H-reflex and compound muscle action potentials (M-waves) were elicited by stimulating the posterior tibial nerve in the popliteal fossa using percutaneous stimulation with single rectangular 1-ms wide pulses (Digitimer DS7AH, Welwyn, Garden City, Hertfordshire, UK). At the start of each experimental testing session, the optimal location for cathode stimulation was determined using a probe (MY0004 Stimulator Probe SS, Neurospec Research, Switzerland) to find the location that elicited the strongest H-reflex response with a fixed submaximal stimulus. Once identified, a cathode electrode (Kendall 130, 30mm diameter disposable gel electrode) was placed in this location with an anode placed over the patella. Prior to electrode placement, the skin underlying the cathode and anode was shaved, abraded, and cleansed with isopropyl alcohol.

The trigger output from a constant-current stimulator (Digitimer DS7AH, Herefordshire, UK) and soleus EMG signals were connected to a terminal block (USB-6218 BNC, National Instruments) linked to a host computer where all signals were 16-bit A/D converted and synchronously sampled at a rate of 2 kHz. For testing, participants lay prone on a plinth, head facing forward with their chin resting on their hands, hips and knees extended, and ankles plantar flexed. Participants were instructed to keep their eyes closed, refrain from moving their hands or head, maintain slow rhythmical breathing, and relax as much as possible for the duration of the testing.

Each experimental testing session commenced by establishing a H-reflex and M-wave recruitment curve following methods of Young et al.,²⁴ using 0.5-2 mA increments where testing ceased as the current delivered was 125% of that required to attain a stable maximal M response (Mmax). The current eliciting 50% of the maximal H-reflex response (H50) on the ascending limb was used for subsequent testing. H50 was determined as an appropriate stimulation intensity for testing as it is sensitive to both spinal facilitation and inhibition²⁵. During testing, five stimuli were delivered at H50 and Mmax intensities at a rate of 0.1 Hz using a connector terminal (USB-6218 BNC, National Instruments, Austin, TX) to capture the stimulator trigger output and soleus EMG signals with the data synchronised and sampled at a

rate of 2kHz using customised LabVIEW software (2015 SP1, National Instruments, Austin, TX). Data processing was performed offline with the mean H50 and Mmax peak-to-peak amplitudes from each test determined using customised MATLAB software (R2022a, MathWorks, Inc. Natick, MA). Spinal reflex excitability was then quantified as the H50/Mmax ratio and used for statistical analysis.

Biomechanical Assessment

A STS task was used to evaluate the effects of condition, sex, and their interaction on functional performance, as it requires considerable neuromuscular control, making it a pertinent task to assess biomechanical responses to experimental conditions²⁶. Participants were instructed to complete 10 STS repetitions at a comfortable pace, with their arms folded across their chest from a chair 44 cm in height. A single repetition of the STS task began when the participant initiated the movement from a fully seated position and ended when the participant returned to a fully seated position. Passive reflective markers were placed on each participants' lower limbs, pelvis and torso, as detailed in Mann et al.,²⁷. During the STS task, participants' three-dimensional (3D) kinematic data (250 Hz; Qualisys AB, Göteborg, Sweden), ground reaction force data (GRF; 2500Hz; Type 9281CA, Kistler, Winterthur, Switzerland; Type9821EA, Kistler, Winterthur, Switzerland) and EMG from all eight muscles were collected (sample rate of 2.5 kHz; Qualisys AB, Göteborg, Sweden) prior to analysis in Visual 3D (C-Motion, Inc., Rockville, USA). Raw kinematic coordinates and GRFs were filtered through a fourth-order zero-phase Butterworth low-pass digital filter, 18Hz and 50Hz, respectively. To support Cartesian local coordinate system sign conventions, all segments were defined: x-axis as medio-lateral, y-axis as anterior-posterior and z-axis as vertical. For each trial, intersegmental joint angles of the knee were calculated using maximal knee flexion marking the beginning and end of each repetition. Vertical stiffness, used to calculate the force-displacement relationship in functional movements^{28,29} was calculated as the peak vertical GRF divided by the maximal centre of mass (CoM) vertical displacement³⁰. As the STS task requires vertical CoM displacement through force production, the spring mass model (30) can be considered generalisable to the STS movement task. STS biomechanical variables were calculated for each repetition and analysed at the repetition level rather than averaged across repetitions.

EMG signals from each STS trial were filtered using a fourth-order zero-phase Butterworth high-pass filter (15 Hz), to remove artefact noise. The signal was then rectified, followed by a second filtering using a fourth-order zero-phase Butterworth low-pass filter (50 Hz), to remove high-frequency noise and smooth the signal. The signal was then rectified again and time-normalised to 101 data points, representing 0 to 100% of the cycle. Once all EMG signals were processed, they were concatenated into single matrices according to muscle group. Principal

Components Analysis (PCA) was then performed on the EMG matrices from each muscle group using covariance matrices. This enabled statistical testing of individual principal components (PCs) via trial PC scores, which represent key characteristics of the EMG waveforms as single numerical values, thereby reducing dimensionality and capturing their essential properties³¹. The number of PCs to extract from the PCA was determined by visual inspection of Scree plots³¹. The first three principal components (PCs) for each muscle group were retained, and their corresponding PC scores, which were derived at the trial level, were entered into the statistical models as the unit of analysis. PCA processing was completed using MATLAB (R2022, MathWorks, Inc. Natick, MA).

