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1 Effects of nociceptive and mechanosensitive afferents sensitization on
2 central and peripheral haemodynamics following exercise-induced
3 muscle damage.

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59 **Abstract**

60 This study aims to test the separated and combined effects of mechanoreflex activation and
61 nociception through exercise-induced muscle damage (EIMD) on central and peripheral
62 haemodynamics before and during single passive leg movement (sPLM). Eight healthy young
63 males undertook four experimental sessions, in which a sPLM was performed on the
64 dominant limb while in each specific session the contralateral was: a) in a resting condition
65 (CTRL), b) stretched (ST), c) resting after EIMD called delayed-onset-muscle-soreness
66 (DOMS) condition, or d) stretched after EIMD (DOMS+ST). EIMD was used to induce
67 DOMS in the following 24-48h. Femoral blood flow (FBF) was assessed using doppler
68 ultrasound while central haemodynamics were assessed via finger photoplethysmography.
69 Leg vascular conductance (LVC) was calculated as FBF/MAP. RR-interval were analyzed in
70 the time (RMSSD) and frequency domain (LF/HF). Blood samples were collected before
71 each condition and gene expression analysis showed increased fold changes for P2X4 and
72 IL1 β in DOMS and DOMS+ST compared with baseline. Resting FBF and LVC were
73 decreased only in the DOMS+ST condition (-26ml/min and -50ml/mmHg/min respectively)
74 with decreased RMSSD and increased LF/HF ratio. MAP, HR, CO, and SV were increased in
75 ST and DOMS+ST compared with CTRL. Marked decreases of delta peaks and AUC for
76 FBF (Δ : -146ml/min and -265ml respectively) and LVC (Δ : -8.66ml/mmHg/min and
77 \pm 1.7ml/mmHg/min respectively) all $p < .05$. These results suggest that combination of
78 mechanoreflex and nociception resulted in decreased vagal tone and concomitant rise in
79 sympathetic drive that led to increases in resting central hemodynamic with reduce limb
80 blood flow before and during sPLM.

81

82

83 **Key Words:** EIMD; Mechanoreflex; Peripheral Sensitization; Haemodynamics; Passive Leg
84 Movement;

85 **Graphic Abstract**

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87

88 **NEW & NOTEWORTHY**

89 Exercise induced muscle damage (EIMD) it is a well-known model to study mechanical
90 hyperalgesia and muscle peripheral nerve sensitizations. The combination of static stretching
91 protocol on the damaged limb extensively increases resting central haemodynamics with
92 reduction in resting limb blood flow and passive leg movement-induced hyperemia. The
93 mechanism underlining these results may be linked to reduction of vagal tone with
94 concomitant increased in sympathetic activity following mechano and nociceptive activation.

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104 **Introduction**

105 Peripheral group III-IV muscle nerve afferents regulate cardiorespiratory response to
106 exercise, composing the afferent arch of the exercise pressor reflex (EPR) (1). Thinly
107 myelinated (group III), and unmyelinated (group IV) muscle afferents mainly respond to
108 mechanical and metabolic stimuli respectively and their activation regulate the increase in
109 mean arterial pressure (MAP), heart rate (HR) and limb blood flow during exercise (2, 3).
110 However, these afferents show polymodal characteristics being sensitive also to other stimuli,
111 such as thermal and nociceptive (4, 5). Despite the exact mechanisms are not fully
112 understood, it has been postulated that the alteration of the muscle chemical milieu after
113 exercise induced muscle damage (EIMD) (6), due to increase metabolites production and
114 accumulation (i.e., prostaglandins, lactate, protons), could sensitize and or increase muscle
115 nerve afferents and related nociceptor activity (4, 7, 8). Indeed, inflammation following
116 muscle damage may cause mechanical hyperalgesia (tenderness and movement induced-pain)
117 (9-11) better known as delayed onset of muscle soreness (DOMS) (12, 13) that is suggested
118 to be linked to an increased activity and sensitization of A δ - (III) and C-fiber (IV) nerve
119 endings (14, 15). Indeed, inflammation, injuries or muscle damage events (as EIMD) are
120 thought to induce afferent nerve sensitization with related increases in expression of
121 purinergic 2X receptor (P2X), acid sensing-ion channel receptor (ASIC), transient receptors
122 potential vanilloid channels receptors (TRPV) (9-11). These receptors are usually present on
123 mechano and metabo sensitive muscle III-IV afferents (16, 17) which seem to being involved
124 in chronic pain condition (18, 19), heart failure (20) and mechanical hyperalgesia following
125 EIMD (11). Specifically, P2X receptors have been suggested as responsible of the increased
126 sympathetic nerve activity (SNA) to mechanical deformation in HF rats (21). Mechanical
127 hyperalgesia from peripheral nerve afferents sensitization following EIMD is linked with
128 autonomic nervous system and may cause an increased in sympathetic activity (22), that in

129 turn has been suggested as one of potential mechanisms of impaired vascular function and
130 increased cardiovascular responses following EIMD (23-26). Considering activation of
131 muscle mechanoreceptors with static or dynamic muscle stretching has shown to activate
132 mechanoreflex (27-30), it is likely that the activity of the sensitized mechanoreceptors would
133 be heightened increasing pain sensations (11, 15, 31) and thereby reducing limb blood flow
134 and vascular hyperemia, even in remote muscle. For instance, sensitization of
135 mechanosensitive afferents and heightened mechanoreflex responses seems to augment
136 peripheral vasoconstriction in patients with heart failure showing decreased vascular
137 responsiveness following passive leg movement (PLM) (32, 33). Mechanoreflex
138 hypersensitivity plays a significant role in cardiovascular diseases where it may lead to
139 dysregulated cardiovascular responses and possible exercise intolerance (34, 35). Different
140 studies have also suggested that mechanoreflex sensitivity may be altered in pain-related
141 diseases where small fiber neuropathy (36, 37) and mechanical hyperalgesia is present (38).
142 This latter may help to explain the abnormal cardiovascular responses to exercise in pain-
143 related diseases (39) which deserves further attention.

144 Yet, no studies have described the singular and combined effects of mechano- and
145 nociceptors sensitization on peripheral and central haemodynamics. Therefore, the aim of this
146 study was to determine the separated and combined effects of mechanoreflex activation and
147 nociceptive stimulus following exercise induced muscle damage on central and peripheral
148 hemodynamic and vascular responsiveness to single passive leg movement executed in
149 remote muscles. We hypothesized that only the combined sensitization of mechano- and
150 nociceptors would result in an autonomic-mediated increase in central haemodynamics and a
151 concurrent reduction of peripheral circulation and vascular responsiveness to single passive
152 leg movement executed in remote muscles.

153

154

155 **Methods**

156 *Participant's characteristics.* Eight healthy, non-smokers active male volunteers (age: $24.2 \pm$
157 2.2 yrs.; body mass 72.4 ± 10.1 kg; and height 179.3 ± 7.7 cm; means \pm SD) took part in the
158 study. Participants self-reported moderately levels of physical activity (3.0 ± 0.5 hours of
159 training per week) with no specific experience of strength training exercise. All procedures
160 conformed to the Declaration of Helsinki standards and were approved by the ethical
161 committee of the University of Verona (CARP) acceptance number (n.14 R2/2021). The
162 participants gave written, informed consent before their participation after full explanation of
163 the purpose and experimental procedures of the study. The participants reported to the
164 laboratory in the morning (8–9 AM) in a fasted state. They were asked to abstain from
165 consuming caffeine 24h and heavy exercise for 48h. Participants were also abstaining from
166 consuming before and 24h after each visits any vitamin supplements, high content vitamin C
167 food, alcohol, or pain medications.

168

169 *Experimental Design.* After a first familiarization visit, the participants reported to the
170 laboratory for 5 distinctive lab visits, in which they performed one of the four experimental
171 sessions in different days: (Control (CTRL); Stretching (ST); DOMS, DOMS with stretching
172 (DOMS+ST)) and the EIMD protocol (Fig 1). Before each of these conditions a blood sample
173 was taken from each participant. The CTRL session consisted of a single passive leg
174 movement (sPLM; described in further details below) of the dominant leg while the
175 contralateral leg remained resting fully extended. ST consisted of the same sPLM on the
176 dominant leg, but with the application of concomitant static stretching-protocol (described in
177 further detail below) in the contralateral leg. DOMS condition consisted of the same sPLM
178 test on the dominant leg while the contralateral leg was resting fully extended after having

179 previously performed the EIMD protocol. DOMS+ST conditions consisted of the same sPLM
180 on the dominant leg while in the contralateral leg a static stretching-protocol was applied
181 after previous application of EIMD protocol. The order of both experiments (CTRL vs ST
182 and DOMS vs DOMS +ST) was randomized and counterbalanced between participants (Fig.
183 1). Each session was performed in a separate day with DOMS and DOMS+ST conditions
184 performed randomly and counterbalanced at 24 or 48h after the EIMD protocol.

185

186 *Experimental protocol.*

187

188 *Blood sampling*

189 The subjects were asked to avoid vigorous physical activity in the 24 h before blood sampling
190 and to fasten from the evening meal until the morning, when samples were obtained. Blood
191 samples were collected before the start of each condition. Blood was sampled from
192 antecubital vein of each subject using a 21-gauge needle. To preserve RNA quality and
193 integrity 3 mL of blood have been collected directly into TEMPUS Blood RNA tubes (ABI,
194 Foster City, CA, USA) containing 6 mL Applied BioSystems RNA stabilization reagent.
195 These samples were immediately frozen at -20°C. A 6 mL K3-EDTA Vacuette was used for
196 hematology.

