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

Fabio Zambolin[1,2], Gaia Giuriato[3], Fabio Giuseppe Laginestra[3,4], Matteo Maria Ottaviani[3,6], Thomas Favaretto[3,5], Elisa Calabria[3], Pablo Duro-Ocana[7,8], Liam Bagley[2,7,8], Azmy Faisal[1,2,9], Tiago Peçanha[1,2], Jamie Stewart McPhee[1,2], Massimo Venturelli[3,4].

[1] Department of Sport and Exercise Sciences. Manchester Metropolitan University, Manchester, UK
[2] Manchester Metropolitan University Institute of Sport. Manchester Metropolitan University, Manchester, UK
[3] Department of Neurosciences, Biomedicine and Movement Sciences. University of Verona. Verona, Italy
[4] Department of Internal Medicine, University of Utah. USA.
[5]Department of Neurosurgery, University Politecnica delle Marche. Ancona, Italy.
[6] Department Medicine, University of Udine, Udine, Italy.
[7] Department of Life Sciences, Manchester Metropolitan University, John Dalton Building, Manchester, UK
[8] Department of Anesthesia, Manchester University NHS Foundation Trust, Manchester, UK
[9] Faculty of Physical Education for Men, Alexandria University, Alexandria, Egypt

Corresponding author

Massimo Venturelli Ph.D.
Associate Professor, Department of Neurosciences, Biomedicine and Movement Sciences. University of Verona.
Via Casorati 43, 37131 Verona, Italy;
e-mail: massimo.venturelli@univr.it

Adjunct Professor, Department of Internal Medicine, University of Utah. USA.

e-mail: massimo.venturelli@utah.edu

OrcID Numbers:

Fabio Zambolin: https://orcid.org/0000-0002-4178-6965

Gaia Giuriato: https://orcid.org/0000-0002-8149-9450

Fabio Giuseppe Laginestra: https://orcid.org/0000-0003-4767-7249

Thomas Favaretto: https://orcid.org/0000-0002-1628-3784

Matteo Maria Ottaviani: https://orcid.org/0000-0002-6354-3278

Elisa Calabria: https://orcid.org/0000-0001-6557-0379

Pablo Duro Ocaña: https://orcid.org/0000-0002-9502-6670

Liam Bagley: https://orcid.org/0000-0001-5538-0870

Azmy Faisal: https://orcid.org/0000-0001-5019-7292

Tiago Peçanha: https://orcid.org/0000-0003-4968-5525

Jamie S. McPhee: https://orcid.org/ 0000-0002-3659-0773

Massimo Venturelli: https://orcid.org/0000-0002-2469-878

Abstract

This study aims to test the separated and combined effects of mechanoreflex activation and nociception through exercise-induced muscle damage (EIMD) on central and peripheral haemodynamics before and during single passive leg movement (sPLM). Eight healthy young males undertook four experimental sessions, in which a sPLM was performed on the dominant limb while in each specific session the contralateral was: a) in a resting condition (CTRL), b) stretched (ST), c) resting after EIMD called delayed-onset-muscle-soreness (DOMS) condition, or d) stretched after EIMD (DOMS+ST). EIMD was used to induce DOMS in the following 24-48h. Femoral blood flow (FBF) was assessed using doppler ultrasound while central haemodynamics were assessed via finger photoplethysmography. Leg vascular conductance (LVC) was calculated as FBF/MAP. RR-interval were analyzed in the time (RMSSD) and frequency domain (LF/HF). Blood samples were collected before each condition and gene expression analysis showed increased fold changes for P2X4 and IL1β in DOMS and DOMS+ST compared with baseline. Resting FBF and LVC were decreased only in the DOMS+ST condition (-26ml/min and -50ml/mmHg/min respectively) with decreased RMSSD and increased LF/HF ratio. MAP, HR, CO, and SV were increased in ST and DOMS+ST compared with CTRL. Marked decreases of delta peaks and AUC for FBF (Δ: -146ml/min and -265ml respectively) and LVC (Δ: -8.66ml/mmHg/min and ±1.7ml/mmHg/min respectively) all $p<.05$. These results suggest that combination of mechanoreflex and nociception resulted in decreased vagal tone and concomitant rise in sympathetic drive that led to increases in resting central hemodynamic with reduce limb blood flow before and during sPLM.