Statistical Analysis

An a priori sample size estimation was conducted using GLIMMPSE (General Linear Multivariate Model Power and Sample Size; <https://glimmpse.samplesizeshop.org/>; ³²) for a within-participant repeated-measures design with two levels for sessions and three levels for time. Although the H50/Mmax ratio was selected as the primary outcome, representing the key indicator of spinal reflex excitability in this study, it was not used to inform sample size estimation because no suitable published data were available to parameterise the required inputs. As such, the Hmax/Mmax ratio was used to parameterise the sample size calculation because it is a well-established, normalised measure of spinal reflex excitability with widely reported normative values and consistent between-session reliability. Furthermore, unlike H50, which is constrained by recruitment curve sampling, Hmax reflects the maximal proportion of the motoneuron pool recruited via Ia afferents, and normalisation to Mmax accounts for total excitable motor units and recording conditions. This makes Hmax/Mmax a physiologically specific and robust measure for informing sample size determinations in this study. Baseline values for the Hmax/Mmax ratio were informed by data reported by Behm et al.,³³ with a mean Hmax/Mmax ratio of 0.50 and a standard deviation of 0.11 in healthy adults. The analysis was powered to detect a conservative Cohen's d effect size of $d = 0.3$, which corresponds to a mean difference in the Hmax/Mmax ratio of 0.033 based on the baseline standard deviation (i.e. $SD \text{ of } 0.11 \times 0.3 = 0.033$). Moderate within-participant correlations of 0.60 for Session and 0.70 for Time and equal variances were applied across factors. Under these assumptions, GLIMMPSE indicated that a sample size of 17 participants would provide approximately 83% power at $P = 0.05$. It should be noted that because the H50/Mmax ratio may exhibit variability characteristics that differ from those of the Hmax/Mmax ratio, the detectable effect size for the H50/Mmax ratio may not align precisely with the assumptions used in the power analysis. Nevertheless, the present study provides new empirical data on H50/

Table 2. Test-retest reliability for pre-condition parameters.

Variable	Session 2	Session 3	ICC (95% CI Limits)	Paired t-test/ Wilcoxon Statistic
TMD (n= 20)	-14.4 ± 9.2	-15.6 ± 10.0	0.64 (0.29–0.84), $p < 0.001$	$Z = -0.65$, $p = 0.52$
Vastus Lateralis Soreness (n = 20)	3.1 ± 2.2	2.5 ± 2.3	0.75 (0.47–0.89), $p < 0.001$	$Z = -1.50$, $p = 0.13$
Soleus Soreness (n = 20)	2.0 ± 1.5	1.5 ± 1.9	0.75 (0.46–0.89), $p < 0.001$	$Z = -1.82$, $p = 0.07$
Mmax Current (mA) (n = 19)	39.9 ± 15.7	42.2 ± 13.8	0.72 (0.42–0.88), $p < 0.001$	$t(18) = 0.83$, $p = 0.42$
Mmax Amplitude (mV) (n = 20)	4.3 ± 1.5	4.1 ± 1.5	0.71 (0.40–0.87), $p < 0.001$	$t(19) = 0.98$, $p = 0.34$
Hmax Current (mA) (n = 20)	15.8 ± 4.7	16.6 ± 5.8	0.88 (0.72–0.95), $p < 0.001$	$t(19) = -1.36$, $p = 0.19$
Hmax Amplitude (mV) (n = 20)	2.1 ± 1.0	2.2 ± 1.1	0.84 (0.62–0.93), $p < 0.001$	$t(19) = -0.33$, $p = 0.74$
Spinal Reflex Excitability (n = 20)	0.26 ± 0.11	0.22 ± 0.10	0.62 (0.27–0.83), $p = 0.002$	$t(19) = 1.67$, $p = 0.11$

CI, confidence interval; Hmax, maximal H-reflex peak-to-peak amplitude; ICC, intraclass correlation coefficient; Mmax, maximal M-wave peak-to-peak amplitude; TMD, total mood disturbance. Values for each session are reported as mean ± SD.

Mmax variability to support more precise sample size planning in future work. All other outcomes were not powered for hypothesis testing and should be interpreted as exploratory.

The reliability of testing conditions between intervention sessions was assessed by comparing pre-condition parameters. Test-retest reliability was evaluated using a single-measure intraclass correlation coefficient (ICC), based on a two-way random-effects model for absolute agreement (ICC(2,1)), with values interpreted according to Koo and Li³⁴. Two-tailed paired sample t-tests (Mmax current, Mmax peak-to-peak, Hmax current, Hmax peak-to-peak, and spinal reflex excitability) and Wilcoxon signed-rank tests (TMD and muscle soreness) were also used to assess the presence of systematic differences between sessions.

Linear Mixed Models (LMM) were used to evaluate the effects of condition (Pre-, Post-PS, Post-EE) and sex (male, female) on dependent measures. LMMs were selected because repeated observations within participants violate the independence assumptions underlying ANOVA and traditional linear models, and this approach appropriately accounts for the hierarchical dependency structure by incorporating participant-specific random effects³⁵. For STS biomechanical outcomes, analyses were conducted at the repetition level. Condition, sex, and condition × sex were specified as fixed effects, with participant included as a random intercept and repeated observations nested within participant. Session, order, and cycle were included to account for the repeated-measures structure of the design, with order included to account for potential sequence-related variance. The experimental design was counterbalanced across sessions; therefore, condition effects were estimated within the full repeated-measures framework rather than assuming independence between post-intervention measurements. Testing

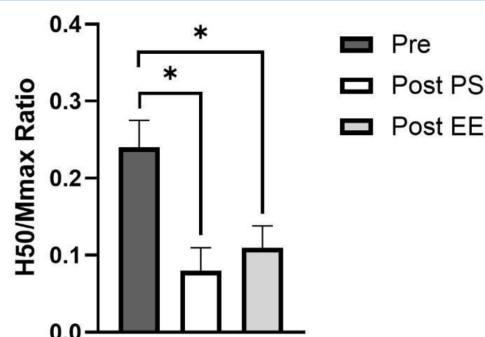


Figure 2. Estimated marginal means of H50/Mmax ratio. Pre-condition (Pre), post-stretching condition (Post-PS), post-explosive effort condition (Post-EE). Error bars = 95% CI. *Indicates a significant difference ($p < 0.001$).

order was included as a repeated within subject factor, with participant as a random effect, applying a diagonal covariance structure. Fixed effects for condition, sex, and their interactions were analysed using Bonferroni-adjusted post-hoc tests. EMG principal component analyses were used to characterise coordinated patterns of muscle activity across the task. As these variables represent interdependent features of the same underlying neuromuscular control system, statistical results were interpreted with emphasis on the consistency and coherence of patterns observed across muscles and components, rather than on isolated outcomes. Descriptive statistics and residuals were generated, and model assumptions were assessed through visual inspection of residual distributions (histograms and Q-Q