197

198 *Hematological testing*

199 All the samples were processed for routine hematological testing immediately after collection
200 (<15min) on the same Sysmex XN-1000 hematology analyzer (Sysmex, USA) using standard
201 local procedures at GB Rossi Hospital, Verona, Italy. The parameters tested included red
202 blood cells count (RBC), white blood cells (WBC) count, and WBC differential, including

203 lymphocytes, monocytes, neutrophils, eosinophils, basophils and large unstained cells,
204 platelet count, mean platelet volume. The instrument was calibrated against appropriate
205 proprietary reference standard material and verified with the use of proprietary controls.

206

207 *Total RNA preparation*

208 Total RNA was isolated from the blood samples using Tempus Spin RNA Isolation Kit
209 (Applied Biosystems) as previously described (40). Quality of the purified RNA from was
210 verified on an Agilent® 2100 Bioanalyzer (Agilent Technologies, CA); RNA concentrations
211 were determined using a Nanodrop® ND-1000 spectrophotometer (NanoDrop Technologies,
212 DE).

213

214 Quantitative real time PCR

215 For quantitative Real Time-PCR assays, total RNA was characterized by electrophoresis
216 (Agilent 2100 Bioanalyzer, CA). 400 ng of RNA was converted to cDNA using random
217 primers and Superscript III (Invitrogen, CA). Amplification was carried out in using SYBR
218 green chemistry (Fast SYBR green master mix Applied Biosystems) and a standard 2-step
219 protocol. The coefficients of variation for gene expression assays triplicates were $0.5 \pm 0.2\%$;
220 min-max [0.1-1.6] on average for all genes analyzed. The primers specific for each gene are
221 reported below. Identity of the amplicons was confirmed by their dissociation profiles and gel
222 analysis. Quantitative PCR experiments were performed in triplicate for each sample. The
223 data were normalized against *Gapdh* housekeeping gene.

224

225 Primers list:

226 P2X4

227 F: TCCGTCTTGGCAAAATAGTG
228 R: AGGTTGCAGTCCCAGTTGAC
229
230 IL1B
231 F CTGTCCTGCGTGTTGAAAGA
232 R TGAAGACAAATCGCTTTTCCA
233
234 IL10
235 F: TGCTGGAGGACTTTAAGGGTTA
236 R: GGGTCTTGGTTCTCAGCTTG
237
238 ASIC3
239 F: TTCTGGAACCGACAGCACTC
240 R: GAGGGGTGGGAGGTCTGG
241
242 TRPV1
243 F: AACTGGACCACCTGGAACAC
244 R: GCCTGAAACTCTGCTTGACC
245
246
247 GAPDH-6
248 F: CAGCCTCAAGATCATCAGCA
249 R: GTCTTCTGGGTGGCAGTGAT
250

251 *Static stretching protocol and range of movement assessment.* During the familiarization visit
252 participant's maximal knee flexion range of movement (ROM) was assessed on the non-
253 dominant limb with the participants in the supine position. All assessments were conducted
254 by the same operator, who moved the participant's non-dominant joint through a 50° range of
255 motion (knee extension) until reaching the point of tolerable maximal flexion where
256 subjective tension-discomfort was rated using Visual Analog Scale (VAS) and Pain Numeric
257 rating scales (P-NRS) (41). VAS was used to self-reported perception of stretching intensity,

258 using a 100-mm scale in which participants rated their perception of stretching intensity from
259 zero (no stretch at all) to ten (maximal stretch as possible). Moreover, subjective feeling of
260 pain was recorded with P-NRS scale, rating the pain arising from the stretching protocol from
261 zero (no pain at all) to ten (pain as bad as it could be). Static stretching consisted of passive
262 single knee flexion of 6 minutes which spanned the 5 minutes of baseline, sPLM maneuver
263 and 1 minute recovery on the contralateral leg to the point of maximal flexion at rest and
264 lasting the entire duration of the time of the sPLM protocol. During stretching protocol, knee
265 joint angle was continuously recorded using a biaxial electro goniometer (Twin axial
266 Goniometer TN1750/ST ADI Instruments Systems, Oxford, UK). During the entire stretching
267 protocol, the knee extensors were stretched to the same range of motion obtained during the
268 initial ROM assessment (42) which was kept identical for all stretching conditions (ST and
269 DOMS+ST respectively). An adjustable load cell was also fixed on the participant's non-
270 dominant ankle and held during the experiment by the same operator measuring the force
271 applied from the flexed stretched non-dominant leg during the entire protocol (Fig. 1).

272

273 *Exercise induced muscle damage protocol.* A warm-up of 10 non-dominant single leg
274 isokinetic knee extensions and knee flexions were carried out through the full test range of
275 motion, ensuring a progressive increase in effort, using an isokinetic dynamometer (Cybex,
276 division of Lumex Inc., Ronkonkoma, NY, USA). A single leg maximal isometric voluntary
277 contraction (MVIC) of the non-dominant limb was assessed prior to the EIMD protocol,
278 where participants were instructed to exert the maximal voluntary isometric contraction
279 during a leg extension movement at 90°. The EIMD protocol, consisted of several blocks of 3
280 sets of 12 maximal voluntary eccentric single knee extensions of the non-dominant limb with
281 30 seconds of recovery. Following each block, a MVIC was performed to determine the loss
282 of muscle strength from baseline. Exercise was stopped once MVIC was reduced by 40% or

283 more from baseline (43). The eccentric phase of the contractions was performed at an angular
284 velocity of $90^{\circ}\cdot\text{s}^{-1}$. The concentric phase was performed sub-maximally at an angular velocity
285 of $90^{\circ}\cdot\text{s}^{-1}$ to minimize fatigue and enhance eccentric damage (44). To ensure the presence of
286 DOMS after EIMD protocol a series of tests were carried out before the starting of each
287 condition (45). Pain Pressure Threshold (PPTS) and indirect measurements of muscle damage
288 were used to assess the entity of DOMS after EIMD (46). PPTS were assessed to underline
289 mechanical hyperalgesia following EIMD using a mark placed on different points of the non-
290 dominant quadriceps using a mechanical pressure algometer (Hilitand, NK-100 Force Gauge,
291 USA) which were standardize between participants and marked to being kept similar between
292 condition (47). Indirect measures of muscle damage were assessed trough MVIC using a
293 Biodex dynamometer (Biodex, Shirley, NY, USA) (48) and a 100-mm visual analog scale
294 (VAS) anchored on the left edge of the scale with the phrase “no pain or soreness” and on the
295 right edge “worst pain/soreness imaginable”(49). Participants were asked to rate their pain-
296 related soreness during their ~~for~~ daily living activities (VAS_{DA})(45) and after performing a
297 squat at approximate 90 degrees of knee angle (VAS_{SQ}) (48).

298

299 *Single Passive Leg Movement Test.* SPLM was implemented as a testing procedure for
300 assessing vascular function during all sessions. Participants remained rested in the upright-
301 seated position for 20 min before the start of data collection and remained in this position
302 throughout the study. The sPLM protocol consisted of 5 min of resting baseline data
303 collection followed by one passive knee flexion and extension, which took 1 s, after which
304 the leg was maintained fully extended for the remaining 59s of post movement data collection
305 (50).

306

307 *Leg blood flow and leg vascular conductance.* Measurements of arterial blood velocity and
308 vessel diameter were performed in the common femoral artery of the dominant leg (i.e.,
309 passively moved leg), distal to the inguinal ligament and proximal to the deep and superficial
310 femoral bifurcation with a Logiq-7 ultrasound Doppler system (General Electric Medical
311 Systems, Milwaukee, WI) (50). The ultrasound Doppler system was equipped with a 12- to
312 14-MHz linear array transducer. Artery diameter was determined at a 90° angle along the
313 central axis of the scanned area. Mean blood velocity (V_{mean}) was measured using the same
314 probe utilizing a frequency of 5 MHz. Measurements of V_{mean} were obtained with the probe
315 positioned to maintain an insonation angle of 60°, and the sample volume was centered and
316 maximized according to vessel size. Utilizing arterial diameter and V_{mean}, femoral blood
317 flow (FBF) was calculated second by second as:

318

$$\text{FBF} = V_{\text{mean}} \times \pi \times \left(\frac{\text{Vessel Diameter}}{2} \right)^2 \times 60$$

319

320 where FBF is in milliliters per minute. All scanning and blinded analyses were performed by
321 experienced and skilled sonographers.

322

323 *Autonomic and central haemodynamics.* HR was assessed using a 3-leads electrocardiogram.
324 Beat-by-beat arterial pressure was determined by finger plethysmography on the non-
325 dominant hand (Finapres model 2300; Ohmeda, Englewood, CO, USA). Automatic
326 calibration was turned off during data collection. The photoplethysmography cuff of the
327 finger pressure device was placed on the third finger of the left hand. The subject's arm was
328 supported by an armrest to avoid arm and finger movement. The Finometer signal was
329 calibrated utilizing the procedure indicated by the manufacturer. All the signals were

330 amplified and recorded through the Power Lab System (PowerLab 16/30; ML880,
331 ADInstruments, Bellavista, NSW, Australia). A non-invasive thoracic impedance cardiograph
332 (Physio Flow®, Manatec, Strasbourg, France) was used to measure heart rate (HR) and
333 estimate stroke volume (SV).