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

Graphic Abstract

NEW & NOTEWORTHY

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

Introduction

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

Methods

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

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

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

Experimental protocol.

Blood sampling

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

Hematological testing

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

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

Total RNA preparation

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

Quantitative real time PCR

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

Primers list:

P2X4

F: TCCGTCTTGGCAAAATAGTG

R: AGGTTGCAGTCCCAGTTGAC

IL1B

F CTGTCCTGCGTGTTGAAAGA
R TGAAGACAAATCGCTTTTCCA

IL10

F: TGCTGGAGGACTTTAAGGGTTA
R: GGGTCTTGGTTCTCAGCTTG

ASIC3

F: TTCTGGAACCGACAGCACTC

R: GAGGGGTGGGAGGTCTGG

TRPV1

F: AACTGGACCACCTGGAACAC
R: GCCTGAAACTCTGCTTGACC

GAPDH-6

F: CAGCCTCAAGATCATCAGCA

R: GTCTTCTGGGTGGCAGTGAT

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

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

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

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

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

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 (Vmean) was measured using the same
314 probe utilizing a frequency of 5 MHz Measurements of Vmean 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 Vmean, femoral blood
317 flow (FBF) was calculated second by second as:
318

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

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 (Finapress 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

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

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

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

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

Statistical Analysis. Normal distribution of the data was assessed with a Shapiro-Wilk test. Student's paired t-test was implemented to determine differences between VAS pain and VAS stretching intensity within stretching measurements (ROM and Force) during ST and DOMS+ST data. One-way repeated measures ANOVA with Tukey-B post-hoc analysis was implemented for gene expressions (P2X4, ASIC3, TRPV1, IL1β, IL10), baselines and sPLM maneuver outcomes for central and peripheral haemodynamics(FBF, MAP, LVC, CO, SV, HR and AUC) within and outcomes of autonomic responses. A Bonferroni-Holm correction was performed for deltas interaction for all peripheral and central haemodynamics outcomes (54). Pearson single correlation analysis were implemented between autonomic responses values of resting and delta peak for FBF and LVC, across all conditions. A sample size of eight participants was selected to ensure a statistical power higher than 0.80 with a type 1 error <0.05 to detect ~15-20% in FBF (main outcome) under stretching conditions (52). All 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 ± 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

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

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

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

Delta Interaction.

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

Single correlation analysis.

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

Discussion

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

We found that only the combination of mechanoreflex and nociceptor activation promotes greater changes on central and peripheral haemodynamics at rest with reduced vagal activity (reduced RMSSD) and concomitant increases of sympathetic drive (increased LF/HF ratio) (Fig 2; Table 2), that inversely and positively correlate with HR, CO, and MAP respectively. Moreover, when both the mechanoreflex and nociceptors were activated, the contralateral leg exhibited a reduction in leg blood flow and vascular responsiveness to sPLM in remote muscles (Fig 3). These results, suggest that the stimulation of mechano- and nociceptive afferents trigger increases in central haemodynamics mediated by vagal tone suppression and increase in sympathetic drive (hyper-additive and additive effects). The sympathetic gain is also responsible, at least in part, for the reduction in resting limb blood flow and decreased vascular responsiveness with additive effects (Fig 4). Interestingly, the singular stimulation of the mechano- and nociceptive afferents via passive static stretching of the skeletal muscle or the DOMS resulted in negligible changes of the peripheral circulation. Furthermore, gene associated with pain and mechanoreceptors activity (P2X4, TRPV1), with marker of increases inflammation (IL1β), increases following EIMD suggesting a possible mechano- and nociceptive sensitization of nerve endings afferents. Therefore, in agreement with our initial hypothesis, only the sensitization of both mechano- and nociceptors resulted in autonomic-mediated increase in central haemodynamics and a concurrent reduction of peripheral circulation and vascular responsiveness in remote skeletal muscles.