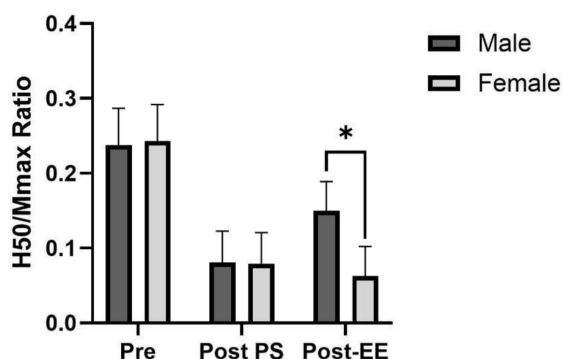


Figure 3. Estimated marginal means of H50/Mmax ratio for females and males. Pre-condition (Pre), post-stretching condition (Post-PS), post-explosive effort condition (Post-EE). Error bars = 95% CI. *Indicates a significant difference ($p = 0.002$).

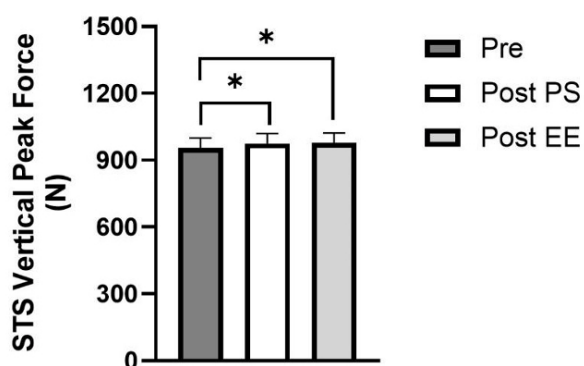


Figure 4. Estimated marginal means of sit-to-stand (STS) vertical peak force. Pre-condition (Pre), post-stretching condition (Post-PS), post-explosive effort condition (Post-EE). Error bars = 95% CI. *Indicates a significant difference (Post-PS: $p = 0.001$; Post-EE: $p < 0.001$).

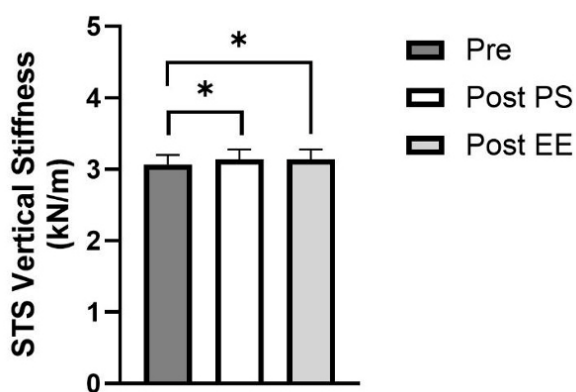


Figure 5. Estimated marginal means of sit-to-stand (STS) vertical stiffness. Pre-condition (Pre), post-stretching condition (Post-PS), post-explosive effort condition (Post-EE). Error bars = 95% CI. *Indicates a significant difference ($p < 0.001$).

plots), indicating approximate normality. All statistical analyses were performed using SPSS version 28.0 (IBM Corp. Released 2021, IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.).

Missing or unusable data points accounted for 3.75% of the total H-reflex observations. These observations were left blank, as linear mixed models are capable of appropriately handling missing data. There were no missing or unusable EMG data points.

Results

Reliability of Testing Conditions

Test-retest reliability data for pre-condition parameters of TMD, muscle soreness, neuromuscular stimulation, and spinal reflex excitability are presented in Table 2. There were no significant differences between sessions for any pre-condition measures ($p \geq 0.068$). The ICCs for all measurements ranged from 0.62 to 0.88 ($p \leq 0.002$), indicating good to excellent test-retest reliability and highly consistent pre-conditions testing across sessions.

Spinal Reflex Excitability

There was a significant main effect of condition [$F(2, 94.956) = 28.030$, $p < 0.001$] on spinal reflex excitability (H50/Mmax ratio). Post-hoc comparisons indicated that spinal reflex excitability was significantly lower Post-PS ($p < 0.001$) and Post-EE ($p < 0.001$) compared to pre-condition (Pre), while spinal reflex excitability was not different ($p = 0.603$) between Post-PS and Post-EE (Figure 2). There was no main effect of sex on spinal reflex excitability (H50/Mmax ratio) [$F(1, 98.097) = 2.535$, $p = 0.115$], indicating that excitability was comparable between males and females. However, a significant condition $\times$ sex interaction for spinal excitability was present [$F(2, 94.956) = 3.140$, $p = 0.048$] with post-hoc comparisons revealing significantly lower excitability Post-EE in females compared with males ($p = 0.002$); while no significant differences ($p \geq 0.880$) between sexes were observed at Pre- or Post-PS (Figure 3).

Sit-to-Stand (STS) Mechanics

For peak vertical GRF during the STS, there was a significant main effect of condition ($F(2, 72.922) = 11.016$, $p < 0.001$), with a mean value in Pre significantly lower than both Post-PS ($p = 0.001$) and Post-EE ($p < 0.001$), and no difference ($p = 1.000$) between Post-PS and Post-EE (Figure 4). A main effect of sex was also observed ($F(1, 37.882) = 9.978$, $p = 0.003$) for peak vertical GRF with males exhibiting significantly higher values than females ($p = 0.003$). A condition $\times$ sex interaction for peak vertical GRF was not present ($F(2, 72.922) = 0.141$, $p = 0.868$).