334

335 *Data Collection and Analysis.* V_{mean} of the femoral artery blood was analyzed for 30 s at
336 baseline and for 60 s during the sPLM test. Before analysis, all hemodynamic data were
337 smoothed using a 3-s rolling average (30). As the response to sPLM is transient and varies
338 between individuals, a peak response was determined for all variables on an individual basis.
339 Maximal absolute peak (peak), relative change calculated as the peak minus the baseline
340 (Δpeak) and area under the curve (AUC) were determined after normalization for baseline for
341 all variables for each subject as the summed response for 60 sec (51) for each subject in all
342 measured variables (52). Mean arterial blood pressure (MAP) was calculated as $(1/3 \text{ SBP} +$
343 $2/3 \text{ DBP})$. Leg vascular conductance (LVC) was calculated as FBF/MAP . Cardiac output
344 (CO) was calculated as $\text{stroke volume} * \text{HR}$ (53). MAP, R-R peaks traces, and knee joint
345 angle and force output were A/D converted using LabChart Pro software (LabChart Pro 8,
346 with HRV Module, ADInstruments, Bellavista, NSW, Australia).

347 Delta for singular effects were calculated deducting CTRL values for all outcomes from ST
348 and DOMS respectively. Delta for interaction were calculated summing previously singular
349 delta ST and delta DOMS and compared with delta between CTRL and DOMS+ST
350 condition. The interaction mode (hypo-additive, hyper-additive or additive) was defined as
351 reported by Wan and colleagues in (54). Briefly, hyper-additive, additive or hypo-additive
352 effects refers to an observed response that during the synergic activation of the reflexes is
353 respectively larger, equal or smaller than the sum of the response evoked by each reflex alone
354 (54).

355 HRV analysis calculations were performed for the 300 seconds at baseline before the sPLM
356 maneuver using (LabChart Pro 8, with HRV Module, ADInstruments, Bellavista, NSW,
357 Australia). RR intervals trace was checked and edited for artifact by visual inspection (55).
358 Root mean squared of successive intervals (RMSSD) was calculated as an index of HRV
359 from the RRi series. Frequency domain analysis for HRV were performed through spectral
360 decomposition of the RRi signal using Fast Fourier Transform via Welch's method with
361 Hanning window in 256 sample segments with 50% overlap (55). Low frequency (LF, 0.04 –
362 0.15 Hz) and high frequency (HF, 0.15 – 1.0 Hz) were calculated as integrals under the
363 respective power spectral density curve, LF/HF was calculated as the ratio between the low
364 and high frequency power (56).

365

366 *Statistical Analysis.* Normal distribution of the data was assessed with a Shapiro-Wilk test.
367 Student's paired t-test was implemented to determine differences between VAS pain and
368 VAS stretching intensity within stretching measurements (ROM and Force) during ST and
369 DOMS+ST data. One-way repeated measures ANOVA with Tukey-B post-hoc analysis was
370 implemented for gene expressions (P2X4, ASIC3, TRPV1, IL1 β , IL10), baselines and sPLM
371 maneuver outcomes for central and peripheral haemodynamics (FBF, MAP, LVC, CO, SV,
372 HR and AUC) within and outcomes of autonomic responses. A Bonferroni-Holm correction
373 was performed for deltas interaction for all peripheral and central haemodynamics outcomes
374 (54). Pearson single correlation analysis were implemented between autonomic responses
375 values of resting and delta peak for FBF and LVC, across all conditions. A sample size of
376 eight participants was selected to ensure a statistical power higher than 0.80 with a type 1
377 error <0.05 to detect ~15-20% in FBF (main outcome) under stretching conditions (52). All
378 data were analyzed using a statistical software package Graph Pad Prism v.9 (GraphPad

379 Software, San Diego, California USA). Data are presented as mean \pm standard deviation (SD)
380 and considered significant when $p < 0.05$.

381

382 **Results**

383 All participants took part in the study and completed all session without reporting any
384 position or postural discomfort during the stretching procedures. Participants were set at the
385 same knee angle during ST and DOMS+ST condition (54.9 ± 4.9 vs 55.1 ± 4.7 degrees;
386 $p > 0.05$), and the force detected were similar between conditions (67.1 ± 22.2 vs 77.4 ± 24.5 N
387 for ST and DOMS+ST, respectively; $p > 0.05$). VAS for stretching intensity were similar
388 between participants and between the two stretching conditions (7.1 ± 1.3 vs 8.1 ± 1.6 cm for
389 ST and DOMS+ST, respectively; $p > 0.05$). VAS for pain intensity was higher for DOMS+ST
390 compared with ST (7.0 ± 2.6 cm vs. 2.6 ± 1.9 , $p < 0.05$).

391

392 *Direct and Indirect measures of DOMS.* Results and comparison for DOMS are reported in
393 Table 1. Mean leg extensor MVC, PPTS, VAS_{DA} and VAS_{SQ} were not different between
394 CTRL and ST conditions ($p > 0.05$). However, MVC decreased significantly in DOMS and
395 DOMS+ST compared with CTRL and ST condition (all $p < 0.05$). PPTS decreased
396 significantly in DOMS and DOMS+ST compared with CTRL and ST condition (all $p < 0.05$).
397 VAS_{DA} was increased in DOMS and DOMS+ST compared with CTRL conditions ($p < 0.05$).
398 VAS_{DA} also increased significantly from ST condition to DOMS and DOMS+ST ($p < 0.05$).
399 VAS_{SQ} increased significantly in DOMS and DOMS+ST compared with CTRL and ST
400 conditions respectively (all $p < 0.05$).

401

402 *Blood Cell Count and Gene expression.* All blood test results and comparison for gene
403 expression across conditions are reported in Table 2. We found P2X4 expression significantly

404 upregulated in DOMS and DOMS+ST condition (all $p < 0.05$) The changes in the expression
405 levels of TRPV1 and ASIC3 follow a similar pattern, although variations reach statistical
406 significance only for TRPV1 in DOMS+ST ($p < 0.05$). IL1B gene shows a sustained increase
407 in expression levels in the responses for DOMS and DOMS+ST condition (all $p < 0.05$). No
408 relevant variation is displayed by IL10 gene. No changes in white blood cells, monocytes,
409 lymphocytes and neutrophils and platelet were found between condition and red however red
410 blood cells count statistically decreased in DOMS and DOMS+ST condition compared with
411 baseline ($p < 0.05$).

412

413 *Resting Measurements.* All results and comparison for central and peripheral haemodynamics
414 at rest are reported in Table 2. Resting FBF and LVC were significantly decreased in
415 DOMS+ST condition (all $p < 0.05$) compared to CTRL. Resting MAP, HR, CO and SV were
416 increased in DOMS+ST (all and ST condition compared to CTRL (all $p < 0.05$). Resting
417 MAP, HR and SV were increased in ST condition compared to DOMS ($p < 0.05$). Moreover,
418 resting MAP, HR, CO, and SV were increased in DOMS+ST compared to DOMS (all
419 $p < 0.05$). Resting MAP and SV increased in DOMS+ST compared with ST (all $p < 0.05$). FBF,
420 LVC, HR, MAP, CO, SV were not different between CTRL and DOMS conditions. A
421 Statistical difference was also found for RMSSD, HF and HF/LF ratio between CTRL and
422 DOMS+ST condition ($p < 0.05$).

423 *Central and Peripheral Haemodynamics during sPLM.* All results and comparison are
424 reported in Table 3. Δ Peak FBF and LVC significantly decreased in DOMS+ST conditions
425 (all $p < 0.05$) compared with CTRL. FBF and LVC AUC decreased significantly from CTRL
426 to ST and DOMS+ST conditions (all $p < 0.05$) (Table 3). Δ peak for MAP, SV, HR, and CO
427 within respectively AUC were not statistically different between all conditions (all $p > 0.05$)
428 (Table 3).

429

430 *Delta Interaction.*

431 All results and comparison are reported in Table 4. The delta interaction (Δ DOMS+ Δ ST vs
432 Δ DOMS+ST) showed no statistical differences in peripheral haemodynamics at rest (FBF,
433 LVC) central haemodynamics(CO, SV) and autonomic responses (RMSSD and LF/HF ratio),
434 resulting in an additive effect of DOMS+ST condition compared with the combination of the
435 singular effects from ST and DOMS condition respectively. However, differences were found
436 in resting HR, CO and MAP in Δ DOMS+ST compared with Δ DOMS+ Δ ST, showing hyper-
437 additive effect for these parameters ($p < 0.05$). No differences were found in vascular
438 responsiveness outcome for the delta interaction.

439

440 *Single correlation analysis.*

441 RMSSD was inversely correlated with increases in HR ($r = -0.46$; $p < 0.05$), MAP ($r = 0.44$;
442 $p < 0.05$) and CO ($r = 0.40$; $p < 0.05$) whereas as LF/HF was found positively correlated with
443 increases in HR ($r = 0.44$; $p < 0.05$), MAP ($r = 0.38$; $p < 0.05$) and CO ($r = 0.40$; $p < 0.05$).
444 Moreover, ASIC3 was inversely correlated with RMSSD ($r = -0.49$; $p < 0.01$) and positively
445 correlated with LF/HF ratio ($r = 0.58$; $p < 0.01$). TRPV1 was found inversely correlated with
446 RMSSD ($r = -0.37$; $p < 0.05$) within LF/HF ratio ($r = -0.37$; $p < 0.05$). P2X4, ASIC3 and TRPV1
447 correlated with MVC ($r = -0.72$; $r = -0.51$; $r = -0.72$; all $p < 0.01$); VAS_{DA} ($r = 0.67$; $r = 0.57$; $r = 0.78$;
448 all $p < 0.01$) and VAS_{SQ} ($r = 0.71$; $r = 0.55$; $r = 0.72$; all $p < 0.01$). PPTS correlated with P2X4 and
449 TRPV1 ($r = -0.38$; $r = -0.36$; all $p < 0.05$).