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

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

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

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

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

IL1β levels. Our findings are in line with Caldwell et al. (26) who found that systemic vascular function measured with FMD, was not affected following EIMD in a remote healthy muscle. However, in some studies was found a close relationship between pro-inflammatory cytokines and decreased vascular function (60-62). Moreover, studies on healthy volunteers following vaccined-induced inflammation found decrease vascular function (decreased FMD and increased arterial stiffness) (63, 64) while in aging population, this effect was not appreciable (65). The discrepancies between these studies have also been attributed to different level of systemic inflammation following influenza-vaccine administration (65). This could have been the case in our current study where the level of inflammation following EIMD may have not been sufficient to cause a level of systemic inflammation necessary to induce systemic vascular function impairment (26). Unfortunately, we can not completely rule out the effect of inflammation to systemic vascular function impairments, so future studies in healthy humans should more extensively monitor inflammation (i.e., TNF-α activity) during DOMS, to elucidate this relationship. Looking at the stretching condition, no significant changes in peripheral haemodynamics were found although an overall reduction in FBF and LVC compared with CTRL. Reduction of peripheral haemodynamics was previously found in the contralateral (resting) leg following static stretching protocol, suggesting an increased sympathetic-mediated vasoconstriction from activation of mechanoreceptors as a potential mechanism for this attenuation (52). The entity of these changes however may differ between the current study due to the distinct stretching protocol implemented, which may have led to different mechanoreflex activation and peripheral haemodynamics changes. Interestingly, one of the major findings of the current study is a decreased FBF and LVC at rest in DOMS+ST conditions. In fact, the singular and distinctive effect of mechanoreflex (ST condition) or nociceptive (DOMS condition) reflexes stimulation seems to not be sufficient to alter peripheral haemodynamics

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

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

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

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

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

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

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

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

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

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

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

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

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

Implication for research and clinical practice.

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

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

Conclusions

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

Limitations:

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

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

Contributions

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

Acknowledgements

We would also like to thank all participants that took part in this project with a special thanks to L. Sacco, D. Sartorio, O. Modica and E. Pujatti for their contribution in the project, the technical support during the data collection and help during the experimental set up.

Fundings:

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 801604 for F.Z. and P.D.O. This project was also partially supported by the Brain Research Foundation Verona Onlus and by the Italian Ministry of Research and University (MIUR) 5-year special funding to strengthen and enhance excellence in research and teaching (https://www.miur.gov.it/dipartimenti-di-eccellenza).

References:

1. **McCloskey DI, and Mitchell JH**. Reflex cardiovascular and respiratory responses originating in exercising muscle. *The Journal of Physiology* 224: 173-186, 1972.
2. **Amann M**. Significance of Group III and IV muscle afferents for the endurance exercising human. *Clinical and Experimental Pharmacology and Physiology* 39: 831-835, 2012.
3. **Amann M, Wan H-Y, Thurston TS, Georgescu VP, and Weavil JC**. On the Influence of Group III/IV Muscle Afferent Feedback on Endurance Exercise Performance. *Exercise and Sport Sciences Reviews* 48: 209-216, 2020.
4. **Jankowski MP, Rau KK, Ekmann KM, Anderson CE, and Koerber HR**. Comprehensive phenotyping of group III and IV muscle afferents in mouse. *Journal Of Neurophysiology* 109: 2374-2381, 2013.
5. **Mense S**. Nervous outflow from skeletal muscle following chemical noxious stimulation. *The Journal of physiology* 267: 75-88, 1977.
6. **Peake JM, Neubauer O, Della Gatta PA, and Nosaka K**. Muscle damage and inflammation during recovery from exercise. *J Appl Physiol (1985)* 122: 559-570, 2017.
7. **Rotto DM, Schultz HD, Longhurst JC, and Kaufman MP**. Sensitization of group III muscle afferents to static contraction by arachidonic acid. *J Appl Physiol (1985)* 68: 861-867, 1990.
8. **Pollak KA, Swenson JD, Vanhaitsma TA, Hughen RW, Jo D, White AT, Light KC, Schweinhardt P, Amann M, and Light AR**. Exogenously applied muscle metabolites synergistically evoke sensations of muscle fatigue and pain in human subjects. *Exp Physiol* 99: 368-380, 2014.
9. **Fujii Y, Ozaki N, Taguchi T, Mizumura K, Furukawa K, and Sugiura Y**. TRP channels and ASICs mediate mechanical hyperalgesia in models of inflammatory muscle pain and delayed onset muscle soreness. *Pain* 140: 292-304, 2008.
10. **Ota H, Katanosaka K, Murase S, Kashio M, Tominaga M, and Mizumura K**. TRPV1 and TRPV4 play pivotal roles in delayed onset muscle soreness. *PLoS One* 8: e65751, 2013.
11. **Matsubara T, Hayashi K, Wakatsuki K, Abe M, Ozaki N, Yamanaka A, Mizumura K, and Taguchi T**. Thin-fibre receptors expressing acid-sensing ion channel 3 contribute to muscular mechanical hypersensitivity after exercise. *European journal of pain (London, England)* 23: 1801-1813, 2019.
12. **Graven-Nielsen T, and Arendt-Nielsen L**. Assessment of mechanisms in localized and widespread musculoskeletal pain. *Nat Rev Rheumatol* 6: 599-606, 2010.