For CoM vertical displacement during the STS, there was no main effect of condition ($F(2, 76) = 1.782$, $p = 0.175$), but there was a main effect of sex ($F(1, 38.013) = 12.822$, $p = 0.001$) with males demonstrating greater

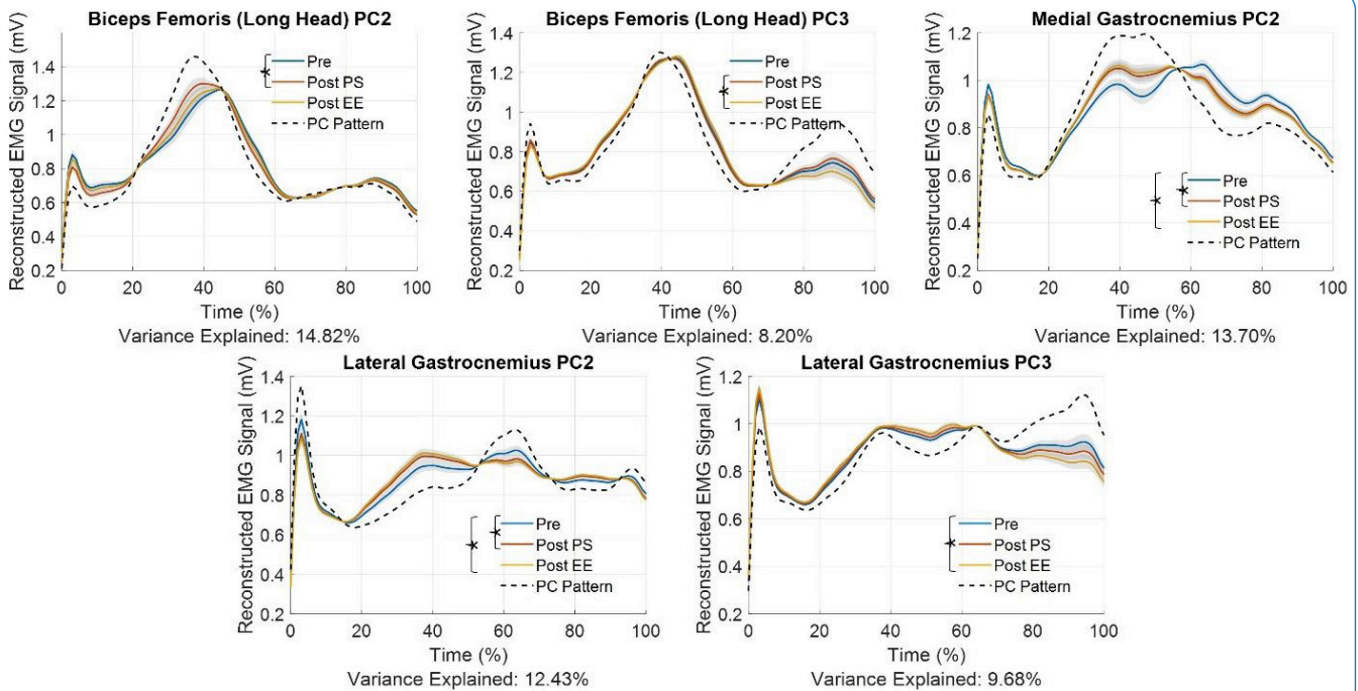


Figure 6. Effect of condition on principal component (PC) reconstructed EMG signals for statistically significant post-hoc differences. Pre-condition (Pre), post-stretching condition (Post-PS), post-explosive effort condition (Post-EE). *Indicates a significant difference between EMG PC patterns ($p < 0.01$).

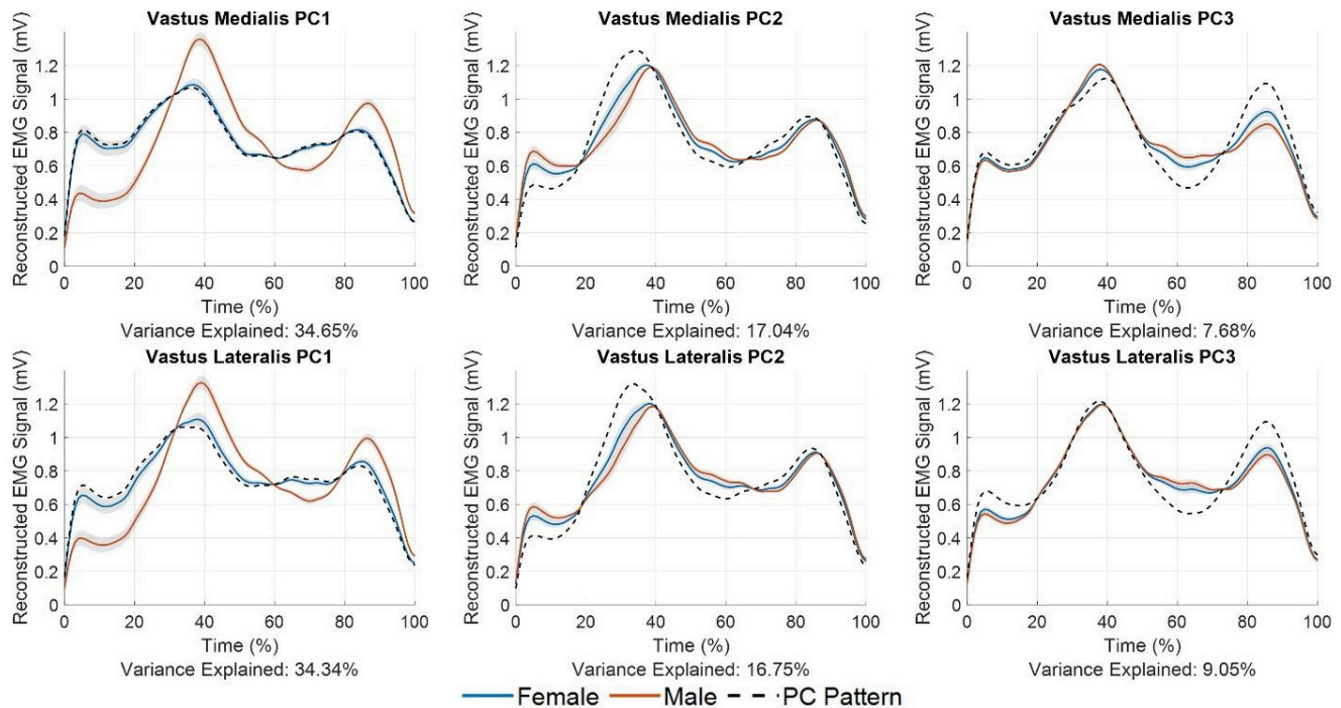


Figure 7. Effect of sex on principal component (PC) reconstructed EMG signals for vastus medialis and vastus lateralis. Statistically significant differences between sexes were observed in all muscle PCs presented in this figure ($p < 0.05$).