450

451 **Discussion**

452 This is the first study investigating the singular and combined effects of mechano- and
453 nociceptor activation on central and peripheral haemodynamics and vascular responsiveness.

454 We found that only the combination of mechanoreflex and nociceptor activation promotes
455 greater changes on central and peripheral haemodynamics at rest with reduced vagal activity
456 (reduced RMSSD) and concomitant increases of sympathetic drive (increased LF/HF ratio)
457 (Fig 2; Table 2), that inversely and positively correlate with HR, CO, and MAP respectively.
458 Moreover, when both the mechanoreflex and nociceptors were activated, the contralateral leg
459 exhibited a reduction in leg blood flow and vascular responsiveness to sPLM in remote
460 muscles (Fig 3). These results, suggest that the stimulation of mechano- and nociceptive
461 afferents trigger increases in central haemodynamics mediated by vagal tone suppression and
462 increase in sympathetic drive (hyper-additive and additive effects). The sympathetic gain is
463 also responsible, at least in part, for the reduction in resting limb blood flow and decreased
464 vascular responsiveness with additive effects (Fig 4). Interestingly, the singular stimulation of
465 the mechano- and nociceptive afferents via passive static stretching of the skeletal muscle or
466 the DOMS resulted in negligible changes of the peripheral circulation. Furthermore, gene
467 associated with pain and mechanoreceptors activity (P2X4, TRPV1), with marker of
468 increases inflammation ($IL1\beta$), increases following EIMD suggesting a possible mechano-
469 and nociceptive sensitization of nerve endings afferents. Therefore, in agreement with our
470 initial hypothesis, only the sensitization of both mechano- and nociceptors resulted in
471 autonomic-mediated increase in central haemodynamics and a concurrent reduction of
472 peripheral circulation and vascular responsiveness in remote skeletal muscles.

473

474 *Exercise induced muscle damage and stretching as a model to study mechanical hyperalgesia*
475 *and mechanoreflex sensitization.*

476 In our experiment we applied EIMD to induce DOMS and mechanical hyperalgesia,
477 with concomitant application of static stretching in the damaged muscle to activate
478 mechanosensitive and nociceptive afferents (Fig. 4). Previous animal studies reported an

479 increased mechanical sensitization of A δ and C-fibers and concomitant mechanical
480 hyperalgesia following EIMD (9, 10, 15). Moreover, other studies revealed a mechanical
481 sensitization of large mechanical fibers in humans (14, 57) after EIMD. In line with these
482 reports, we found an increased mechanical hyperalgesia (from reduced PPTs), within
483 increased in self-reported pain during DOMS+ST condition compared with ST alone,
484 suggesting an increased mechano- and nociceptive activation. Moreover, we found an
485 increased gene expression in P2X4 channel, and a positive trend in ASIC3 and TRV1 in
486 DOMS and DOMS+ST. These data are in line with previous investigations on chronic pain,
487 and mechanical hyperalgesia in different patient's population (18, 21) and exercise induced
488 muscle damage (10, 11, 15, 58). From the single correlation analysis, we found that these
489 genes nicely correlated with marker of EIMD (PPTS, VAS and MVC), suggesting that the
490 entity of the soreness and hyperalgesia following EIMD was correlated with the higher gene
491 expression.

492

493 *Evidence of mechano- and nociceptors activation on the peripheral and central*
494 *haemodynamics at rest.*

495 Vascular function within peripheral haemodynamics has been previously found
496 impaired after EIMD (24). Indeed, despite the big impact EIMD has on muscle function, it
497 also seems to impair the cardiovascular system, particularly endothelial and microvascular
498 function (59) with increases in arterial stiffness (24). It seems that this impairments have been
499 linked to the increased inflammatory response following EIMD in the damaged limb (24).
500 Although several studies found an impaired vascular function in the skeletal muscle directly
501 affected by the DOMS, limited studies investigate the possible cross-over effects of DOMS
502 on remote skeletal muscle (23, 26). In the current study we did not find reduced blood flow in
503 the contralateral leg at rest during DOMS compared with CTRL condition, despite higher

504 IL1 β levels. Our findings are in line with Caldwell et al. (26) who found that systemic
505 vascular function measured with FMD, was not affected following EIMD in a remote
506 healthy muscle. However, in some studies was found a close relationship between pro-
507 inflammatory cytokines and decreased vascular function (60-62). Moreover, studies on
508 healthy volunteers following vaccinated-induced inflammation found decrease vascular
509 function (decreased FMD and increased arterial stiffness) (63, 64) while in aging population,
510 this effect was not appreciable (65). The discrepancies between these studies have also been
511 attributed to different level of systemic inflammation following influenza-vaccine
512 administration (65). This could have been the case in our current study where the level of
513 inflammation following EIMD may have not been sufficient to cause a level of systemic
514 inflammation necessary to induce systemic vascular function impairment (26). Unfortunately,
515 we can not completely rule out the effect of inflammation to systemic vascular function
516 impairments, so future studies in healthy humans should more extensively monitor
517 inflammation (i.e., TNF- α activity) during DOMS, to elucidate this relationship. Looking at
518 the stretching condition, no significant changes in peripheral haemodynamics were found
519 although an overall reduction in FBF and LVC compared with CTRL. Reduction of
520 peripheral haemodynamics was previously found in the contralateral (resting) leg following
521 static stretching protocol, suggesting an increased sympathetic-mediated vasoconstriction
522 from activation of mechanoreceptors as a potential mechanism for this attenuation (52). The
523 entity of these changes however may differ between the current study due to the distinct
524 stretching protocol implemented, which may have led to different mechanoreflex activation
525 and peripheral haemodynamics changes. Interestingly, one of the major findings of the
526 current study is a decreased FBF and LVC at rest in DOMS+ST conditions. In fact, the
527 singular and distinctive effect of mechanoreflex (ST condition) or nociceptive (DOMS
528 condition) reflexes stimulation seems to not be sufficient to alter peripheral haemodynamics

529 (Table 4). Recently, studies of cardiovascular reflexes have brought attention to the
530 importance of the individual and interactive relationships between cardiovascular reflexes,
531 suggesting hypo-additive, hyper-additive, or additive effects on peripheral and central
532 haemodynamics(54). Reduction of peripheral and central haemodynamics in DOMS+ST
533 condition may have been a consequence of a hypo-additive effect (54) after mechanoreflex
534 and nociceptive activation due to nerve peripheral sensitization.

535 Indeed, we found increases in blood gene expression for P2X4, and TRPV1 that have
536 been linked to cardiovascular function (66), with P2X4 receptors be linked to nociceptive and
537 mechanoreflex sensitization (58, 67). Purinergic receptors seem to act as mediators in
538 peripheral vasoconstriction by ATP released from sympathetic nerve activation (68), their
539 sensitization after EIMD may have led to an increase sympathetic nerve activation. So, it may
540 be concluded that the stimulation of mechano- and nociceptive reflexes leads to additive
541 effects in reducing peripheral haemodynamics at rest compared with the singular effects
542 alone.

543

544 Regarding changes on central haemodynamics, previous studies suggest a possible
545 alteration following EIMD where an increased blood pressure and HR responses was found
546 during isometric exercise (23, 69). Interestingly, resting HR and MAP appeared to not be
547 impacted by EIMD at baseline (24, 69, 70). In line with these studies, we did not find any
548 differences in central haemodynamics at rest between CTRL and DOMS conditions. On the
549 other hand, central haemodynamics increased in ST conditions compared with CTRL, as
550 previously reported, following static and dynamic quadriceps stretching protocols (28, 29,
551 42). This has been suggested by different authors to be linked to increase parasympathetic
552 withdrawal after the onset of muscle stretch (71, 72) combined with an increased vascular
553 resistance within the stretched limb. This mechanism has been attributed to the muscle

554 lengthening that increase activation of perivascular sympathetic nerves, or cell to cell
555 signaling, resulting in norepinephrine release (73, 74), with increased vasculature resistance
556 and decreased blood flow (75). Furthermore, HR, MAP and SV were significantly different in
557 DOMS+ST conditions compared to all conditions, moreover for delta's interaction for resting
558 HR and MAP showed hyper-additive effect of mechano- and nociceptors sensitization on
559 central haemodynamics.