13. **Courtney CA, Aoyagi K, Fernández-de-Las-Peñas C, and Madeleine P**. BILATERAL SENSORY DEFICITS AND WIDESPREAD HYPERALGESIA OCCUR FOLLOWING INDUCED DELAYED ONSET MUSCLE SORENESS OF THE QUADRICEPS. *Int J Sports Phys Ther* 15: 12-21, 2020.
14. **Queme F, Taguchi T, Mizumura K, and Graven-Nielsen T**. Muscular Heat and Mechanical Pain Sensitivity After Lengthening Contractions in Humans and Animals. *The Journal of Pain* 14: 1425-1436, 2013.
15. **Mizumura K, and Taguchi T**. Delayed onset muscle soreness: Involvement of neurotrophic factors. *The Journal of Physiological Sciences* 66: 43-52, 2016.
16. **Light AR, Hughen RW, Zhang J, Rainier J, Liu Z, and Lee J**. Dorsal Root Ganglion Neurons Innervating Skeletal Muscle Respond to Physiological Combinations of Protons, ATP, and Lactate Mediated by ASIC, P2X, and TRPV1. *Journal of Neurophysiology* 100: 1184-1201, 2008.
17. **Mense S**. Functional Anatomy of Muscle: Muscle, Nociceptors and Afferent Fibers. In: *Muscle Pain: Understanding the Mechanisms*, edited by Mense S, and Gerwin RD. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010, p. 17-48.
18. **Light AR, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, and Light KC**. Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. *J Intern Med* 271: 64-81, 2012.
19. **Light AR, White AT, Hughen RW, and Light KC**. Moderate exercise increases expression for sensory, adrenergic, and immune genes in chronic fatigue syndrome patients but not in normal subjects. *J Pain* 10: 1099-1112, 2009.
20. **Antunes-Correa LM, Nobre TS, Groehs RV, Alves MJ, Fernandes T, Couto GK, Rondon MU, Oliveira P, Lima M, Mathias W, Brum PC, Mady C, Almeida DR, Rossoni LV, Oliveira EM, Middlekauff HR, and Negrao CE**. Molecular basis for the improvement in muscle metaboreflex and mechanoreflex control in exercise-trained humans with chronic heart failure. *Am J Physiol Heart Circ Physiol* 307: H1655-1666, 2014.
21. **Wang H-J, Li Y-L, Gao L, Zucker IH, and Wang W**. Alteration in skeletal muscle afferents in rats with chronic heart failure. *The Journal of physiology* 588: 5033-5047, 2010.
22. **Fleckenstein J, Neuberger EWI, Bormuth P, Comes F, Schneider A, Banzer W, Fischer L, and Simon P**. Investigation of the Sympathetic Regulation in Delayed Onset Muscle Soreness: Results of an RCT. *Front Physiol* 12: 697335, 2021.
23. **Ray CA, Mahoney ET, and Hume KM**. Exercise-induced muscle injury augments forearm vascular resistance during leg exercise. *Am J Physiol* 275: H443-447, 1998.
24. **Barnes JN, Trombold JR, Dhindsa M, Lin H-F, and Tanaka H**. Arterial stiffening following eccentric exercise-induced muscle damage. *Journal of Applied Physiology* 109: 1102-1108, 2010.
25. **Stacy MR, Bladon KJ, Lawrence JL, McGlinchy SA, and Scheuermann BW**. Serial assessment of local peripheral vascular function after eccentric exercise. *Appl Physiol Nutr Metab* 38: 1181-1186, 2013.
26. **Caldwell JT, Wardlow GC, Branch PA, Ramos M, Black CD, and Ade CJ**. Effect of exercise-induced muscle damage on vascular function and skeletal muscle microvascular deoxygenation. *Physiological Reports* 4: e13032, 2016.
27. **Kruse NT, and Scheuermann BW**. Cardiovascular Responses to Skeletal Muscle Stretching: "Stretching" the Truth or a New Exercise Paradigm for Cardiovascular Medicine? *Sports Medicine* 47: 2507-2520, 2017.
28. **Nakamura N, Ikeda N, Heng P, and Muraoka I**. Muscle stiffening is associated with muscle mechanoreflex-mediated cardioacceleration. *European Journal of Applied Physiology* 122: 781-790, 2022.
29. **Venturelli M, Rampichini S, Coratella G, Limonta E, Bisconti AV, Cè E, and Esposito F**. Heart and musculoskeletal hemodynamic responses to repetitive bouts of quadriceps static stretching. *J Appl Physiol (1985)* 127: 376-384, 2019.