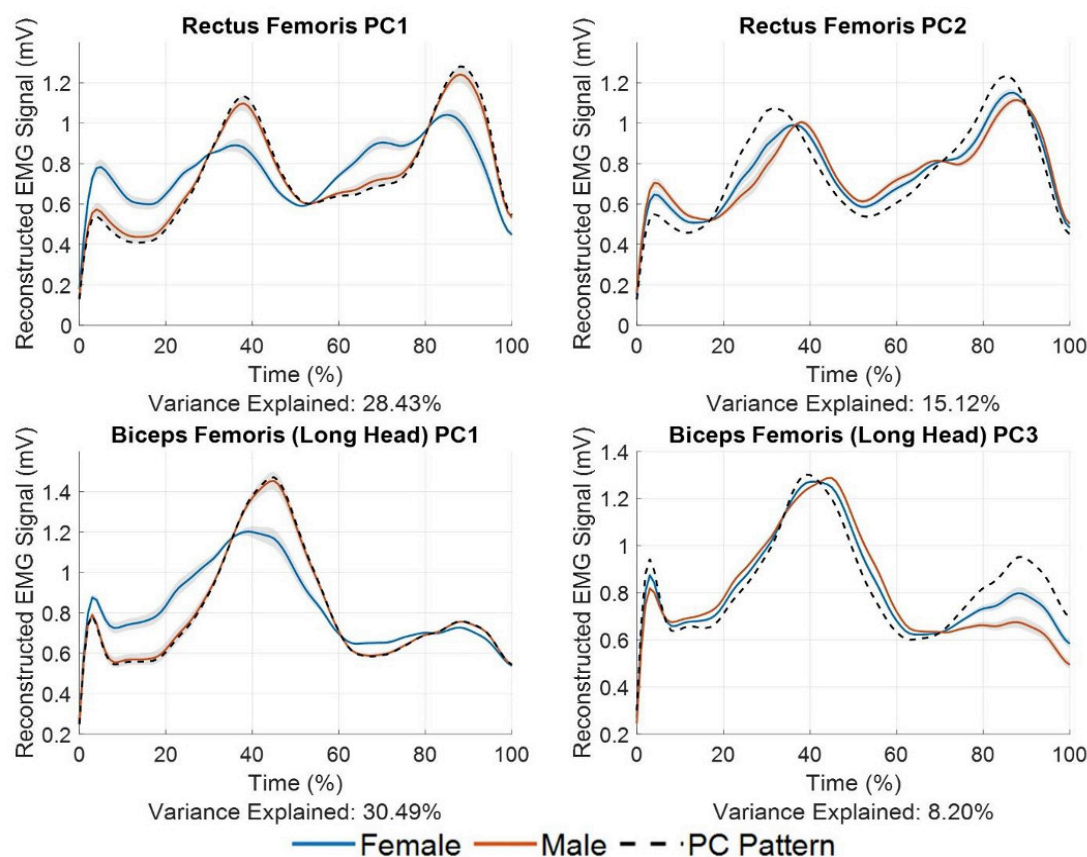


Figure 8. Effect of sex on principal component (PC) reconstructed EMG signals for rectus femoris and biceps femoris. Statistically significant differences between sexes were observed in all muscle PCs presented in this figure ($p < 0.05$).

displacement than females. No condition $\times$ sex interaction was apparent ($F(2, 76) = 1.179, p = 0.313$).

For peak vertical stiffness during the STS, there was a main effect of condition ($F(2, 72.662) = 10.953, p < 0.001$) with lower stiffness in Pre compared to both Post-PS ($p < 0.001$) and Post-EE ($p < 0.001$) conditions; while stiffness was similar ($p = 1.000$) Post-PS and Post-EE (Figure 5). There was no main effect of sex ($F(1, 37.690) = 0.535, p = 0.469$) on peak vertical stiffness nor a condition $\times$ sex interaction ($F(2, 72.662) = 0.139, p = 0.870$).

Sit-to-Stand (STS) Electromyography

Significant main effects for condition for STS EMG PC scores (Figure 6) were observed for the long head of biceps femoris PC2 and PC3 [$F(2, \geq 971.462) \geq 4.387, p \leq 0.01$], the medial head of gastrocnemius PC2 [$F(2, 913.048) = 10.825, p < 0.001$], and the lateral head of gastrocnemius PC2 and PC3 [$F(2, \geq 961.032) \geq 6.295, p \leq 0.005$]. A significant main effect for condition was observed for STS EMG PC1 for the lateral head of gastrocnemius [$F(2,$

$817.668) = 3.080, p = 0.047$], however post-hoc analyses were not significant ($p \geq 0.076$). No significant main effects for condition were found for the long head of biceps femoris PC1, medial head of gastrocnemius PC1 and PC3, soleus, vastus medialis, vastus lateralis, tibialis anterior, or rectus femoris muscles [$F(2, \geq 627.190) \leq 2.590, p \geq 0.076$].

Significant effects for sex were observed for most STS EMG PC scores across all muscles (Figures 7-10). Specifically, vastus medialis, PC1, PC2 and PC3 [$F(1, \geq 976) \geq 11.72, p \leq 0.001$], vastus lateralis PC1, PC2 and PC3 [$F(1, \geq 707) \geq 5.23, p \leq 0.023$], tibialis anterior PC1 and PC2 [$F(1, \geq 1073) \geq 14.16, p \leq 0.001$], long head of biceps femoris PC1 and PC3 [$F(1, \geq 1075) \geq 45.84, p \leq 0.001$], lateral head of gastrocnemius PC1, PC2 and PC3 [$F(1, \geq 929) \geq 15.01, p \leq 0.001$], medial head of gastrocnemius PC1 and PC2 [$F(1, \geq 1016) \geq 27.88, p \leq 0.001$], rectus femoris PC1 and PC2 [$F(1, \geq 1052) \geq 11.80, p \leq 0.001$], and soleus PC1, PC2 and PC3 [$F(1, \geq 1058) \geq 12.82, p \leq 0.001$]. No significant differences were observed between