560

561 *Evidence of mechano- and nociceptors activation on sPLM-induced hyperemia.*

562 Previous studies have shown that EIMD may cause a reduction in vascular hyperemia
563 in the leg impacted by DOMS (25). However, no differences were found in the systemic
564 vascular responsiveness in a remote muscle (brachial artery) 48h post-EIMD (26). In line
565 with this result, we did not find any statistical difference in sPLM-induced hyperemia in
566 DOMS condition in a remote skeletal muscle, suggesting that systemic inflammation
567 following EIMD did not exert a sufficient effect to decrease systemic vascular function, as
568 suggested in previous model of inflammation (60-62). Interestingly, the major finding of the
569 current study was a decreased vascular responsiveness (FBF and LVC peaks within related
570 AUCs) following sPLM in DOMS+ST condition (Fig. 3). These results could be explained
571 by an increased systemic vasoconstriction, following mechanoreceptors sensitization (Fig. 4).
572 For instance, previous studies revealed that heightened mechanoreflex sensitivity seems to
573 augment peripheral sympathetic vasoconstriction in response to PLM (32, 33). Moreover, a
574 recent study reported a reduced LVC after superimposed PLM during concomitant exercise
575 executed in different muscles (76), underlining the role that sympathetic vasoconstriction has
576 in attenuating the PLM-induced hyperemia. Indeed, previous investigators have reported an
577 increased sympathetic activity was linked to suppressed vasodilatory responses following
578 exercise or negative pressure stimulation (77), linking the role of sympathetic drive in

579 reducing vascular vasodilatory responses. Despite singular effects of mechano- and
580 nociceptive reflexes were not sufficient to alter vascular responsiveness following sPLM, we
581 found an additive effects in DOMS+ST condition without differences in central
582 haemodynamics response. However we can not completely exclude that inflammation
583 following EIMD had exert a possible role in decrease vascular responsiveness. Changes in
584 central haemodynamics are usually associated to continues PLM, rather than sPLM, where
585 only a single limb movement is performed, avoiding increases in mechanoreflex activation
586 and central haemodynamics, that is usually associated with continues PLM (50).

587

588 *Mechanisms of mechanoreflex and nociceptor activation as mediators of peripheral and*
589 *central haemodynamics alterations at rest and during sPLM.*

590 The intensity and modality of static stretching has suggested to play an important role
591 on mechanoreflex activation and central haemodynamics responses (27). Indeed, constant
592 angle at low/to moderate intensity static stretching have shown to activate mechanoreflex
593 only in the early phase of the stretching protocol increasing slightly the central
594 haemodynamics (28, 42, 78) showing that mechanical tension play an important role on
595 maintaining mechanoreflex discharge. For these reasons in our protocol, we decided to adopt
596 a high intensity static stretching protocol to maintain mechanoreflex activation within higher
597 parasympathetic withdrawal that in turn has elicited strong increasing in resting central
598 haemodynamics. This effect was amplified during DOMS+ST protocol, presumably due to an
599 increased stiffness of the muscle (increased in mechanoreceptors discharge) and increased
600 nociceptive activity coupled with higher rating of self-reported perceived pain and P2X4,
601 TRPV1 gene expression following EIMD.

602 Although no difference was found in HR, MAP, SV, and CO at rest between CTRL
603 and DOMS conditions, increased central haemodynamics in ST was detected (28, 29, 79)

604 with further increased in DOMS+ST conditions, suggesting an increased parasympathetic
605 withdrawal from heightened mechanoreflex activation. Indeed, previous study has reported a
606 decreased RMSSD in subjects with low flexibility following stretching, finding an impaired
607 sympatho-vagal balance, and increased parasympathetic withdrawal (80). Moreover, in line
608 with this hypothesis, we found a reduced HRV (i.e., RMSSD) during DOMS+ST condition,
609 with significant correlation in increased HR, CO and MAP, linking a suppression of vagal
610 tone as the main drive to the increased central haemodynamics (71, 72). Decrease in RMSSD
611 was also correlated with increases in ASIC3 and TRPV1 possibly linking increase in
612 parasympathetic withdrawal and mechanical hyperalgesia.

613 In the current studies we also recorded an increased LF/HF ratio in DOMS+ST, and a
614 positive correlation between LF/HF ratio and increases in central haemodynamics. LF/HF
615 ratio has been proposed as metric to measure sympatho-vagal balance (81) and reflecting an
616 increase in sympathetic activity (56). This result could be explained by an increased
617 stimulation of nociceptors following EIMD due to afferents sensitization. Thus, seen that
618 muscle afferents has been reported to become sensitized after EIMD due to increase
619 inflammation (82) that in turns increases in sympathetic mediated pain activity (22), and seen
620 that LF/HF has shown to be correlated with an increased inflammatory response (83), it may
621 be hypothesize that sympathetic mediated pain from increased nociceptors sensitization may
622 have led to increases in LF/HF ratio during DOMS+ST condition, within concomitant
623 increases in central haemodynamics. However, critiques were raised on the LF frequency and
624 increased LF/HF ratio as a marker of increased sympathetic nerve activity (84), so future
625 studies are needed to confirm its validity as “sympathetic biomarker”. From these results it
626 could be hypothesized that the singular effects of mechano- and nociceptive stimulation are
627 not sufficient to elicit strong changes in peripheral haemodynamics and vascular
628 responsiveness while their combination exert an additive effect in DOMS+ST condition.

629 Indeed, increased sympathetically mediated-pain activity, coupled with increased
630 parasympathetic withdrawal, from heightened nociceptors and mechanoreflex activation,
631 resulted in 1) increased resting central haemodynamics (hyper-additive and additive effect);
632 2) reduced resting peripheral haemodynamics (additive effect); 3) reduced sPLM-induced
633 hyperemia (additive effect).

634

635 *Implication for research and clinical practice.*

636 Our study highlights the role of mechanoreflex sensitisation on the autonomic nervous system
637 and blood flow regulation in a remote non affected muscle. The current results may shed light
638 on possible mechanisms of increase cardiovascular response and exercise intolerance for
639 people experiencing DOMS. Indeed, previous research has highlighted the cardiovascular and
640 neuromuscular impairments following EIMD (43, 69), and proposed the increased muscle
641 nerve afferents activity as a potential mechanism underlining these impairments (82).
642 Moreover increase in muscle nerve afferent feedback from mechanoreceptors activity has
643 been suggested as a possible cause of elevated cardiorespiratory responses and hyperpnea
644 during exercises after EIMD (85), leading to exaggerated ventilation at the onset of exercise.
645 Similar results were found recently in patients with chronic fatigue, where an increased
646 VE/VO_2 , VE/VCO_2 and tidal volume were found (86). Interestingly these outcomes were
647 previously associated with group III-IV muscle nerve afferents activation (87). Indeed,
648 muscle nerve afferents sensitisation seem to play a significant role in cardiovascular diseases,
649 such as heart failure, where a mechanoreflex sensitisation led to dysregulated cardiovascular
650 and cardiorespiratory responses and exercise intolerance (88). More recently, studies have
651 also suggested that mechanoreflex sensitivity may be altered in diseases such as Rheumatoid
652 Arthritis, Myofascial Pain Syndromes and Fibromyalgia (36, 37, 89). From all these evidence
653 it seems that nerve afferent sensitisation is responsible of the dysregulation of different

654 physiological mechanisms linked to exercise intolerance and performance (3). Future
655 research should focus on the role that muscle peripheral sensitisation may have in exercise
656 intolerance and find targeted intervention to restore its correct functioning.

657

658 **Conclusions**

659 All these findings, suggest that only the combined effects of sensitized mechanosensitive and
660 nociceptive fibers led to a parasympathetic withdrawal within possible increase in
661 sympathetic drive, increasing central haemodynamics with concomitant decreasing in blood
662 flow at rest and reduced vascular responsiveness to sPLM in a contralateral non-damaged
663 limb. These findings may help to underline the additive interactive effects of mechano- and
664 nociceptors sensitization on blood flow supply in remote muscle, improving the existing
665 knowledge on the effects of a heightened mechano- and nociceptors feedback in pain-related
666 diseases and syndromes.

667

668 *Limitations:*

669 One possible limitation of the present study is the lack of direct sympathetic measures
670 as usually assessed through muscle nerve sympathetic activity (MSNA). However, this
671 technique is extremely difficult to implement in a such experimental study design, so we
672 decided to use HRV as a surrogate of autonomic nervous system activity. Another possible
673 limitation is represented by the white blood cells (WBC) gene expression analysis. Despite it
674 is very well known about the interaction of immune-system and nerve afferents sensitization
675 (58) and that previous studies adopted WBC gene expression for studying chronic conditions
676 and nerve afferents (18, 19), this technique may lack of specificity. Indeed, it may be difficult
677 to define in which region of the human body (e.g., skeletal muscle) there was an increased

678 expression of these receptors. However, seen that the musculoskeletal system was more
679 impacted from EIMD we may infer that most of the inflammation and related sensitization
680 was located on the damaged limb and not elsewhere.

681

682 **Contributions**

683 All the authors played a role in the content and writing of the manuscript. In addition,
684 M.V was the principal investigator; M.V, F.Z., and J.S.M. had input into the original idea,
685 study design, and conduct of the study. F.Z., M.V., G.G., T.F., M.M.O. collected the data;
686 F.Z., M.V., F.G.L., and P.D.O. performed data analysis and statistics, and F.Z., F.G.L., E.C.
687 prepared it for presentation. F.Z. and M.V wrote the manuscript. J.S.M., M.V. T.P., A.F, L.B,
688 E.C. and P.D.O reviewed the manuscript.

689

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959 Rheumatoid Arthritis. *The FASEB Journal* 33: 696.613-696.613, 2019.