30. **Venturelli M, Ce E, Limonta E, Bisconti AV, Devoto M, Rampichini S, and Esposito F**. Central and peripheral responses to static and dynamic stretch of skeletal muscle: mechano- and metaboreflex implications. *J Appl Physiol (1985)* 122: 112-120, 2017.
31. **Zimmermann K, Leidl C, Kaschka M, Carr RW, Terekhin P, Handwerker HO, and Forster C**. Central projection of pain arising from delayed onset muscle soreness (DOMS) in human subjects. *PLoS One* 7: e47230, 2012.
32. **Ives SJ, Amann M, Venturelli M, Witman MAH, Groot HJ, Wray DW, Morgan DE, Stehlik J, and Richardson RS**. The Mechanoreflex and Hemodynamic Response to Passive Leg Movement in Heart Failure. *Medicine & Science in Sports & Exercise* 48: 2016.
33. **Witman MAH, Ives SJ, Trinity JD, Groot HJ, Stehlik J, and Richardson RS**. Heart failure and movement-induced hemodynamics: partitioning the impact of central and peripheral dysfunction. *International journal of cardiology* 178: 232-238, 2015.
34. **Angius L, and Crisafulli A**. Exercise intolerance and fatigue in chronic heart failure: is there a role for group III/IV afferent feedback? *Eur J Prev Cardiol* 27: 1862-1872, 2020.
35. **Smith SA, Mitchell JH, and Garry MG**. The mammalian exercise pressor reflex in health and disease. *Exp Physiol* 91: 89-102, 2006.
36. **Grayston R, Czanner G, Elhadd K, Goebel A, Frank B, Üçeyler N, Malik RA, and Alam U**. A systematic review and meta-analysis of the prevalence of small fiber pathology in fibromyalgia: Implications for a new paradigm in fibromyalgia etiopathogenesis. *Seminars in Arthritis and Rheumatism* 48: 933-940, 2019.
37. **Serra J, Collado A, Solà R, Antonelli F, Torres X, Salgueiro M, Quiles C, and Bostock H**. Hyperexcitable C nociceptors in fibromyalgia. *Annals of Neurology* 75: 196-208, 2014.
38. **Staud R, Weyl EE, Price DD, and Robinson ME**. Mechanical and heat hyperalgesia highly predict clinical pain intensity in patients with chronic musculoskeletal pain syndromes. *The journal of pain* 13: 725-735, 2012.
39. **Peçanha T, Meireles K, Pinto AJ, Rezende DAN, Iraha AY, Mazzolani BC, Smaira FI, Sales ARK, Bonfiglioli K, Sá-Pinto ALd, Lima FR, Irigoyen MC, Gualano B, and Roschel H**. Increased sympathetic and haemodynamic responses to exercise and muscle metaboreflex activation in post-menopausal women with rheumatoid arthritis. *The Journal of physiology* 599: 927-941, 2021.
40. **Calabria E, Mazza EMC, Dyar KA, Pogliaghi S, Bruseghini P, Morandi C, Salvagno GL, Gelati M, Guidi GC, Bicciato S, Schiaffino S, Schena F, and Capelli C**. Aging: a portrait from gene expression profile in blood cells. *Aging* 8: 1802-1821, 2016.
41. **Karcioglu O, Topacoglu H, Dikme O, and Dikme O**. A systematic review of the pain scales in adults: Which to use? *The American Journal of Emergency Medicine* 36: 707-714, 2018.
42. **Kruse NT, Silette CR, and Scheuermann BW**. Influence of passive stretch on muscle blood flow, oxygenation and central cardiovascular responses in healthy young males. *American Journal of Physiology-Heart and Circulatory Physiology* 310: H1210-H1221, 2016.
43. **Byrne C, Twist C, and Eston R**. Neuromuscular Function After Exercise-Induced Muscle Damage. *Sports Medicine* 34: 49-69, 2004.
44. **Hicks KM, Onambélé GL, Winwood K, and Morse CI**. Muscle Damage following Maximal Eccentric Knee Extensions in Males and Females. *PLOS ONE* 11: e0150848, 2016.
45. **Warren GL, Lowe DA, and Armstrong RB**. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43-59, 1999.
46. **da Silva W, Machado ÁS, Lemos AL, de Andrade CF, Priego-Quesada JI, and Carpes FP**. Relationship between exercise-induced muscle soreness, pain thresholds, and skin temperature in men and women. *Journal of Thermal Biology* 100: 103051, 2021.
47. **Baker SJ, Kelly NM, and Eston RG**. Pressure pain tolerance at different sites on the quadriceps femoris prior to and following eccentric exercise. *European Journal of Pain* 1: 229-233, 1997.