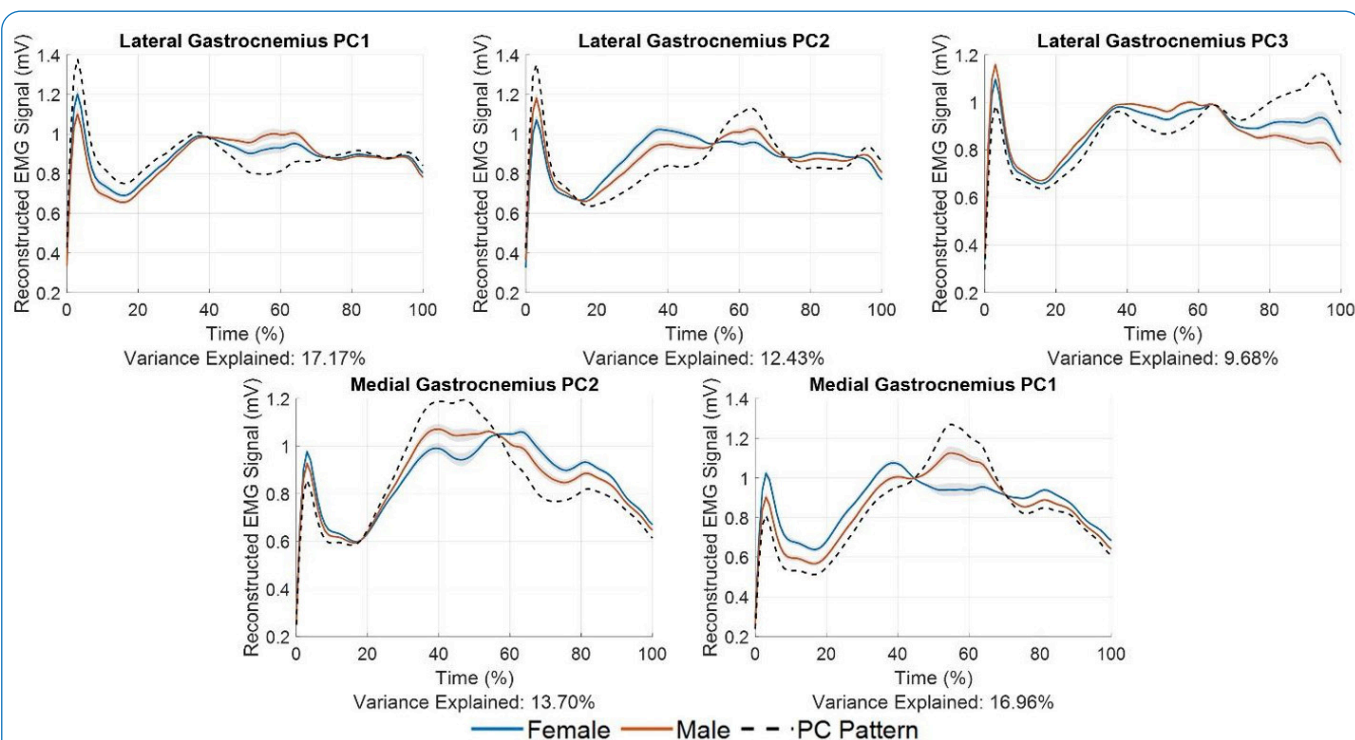


Figure 9. Effect of sex on principal component (PC) reconstructed EMG signals for lateral gastrocnemius and medial gastrocnemius. Statistically significant differences between sexes were observed in all muscle PCs presented in this figure ($p < 0.05$).

sexes across conditions for tibialis anterior PC3, biceps femoris PC2, medial head of gastrocnemius PC3, or rectus femoris PC3 ($F(1, \geq 933) \leq 2.18$, $p \geq 0.140$).

Discussion

This study examined the acute effects of PS and EE on spinal reflex excitability and lower limb STS EMG patterns and vertical stiffness in healthy physically active adults. Results demonstrated that triceps surae stretching (Post-PS) and explosive CMJs (Post-EE) protocols both significantly decreased spinal reflex excitability, as measured through the H50/Mmax ratio, and influenced EMG activity patterns and increased vertical stiffness during an STS task. The reduction in H50/Mmax ratio, relative to the pre-condition, observed following both stretching^{7,8} and repeated explosive efforts, which likely produced transient exercise-induced fatigue^{9,10,36}, is broadly consistent with previous studies. This indicates that both PS and repeated explosive actions have an acute inhibitory effect on spinal reflex excitability. Notably, results of the current study demonstrated similar reductions in acute spinal reflex excitability, with considerably shorter duration (three minutes) of PS compared to Avela et al.⁷. Regarding the

explosive CMJ protocol, the findings are consistent with previous investigations that utilised sustained voluntary isometric contraction protocols¹⁰. However, given the explosive CMJ protocol used here is a multi-joint functional activity (which more closely replicates real-life functional movements typical in sports and daily activities), as opposed to a single-joint sustained isometric contraction, our findings are likely more ecologically valid to changes in spinal reflex excitability occurring during sustained physical performance.

Mechanistically, the alterations in spinal reflex excitability observed may be due to increased activation of group III and IV afferent fibres. Mechanical compression during PS reduces muscle blood flow and oxygenation^{37,38}, resulting in accumulation of metabolites and subsequent activation of group III and IV afferent fibres, which are sensitive to mechanical and metabolic changes within muscle, respectively^{39,40}. Similarly, sustained exercise bouts alter the metabolic state of muscle tissue, activating metabosensitive group IV afferent fibres, whereas the mechanical requirements of the motor task activate mechanosensitive group III afferent fibres^{39,40}. This increased activity of group III and IV afferents, as a result of either PS or sustained exercise, likely reduces motor activity through inhibition of spinal reflex excitability, as