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962 **Figures legend**

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965 Fig 1. Study design and experimental procedures. On the top center the study design and randomization procedure.
966 On the bottom left is represented with an outlined lined the force registered in the load cell applied to the stretched leg, while
967 the solid line represents the knee angle measured with the electrical goniometer during stretching protocol. On the bottom
968 right is represented the experimental procedures during the different sessions. Abbreviations: Control Condition (CTRL);
969 Stretching condition (ST); DOMS condition (DOMS); and DOMS with stretching condition (DOMS+ST); Exercise Induced
970 Muscle Damage (EIMD).

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974 Fig 2. Changes in peripheral hemodynamic responses at rest during control condition (CTRL), Stretching condition (ST),
975 DOMS condition (DOMS) and DOMS with stretching condition (DOMS+ST) respectively. A: Femoral Blood Flow (FBF);
976 B: Leg Vascular Conductance (LVC); *significantly different from CTRL.

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978

979 Fig 3. Changes in central and peripheral haemodynamics responses to sPLM maneuver. A: Femoral Blood Flow
980 Haemodynamics (FBF); B: Leg Vascular Conductance Haemodynamics (LVC); C: Peak Δ FBF; D: Peak Δ LVC; E: FBF
981 Area Under the Curve (AUC); F: LVC Area Under the Curve (AUC); *significantly different from CTRL vs DOMS+ST.

982

983 Fig. 4. After performing the exercise-induced muscle damage protocol (1) a state of muscle inflammation was initiated in the
984 non-dominant limb that in turn sensitized muscle nerve afferents within nociceptors gene expression (P2X4) present on the A δ
985 and C-fibers nerve endings, causing delayed onset muscle soreness (DOMS). Furthermore, static stretching protocol (2) was
986 applied to the sensitized muscle to activate the mechanoreflex. These combining effects resulted in an increased activation of
987 the muscle nerve afferents and nociceptors, leading to heightened responses from the cardiovascular centers and concomitant
988 decreased of vagal activity and increase in sympathetic tone, leading to systemic vasoconstriction. The increased activation
989 of the muscle nerve afferents induced a decrease in femoral blood flow at rest (top left) and blunted vasodilation response
990 following sPLM in DOMS+ST condition compared with CTRL.

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Table n.1. Direct and Indirect measurements of DOMS.				
Variable	CTRL	ST	DOMS	DOMS+ST
MVC (N)	686 ± 121	682 ± 130	422 ± 170*	432 ± 197*
PPTS (kg)	6.05 ± 1.30	5.89 ± 1.46	3.99 ± 1.19*	3.92 ± 1.49*
VAS daily activities (mm)	0.41 ± 0.27	0.67 ± 0.51	53.88 ± 28.11*	54.25 ± 27.37*
VAS squat (mm)	0.49 ± 0.36	0.70 ± 0.28	46.50 ± 28.07*	51.00 ± 20.45*

Data are presented as mean ± standard deviation. MVC = Maximal Voluntary Contraction; PPTS = Pain Pressure Thresholds; VAS = Visual Analog Scale; CTRL = Control Condition; DOMS= delayed onset muscle soreness condition; ST= Stretching condition; DOMS +ST = delayed onset muscle soreness with stretching, condition; *p<0.05 respect to CTRL.

Table n.2. Resting peripheral and central haemodynamics with autonomic responses and blood gene expression.

Variable	CTRL	DOMS	ST	DOMS+ST
FBF (ml/min)	316 ± 80	249 ± 135	246 ± 106	198 ± 72 [§]
LVC (ml/min/mmHg)	3.5 ± 1.0	2.6 ± 0.8	2.5 ± 1.1	1.8 ± 0.3 [§]
MAP (mmhg)	92 ± 3	92 ± 4	107 ± 5 ^{§†}	117 ± 3 ^{§†*}
Heart Rate (bpm)	68 ± 3	60 ± 2	81 ± 4 ^{§†}	99 ± 4 ^{§†*}
Cardiac Output (l/min)	5.9 ± 0.6	5.8 ± 0.7	8.0 ± 0.8 [§]	10.7 ± 0.9 ^{§†}
Stroke Volume (ml)	88 ± 7	91 ± 6	99 ± 6 ^{§†}	109 ± 5 ^{§†*}
RMSSD (ms)	52 ± 21	38 ± 25	41 ± 18	31 ± 16 [§]
LF/HF (ms²)	1.7 ± 0.7	2.5 ± 1.2	2.7 ± 1.2	2.9 ± 1.2 [§]
P2X4 (FC)	0.9 ± 0.1	1.4 ± 0.4 [§]	0.8 ± 0.2	1.5 ± 0.6 [§]
ASIC3 (FC)	0.9 ± 0.1	1.4 ± 1.1	0.9 ± 0.2	1.4 ± 0.5
TRPV1 (FC)	0.8 ± 0.1	1.5 ± 0.6	0.9 ± 0.2	1.5 ± 0.4 [§]
IL1β (FC)	0.8 ± 0.2	1.7 ± 0.5 [§]	1.0 ± 0.4	1.6 ± 0.4 [§]
IL10 (FC)	0.9 ± 0.2	1.3 ± 0.8	1.3 ± 0.5	1.3 ± 0.5
RBCW (10¹²cell*L⁻¹)	5.04 ± 2.7	4.7 ± 2.4 ^{§*}	4.98 ± 1.9	4.7 ± 1.8 ^{§*}
WBC (10⁹cell*L⁻¹)	6.8 ± 2.2	6.8 ± 2.4	6.8 ± 2.6	6.8 ± 2.5
PLT (10⁹cell*L⁻¹)	2.8 ± 0.2	2.8 ± 0.4	2.4 ± 0.6	2.5 ± 0.5
LYMPH (10⁹cell*L⁻¹)	2.8 ± 0.1	2.8 ± 0.4	2.4 ± 0.3	2.5 ± 0.5
MONO (10⁹cell*L⁻¹)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
NEU (10⁹cell*L⁻¹)	4.0 ± 0.7	3.9 ± 1.4	4.1 ± 1.1	3.7 ± 1.2

Data are presented as mean ± standard deviation. FBF= Femoral Blood Flow; LVC= Leg Vascular Conductance; bpm= beat per minute; MAP= mean arterial pressure; DOMS= delayed onset muscle soreness condition; ST= Stretching condition; DOMS +ST = delayed onset muscle soreness with stretching, condition; RMSSD = Root mean squared of successive differences; LF/HF = ratio between Low frequency and high frequency of the heart rate variability; P2X4 = purinergic-2X4 receptor; ASIC3 = acid sensing ion channel 3; TRPV1 = transient receptor potential cation channel subfamily V member 1; IL1β = interleukin 1β ; IL10 = interleukin 10; RBC~~W~~ = red blood cell count; WBC = white blood cells counts; PLT = Platelet; LYMPH = lymphocytes; MONO = Monocytes ; NEU = Neutrophil; § p<0.05 respect to CTRL, †p<0.05 respect to DOMS. *p<0.05 compared to ST.

Table n.3. Peripheral and central haemodynamics during sPLM.

Variable	CTRL		DOMS		ST		DOMS+ST	
	Δ Peak	AUC	Δ Peak	AUC	Δ Peak	AUC	Δ Peak	AUC
FBF (ml/min)	802 \pm 250	1322 \pm 377	545 \pm 228	1113 \pm 542	423 \pm 202	956 \pm 327	390 \pm 146 [§]	745 \pm 192 [§]
LVC (ml/min/mmHg)	8.6 \pm 3.6	15 \pm 5.1	6.1 \pm 3.3	11.8 \pm 6.6	4.4 \pm 1.8	9.7 \pm 3.4	2.7 \pm 1.1 [§]	6.9 \pm 1.7 [§]
MAP (mmhg)	-0.2 \pm 0.1	0.1 \pm 0.1	-0.2 \pm 0.1	0.1 \pm 0.2	-0.2 \pm 0.1	0.1 \pm 0.1	-0.2 \pm 0.1	0.1 \pm 0.1
Heart Rate (bpm)	2.0 \pm 0.7	1.0 \pm 0.3	2.1 \pm 0.5	1.0 \pm 0.4	1.9 \pm 1.2	0.9 \pm 0.6	1.8 \pm 1.2	0.9 \pm 0.6
Cardiac Output (l/min)	0.2 \pm 0.1	0.19 \pm 0.04	0.4 \pm 0.2	0.2 \pm 0.1	0.4 \pm 0.2	0.2 \pm 0.1	0.4 \pm 0.2	0.2 \pm 0.1
Stroke Volume (ml)	0.6 \pm 0.2	0.3 \pm 0.1	0.6 \pm 0.3	0.3 \pm 0.1	0.6 \pm 0.3	0.3 \pm 0.2	0.5 \pm 0.4	0.3 \pm 0.2