48. **Burt D, Lamb K, Nicholas C, and Twist C**. Effects of muscle-damaging exercise on physiological, metabolic, and perceptual responses during two modes of endurance exercise. *Journal of Exercise Science & Fitness* 10: 70-77, 2012.
49. **Szczyglowski MK, Ade CJ, Campbell JA, and Black CD**. The effects of exercise-induced muscle damage on critical torque. *Eur J Appl Physiol* 117: 2225-2236, 2017.
50. **Venturelli M, Layec G, Trinity J, Hart CR, Broxterman RM, and Richardson RS**. Single passive leg movement-induced hyperemia: a simple vascular function assessment without a chronotropic response. *J Appl Physiol (1985)* 122: 28-37, 2017.
51. **Broxterman RM, Trinity JD, Gifford JR, Kwon OS, Kithas AC, Hydren JR, Nelson AD, Morgan DE, Jessop JE, Bledsoe AD, and Richardson RS**. Single passive leg movement assessment of vascular function: contribution of nitric oxide. *Journal of Applied Physiology* 123: 1468-1476, 2017.
52. **Venturelli M, Rampichini S, Coratella G, Limonta E, Bisconti AV, Ce E, and Esposito F**. Heart and musculoskeletal hemodynamic responses to repetitive bouts of quadriceps static stretching. *J Appl Physiol (1985)* 127: 376-384, 2019.
53. **Richard R, Lonsdorfer-Wolf E, Charloux A, Doutreleau S, Buchheit M, Oswald-Mammosser M, Lampert E, Mettauer B, Geny B, and Lonsdorfer J**. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *European Journal of Applied Physiology* 85: 202-207, 2001.
54. **Wan H-Y, Weavil JC, Thurston TS, Georgescu VP, Hureau TJ, Bledsoe AD, Buys MJ, Jessop JE, Richardson RS, and Amann M**. The exercise pressor reflex and chemoreflex interaction: cardiovascular implications for the exercising human. *The Journal of physiology* 598: 2311-2321, 2020.
55. **Force T**. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *European heart journal* 17: 354-381, 1996.
56. **Shaffer F, and Ginsberg JP**. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health* 5: 258-258, 2017.
57. **Weerakkody NS, Whitehead NP, Canny BJ, Gregory JE, and Proske U**. Large-fiber mechanoreceptors contribute to muscle soreness after eccentric exercise. *The Journal of Pain* 2: 209-219, 2001.
58. **Oliveira-Fusaro MC, Gregory NS, Kolker SJ, Rasmussen L, Allen LH, and Sluka KA**. P2X4 Receptors on Muscle Macrophages Are Required for Development of Hyperalgesia in an Animal Model of Activity-Induced Muscle Pain. *Mol Neurobiol* 57: 1917-1929, 2020.
59. **Kano Y, Padilla DJ, Behnke BJ, Hageman KS, Musch TI, and Poole DC**. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. *J Appl Physiol (1985)* 99: 1516-1522, 2005.
60. **Wimalasundera R, Fexby S, Regan L, Thom SA, and Hughes AD**. Effect of tumour necrosis factor-alpha and interleukin 1beta on endothelium-dependent relaxation in rat mesenteric resistance arteries in vitro. *Br J Pharmacol* 138: 1285-1294, 2003.
61. **Sprague AH, and Khalil RA**. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol* 78: 539-552, 2009.
62. **Palma CD, Meacci E, Perrotta C, Bruni P, and Clementi E**. Endothelial Nitric Oxide Synthase Activation by Tumor Necrosis Factor α Through Neutral Sphingomyelinase 2, Sphingosine Kinase 1, and Sphingosine 1 Phosphate Receptors. *Arteriosclerosis, Thrombosis, and Vascular Biology* 26: 99-105, 2006.
63. **Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, Donald AE, Palacios M, Griffin GE, Deanfield JE, MacAllister RJ, and Vallance P**. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 102: 994-999, 2000.
64. **Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, Toutouza M, and Stefanadis C**. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation* 112: 2193-2200, 2005.