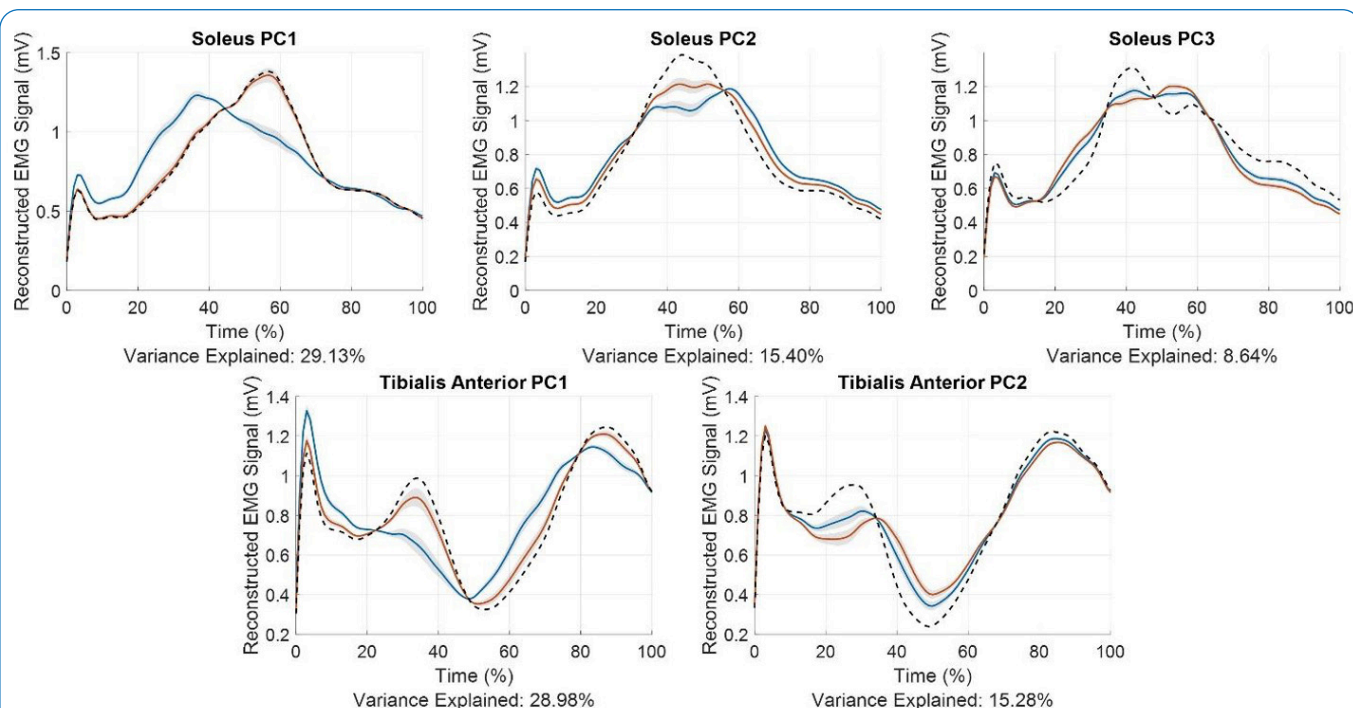


Figure 10. Effect of sex on principal component (PC) reconstructed EMG signals for soleus and tibialis anterior. Statistically significant differences between sexes were observed in all muscle PCs presented in this figure ($p < 0.05$).

reflected in a reduced H50/Mmax ratio.

Results also demonstrated that both PS and EE influence multiple STS EMG activation patterns. Few studies have isolated the effects of PS or sustained exercise on muscle activity during functional movements. Notably, da Silva et al.⁴¹ observed lower limb muscle activity pattern differences between controls and gymnasts after fatiguing single-leg isometric contractions. These findings align with our results, indicating that the neuromuscular system adapts muscle activity in response to localised perturbation (PS and EE interventions), reflecting elements of motor equivalence; the central nervous system's capacity to maintain functional movement through adaptable recruitment strategies^{1,42}. In our study, STS EMG PC changes were limited to posterior chain muscles, possibly due to their stabilising role during the STS, with adjustments likely managed through antagonist co-activation⁴³. Visual inspection of reconstructed PC waveforms (Figure 6) suggests similar amplitude and timing across muscles between conditions, with observed differences potentially reflecting subtle shifts in motor unit recruitment and firing. The PCs from surface EMG may reflect spinal pattern generator output⁴⁴; modest PC variances (14-23%) in posterior muscles suggest multiple spinal control mechanisms integrate sensory and motor inputs to stabilise muscle activation patterns

post-perturbation⁴⁵. The EMG principal component results should be interpreted as reflecting coordinated, system-level adaptations in muscle activation rather than as a series of independent statistical tests. Accordingly, emphasis is placed on the consistency of patterns across muscles and components, rather than on isolated statistical outcomes. These patterns likely reflect coordinated modulation of muscle activity consistent with the functional demands of the STS task.

An additional perspective focuses on the regulation of joint stiffness through muscle coactivation. Humans exhibit increased stiffness via muscle coactivation during challenging conditions, a phenomenon that can be understood within the framework of the equilibrium-point (EP) hypothesis⁴³. According to this hypothesis, the central nervous system specifies the magnitude of the stretch reflex threshold, effectively defining the subthreshold depolarisation of the corresponding motoneuronal pool, so that muscle state variables (force, length, and activation) emerge from interactions between the muscle, its reflex connections, and external loads^{46,47}. Agonist-antagonist coactivation likely reflects the CNS's strategy to preserve task-level control, thereby avoiding the necessity to shift to muscle-level control⁴³. The contribution of spinal cord circuitry to muscle coactivation appears to be minimal, suggesting that joint stiffness is predominantly mediated by

supraspinal processes⁴⁸. Notably, a study using stability-based optimisation demonstrated that increased stiffness reduced the whole-body response to self-generated perturbations and diminished reflex effects from muscle spindles⁴⁹, suggesting a role for stiffness modulation in stability. However, the benefits of increased joint stiffness appear to be task dependent. While enhanced stiffness can improve movement stability in kinematic chains with fixed origins (typically observed in laboratory settings where participants are secured to supporting surfaces), stiffening joints does not seem to offer an advantage during non-fixed tasks such as standing⁴³. In our sit-to-stand task, where the body's base of support is not fixed, increased vertical stiffness may theoretically indicate reduced stability. Alternatively, increased stiffness may facilitate proprioception by enhancing Ia afferent signalling, mediated by gamma efferent drive, potentially improving motor accuracy^{50,51}. This functional tuning of joint stiffness can also be interpreted through the lens of Adaptive Model Theory^{52,53}, which proposes that the CNS forms task-specific synergies and internal models to optimise control in reduced-dimensionality spaces. Within AMT, stiffness modulation emerges as a learned, feedforward strategy embedded within preformed synergies that stabilise performance under variable conditions^{52,53}. Within the context of the EP hypothesis and AMT, this role of stiffness modulation aligns with the uncontrolled manifold framework^{43,53}, which posits that the CNS maintains task-level stability by minimising variability in task-relevant dimensions while permitting flexibility in redundant degrees of freedom⁵⁴, a mechanism that future analyses may further elucidate.