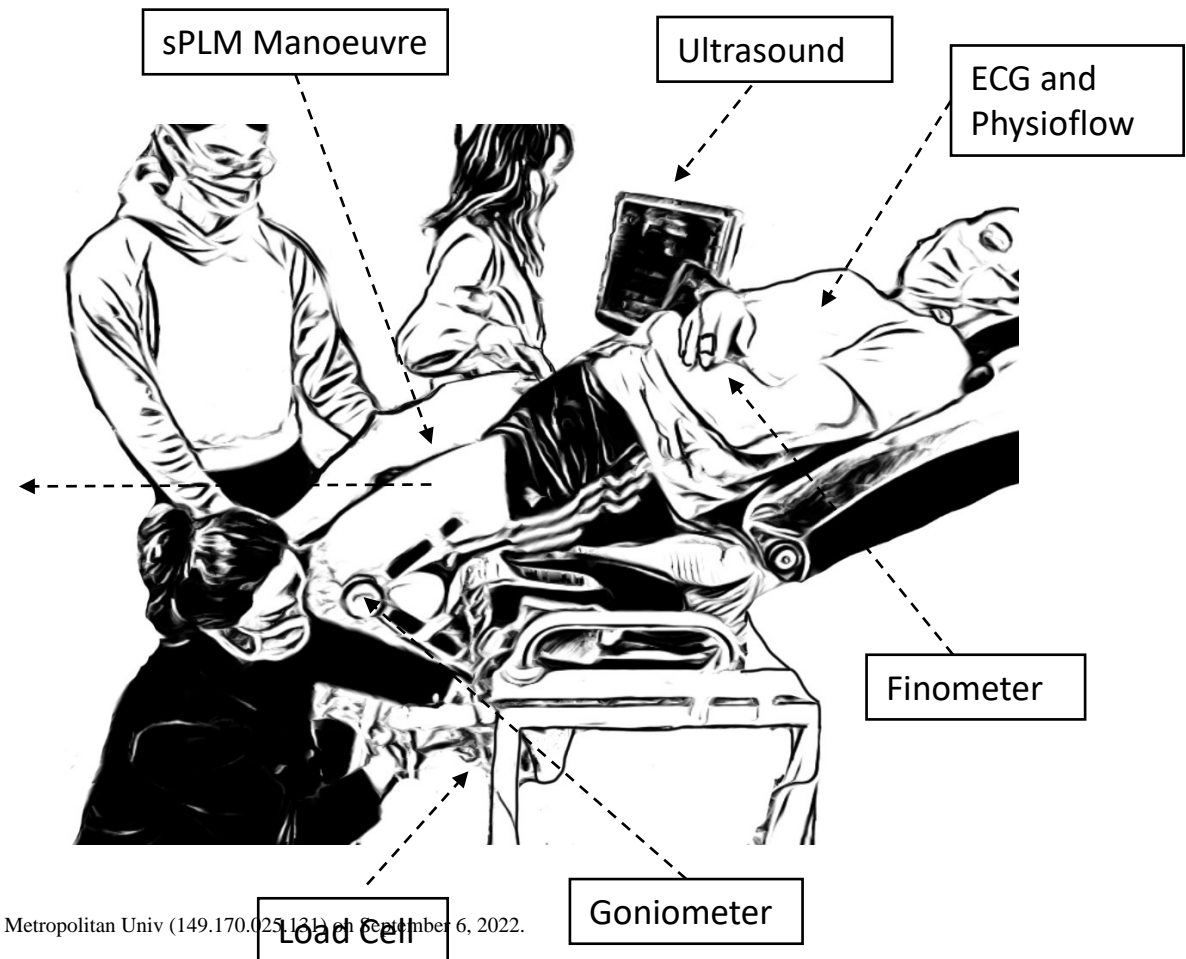
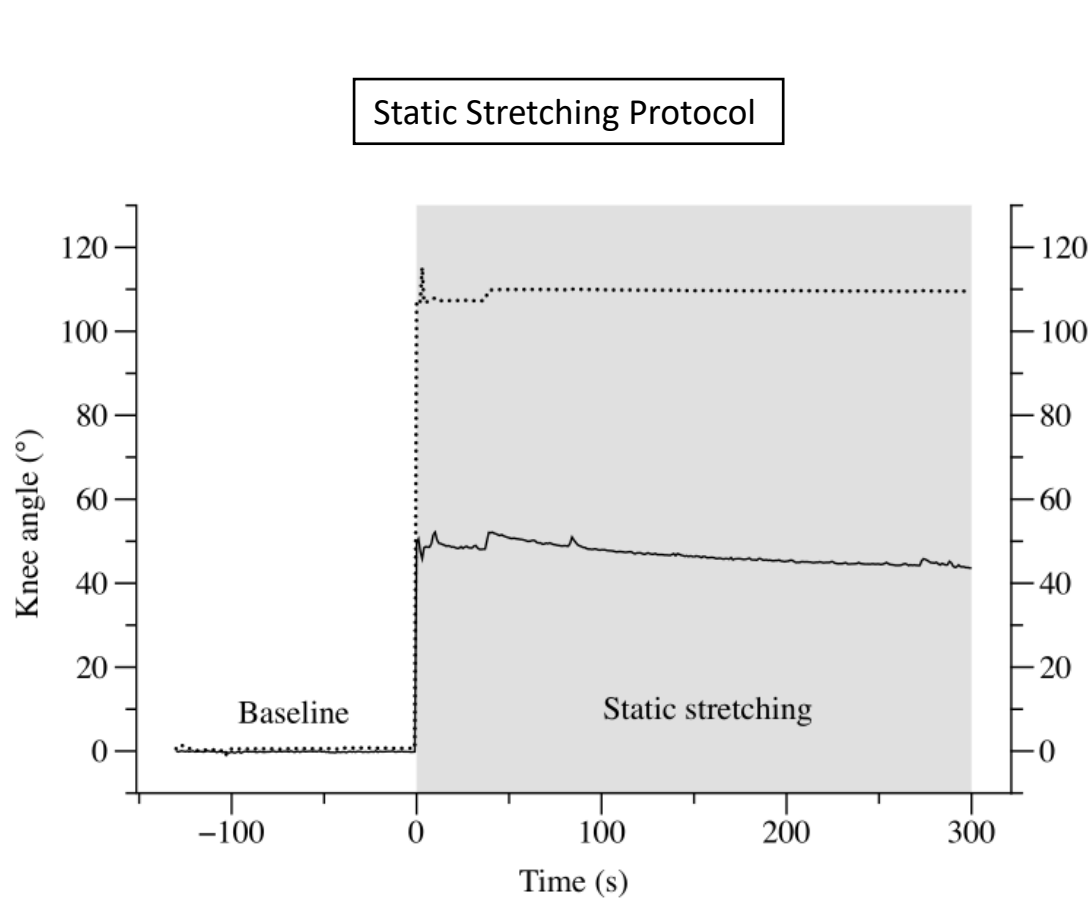
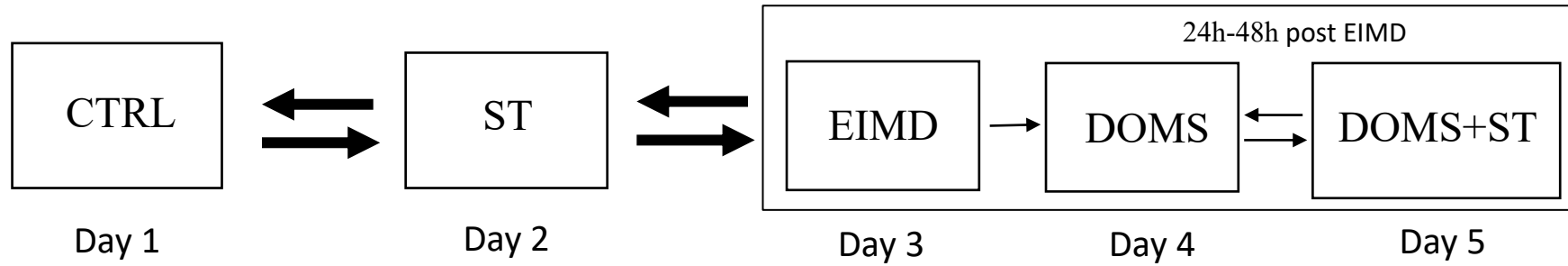
Data are presented as mean \pm standard deviation. FBF = Femoral Blood Flow; LVC = Leg Vascular Conductance; MAP = mean arterial pressure; Δ peak = delta peak; AUC = area under the curve; CTRL = Control Condition; DOMS= delayed onset muscle soreness condition; ST= Stretching condition; DOMS + ST = delayed onset muscle soreness with stretching, condition; \S = p <0.05 respect to CTRL; *= p <0.05 respect to ST.

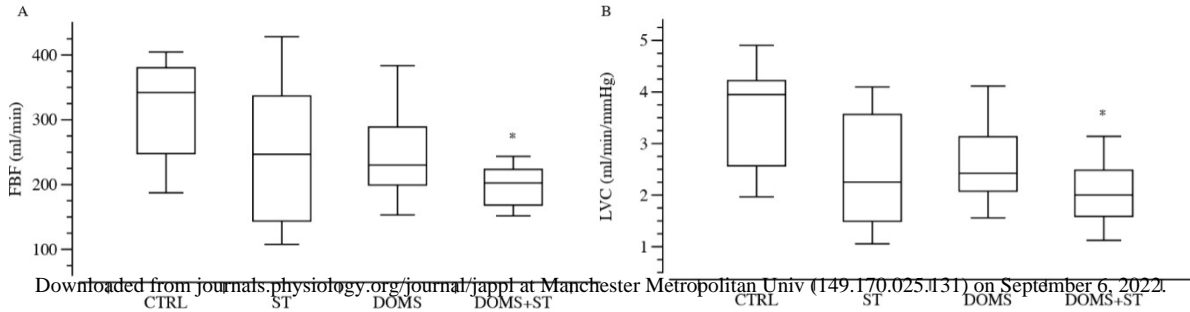
Table n.4. Effects of singular and combined reflex effects

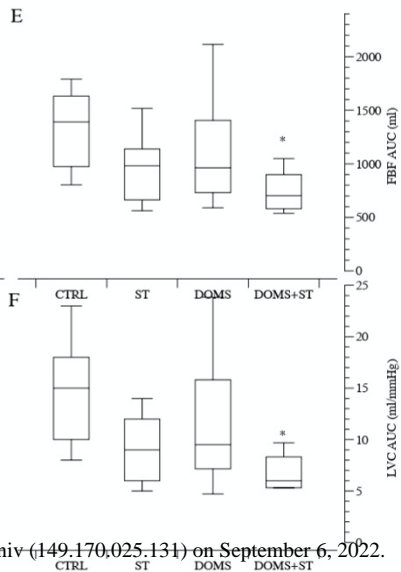
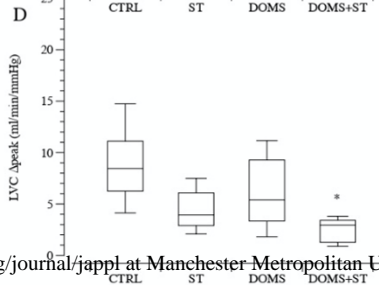
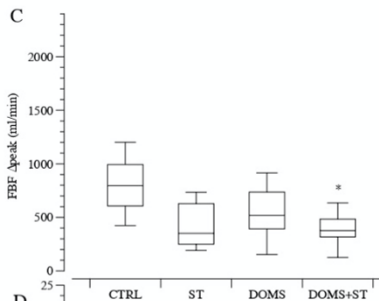
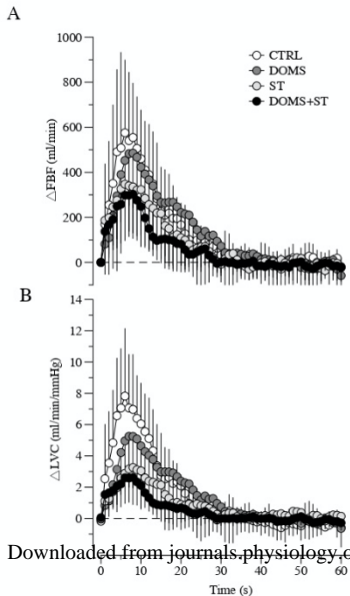
Variable	Δ ST	Δ DOMS	Δ DOMS+ Δ ST	Δ DOMS+ST	Effect
LVC (ml/min/mmHg)	-1.1 \pm 1.3	-0.9 \pm 1.0	-2.0 \pm 2.2	-1.7 \pm 0.9	Additive
FBF (ml/min)	-68 \pm 133	-70 \pm 92	-138 \pm 210	-1.7 \pm 0.9	Additive
MAP (mmhg)	13.5 \pm 1.8	-3.0 \pm 7.4	10.4 \pm 8.8	31.8 \pm 2.9*	Hyper-additive
Heart Rate (bpm)	15.5 \pm 2.4	0.0 \pm 3.6	15.5 \pm 4.4	25.8 \pm 3.6*	Hyper-additive
Cardiac Output (l/min)	-2.17 \pm 0.5	-0.3 \pm 1.0	-2.4 \pm 0.9	-5.0 \pm 0.8*	Hyper-Additive
Stroke Volume (ml)	12.5 \pm 8.7	9.1 \pm 15.1	21.7 \pm 18.3	22.8 \pm 9.0	Additive
RMSSD (ms)	-10.3 \pm 6.0	-13.6 \pm 16.3	-23.8 \pm 17.1	-20.9 \pm 14.1	Additive
LF/HF (ms²)	0.8 \pm 0.8	0.9 \pm 1.5	1.8 \pm 1.4	1.2 \pm 0.9	Additive
FBF Δpeak (ml/min)	-378 \pm 288	-257 \pm 263	-636 \pm 494	-411 \pm 281	Additive
LVC Δpeak (ml/min)	-4.4 \pm 3.2	-2.7 \pm 3.4	-7.1 \pm 5.7	-6.09 \pm 3.64	Additive
MAP Δpeak (mmhg)	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	Additive
Heart Rate Δpeak (bpm)	0.0 \pm 0.5	0.1 \pm 0.9	0.1 \pm 1.2	0.2 \pm 1.0	Additive
Cardiac Output Δpeak (l/min)	0.0 \pm 0.1	0.0 \pm 0.2	0.0 \pm 0.2	0.0 \pm 0.2	Additive
Stroke Volume Δpeak (ml)	0.0 \pm 0.2	0.0 \pm 0.3	0.0 \pm 0.4	0.0 \pm 0.3	Additive
FBF AUC (ml)	-367 \pm 334	-210 \pm 493	-578 \pm 735	-579 \pm 335	Additive
LVC AUC (ml/m)	-5.1 \pm 3.6	-3.1 \pm 5.4	-8.2 \pm 7.7	-7.7 \pm 4.1	Additive
MAP AUC (mmHg)	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	Additive
Heart Rate AUC (bpm)	0.0 \pm 0.5	0.1 \pm 0.4	0.0 \pm 0.6	0.1 \pm 0.5	Additive
Cardiac Output AUC (l)	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	Additive
Stroke Volume AUC (ml)	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	0.0 \pm 0.1	Additive

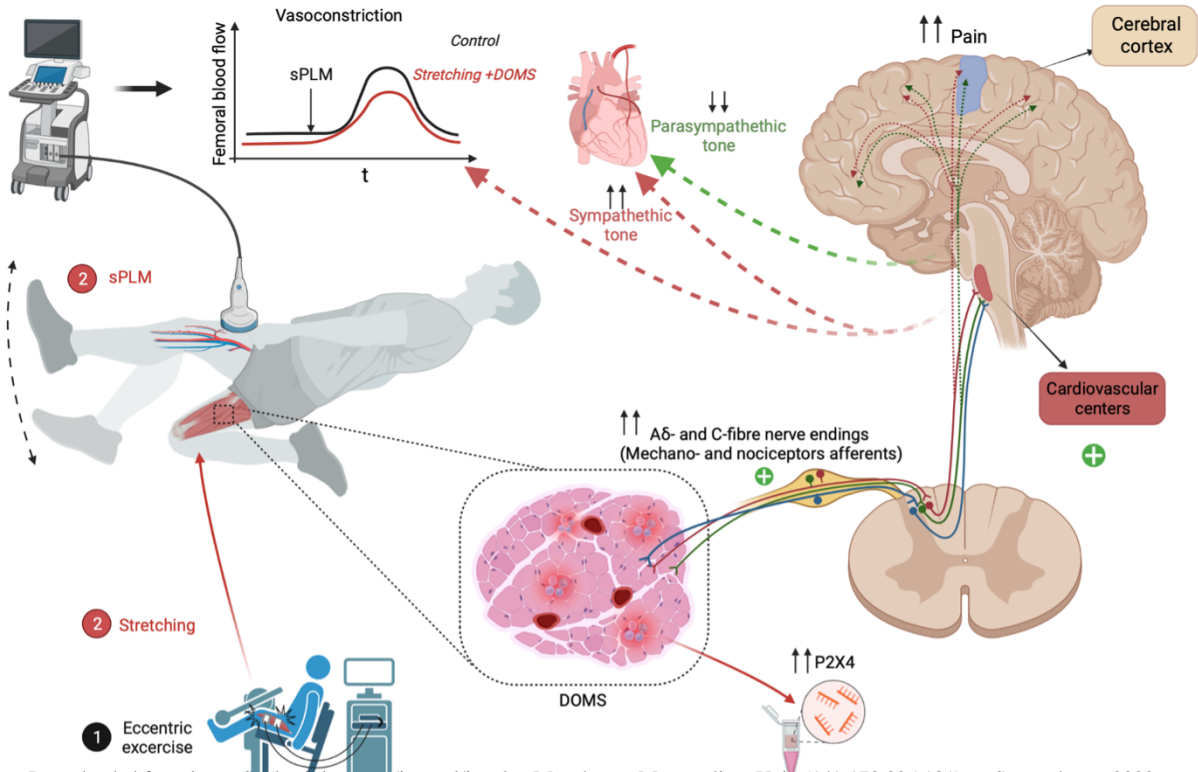
Data are presented as mean \pm standard deviation. FBF= Femoral Blood Flow; LVC= Leg Vascular Conductance; bpm= beat per minute; MAP= mean arterial pressure; AUC = area under the curve; Δ peak = delta peak; Δ DOMS= delta between delayed onset muscle soreness and control conditions; Δ ST= delta between stretching and control conditions; Δ DOMS + Δ ST = delta delayed onset muscle soreness summed with delta stretching; Δ DOMS +ST= delta between DOMS+ST and control conditions; RMSSD = Root mean squared of successive differences; LF/HF = ratio between Low frequency and high frequency of the heart rate variability; *= p <0.05 compared to Δ DOMS+ Δ ST.

Study Design









Effects of nociceptive and mechanosensitive afferents sensitization on central and peripheral haemodynamics following exercise-induced muscle damage.

METHODS

This study aims to test the separated and combined effects of mechanoreflex activation and nociception through exercise-induced muscle damage (EIMD) (1) on central and peripheral haemodynamics at rest and during single passive leg movement (sPLM). (2)

OUTCOMES

The combination of static stretching protocol on the damaged limb (2) extensively increases resting central haemodynamics with reduction in resting limb blood flow and passive leg movement-induced hyperemia.