Another notable finding was the observation that females had a significantly lower spinal reflex excitability in the Post-EE condition when compared to males (Figure 3). This is consistent with previous research that has shown differences in H-reflex responses between sexes, though a clear explanation for these variances is yet to be identified¹³. Nevertheless, there are physiological differences, beyond those of serum sex hormones alone, that contribute to the differences in spinal reflex excitability between sexes¹³. Interestingly, we also observed systematic EMG pattern differences between sexes across all muscle groups (Figures 7-10). These sex differences suggest that females may employ different neuromuscular control strategies compared to males. These altered motor control patterns may align with sex-specific morphological factors, such as differences in muscle distribution and limb structure. The H50/Mmax ratio and the EMG PC pattern differences observed between sexes provide insight into the neural interplay between muscular fatigue, sex, spinal reflex excitability and potential injury risk, particularly within females, however further research is required to explore this hypothesis.

A myriad of research has shown that females are at an increased risk of anterior cruciate ligament (ACL) injury due to biomechanical, anatomical, neuromuscular, and

hormonal variances between the sexes⁵⁵. There is also evidence that ligamentous injury risk is increased among females at the ankle joint¹⁶. Our results suggest a higher level of activation of the quadriceps in female participants during the initiation of standing within an STS task (Figure 7). Although the implications of this finding are currently unknown, there is evidence indicating that higher maximal quadriceps activation in females predisposes them to an increased risk of ACL rupture⁵⁶. Whilst further investigation is required, it is possible that greater activation of the quadriceps in females during submaximal functional tasks contributes to their increased risk of ACL injury. Taken together, our observed reduction in spinal reflex excitability, varied muscle activation patterns and increased vertical stiffness following PS and EE may hypothetically impact joint stability^{57,58}. Therefore, it is possible that this provides insight into a potential mechanistic explanation contributing to the known sex-based differences in lower limb injury risk, however this is a conjecture and requires further investigation.

This study has a number of limitations that should be acknowledged. Firstly, the menstrual cycle phase, hormonal contraceptive use, menopausal status, or other hormone-related factors were not controlled across all testing sessions. These uncontrolled variables may have implications for the interpretation of the H50/Mmax ratio and sensitivity to fatigue. Our exploratory findings suggest future research could specifically investigate the effect of menstrual cycle phase or hormone status on neurophysiological and biomechanical measures. A further limitation of the present design is that both interventions were completed within the same session, such that the second post-intervention assessment may include residual effects of the first intervention. Although the counterbalanced design and mixed modelling appropriately account for the dependency among repeated observations within participants, they do not eliminate the possibility of acute physiological carryover. Accordingly, post-intervention effects should be interpreted as condition-related responses estimated within the sequential experimental context rather than as fully isolated, carryover-free effects. Further to this, it is acknowledged that although the LMM accounts for individual variation, the STS task was self-paced and thus changes in movement velocity may impact upon joint stiffness calculations. Finally, given the number of EMG-derived variables, there remains a risk of inflated type I error if individual statistical outcomes are considered in isolation. Interpretation therefore focuses on convergent patterns across related variables, reflecting the interdependent nature of neuromuscular coordination.

Conclusions

In conclusion, our results demonstrated that triceps surae stretching and repeated explosive CMJ protocols

both significantly decreased spinal reflex excitability, and within STS altered EMG activation patterns and increased vertical stiffness. Females displayed significantly lower spinal reflex excitability in the Post-EE condition and systematically different lower limb STS EMG patterns, when compared to males. Considering the role of spinal reflexes in ensuring effective motor control and regulation of joint stiffness and stability, we speculate this mechanism may contribute to ligamentous injury risk. We emphasise, however, the link between spinal reflexes, joint stiffness and stability and ligamentous injury risk is a conjecture, and not directly examined here. Future studies should investigate the role of spinal reflex responses, joint stiffness and stability, and ligamentous injury risk, particularly among females. Our findings strengthen the proposition of an interaction between neurophysiological function and biomechanical performance. This may have broad implications for enhancing our understanding of the neural control of movement, exercise rehabilitation, and injury prevention, and warrants further investigation.

Ethics Approval

Ethical approval for this study was provided by the Charles Sturt University Human Research Ethics Committee (Protocol Number: H24030). The procedures used in this study adhere to the tenets of the Declaration of Helsinki and comply with all relevant laws and institutional guidelines.

Consent to Participate

Written informed consent was obtained from all individual participants included in the study.

Authors' Contributions

Sophie McCauley: Methodology, Formal analysis, Investigation, Writing—Original Draft, Visualisation; Kerry Mann: Conceptualisation, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing—Review and Editing, Visualisation, Supervision; Jack Cannon: Conceptualisation, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing—Review and Editing, Visualisation, Supervision; Michael Chang: Conceptualisation, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing—Review and Editing, Visualisation, Supervision; Nnamdi Mgbemena: Conceptualisation, Methodology, Writing—Review and Editing; Mehdi Rassafiani: Conceptualisation, Methodology, Writing—Review and Editing; Timothy Miller: Conceptualisation, Methodology, Formal analysis, Investigation, Writing—Original Draft, Writing—Review and Editing, Supervision, Project administration. All authors read and approved the final version of the manuscript.

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