65. **Ranadive SM, Kappus RM, Cook MD, Yan H, Lane AD, Woods JA, Wilund KR, Iwamoto G, Vanar V, Tandon R, and Fernhall B**. Effect of acute moderate exercise on induced inflammation and arterial function in older adults. *Experimental Physiology* 99: 729-739, 2014.
66. **Premkumar LS, and Raisinghani M**. Nociceptors in cardiovascular functions: complex interplay as a result of cyclooxygenase inhibition. *Mol Pain* 2: 26-26, 2006.
67. **Yang A, Sonin D, Jones L, Barry WH, and Liang BT**. A beneficial role of cardiac P2X4 receptors in heart failure: rescue of the calsequestrin overexpression model of cardiomyopathy. *American journal of physiology Heart and circulatory physiology* 287: H1096-1103, 2004.
68. **Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA, and Elde R**. Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. *Proceedings of the National Academy of Sciences* 93: 8063-8067, 1996.
69. **Miles MP, Li Y, Rinard JP, Clarkson PM, and Williamson JW**. Eccentric exercise augments the cardiovascular response to static exercise. *Med Sci Sports Exerc* 29: 457-466, 1997.
70. **Hotta N, Sato K, Sun Z, Katayama K, Akima H, Kondo T, and Ishida K**. Ventilatory and circulatory responses at the onset of exercise after eccentric exercise. *Eur J Appl Physiol* 97: 598-606, 2006.
71. **Gladwell VF, and Coote JH**. Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *The Journal of Physiology* 540: 1095-1102, 2002.
72. **Drew RC, Bell MPD, and White MJ**. Modulation of spontaneous baroreflex control of heart rate and indexes of vagal tone by passive calf muscle stretch during graded metaboreflex activation in humans. *Journal of Applied Physiology* 104: 716-723, 2008.
73. **Welsh DG, and Segal SS**. Muscle length directs sympathetic nerve activity and vasomotor tone in resistance vessels of hamster retractor. *Circ Res* 79: 551-559, 1996.
74. **Haug SJ, Welsh DG, and Segal SS**. Sympathetic nerves inhibit conducted vasodilatation along feed arteries during passive stretch of hamster skeletal muscle. *J Physiol* 552: 273-282, 2003.
75. **McDaniel J, Ives SJ, and Richardson RS**. Human muscle length-dependent changes in blood flow. *Journal of Applied Physiology* 112: 560-565, 2012.
76. **Venturelli M, Rossman MJ, Ives SJ, Weavil JC, Amann M, Wray DW, and Richardson RS**. Passive leg movement-induced vasodilation and exercise-induced sympathetic vasoconstriction. *Autonomic Neuroscience* 239: 102969, 2022.
77. **Atkinson CL, Lewis NC, Carter HH, Thijssen DH, Ainslie PN, and Green DJ**. Impact of sympathetic nervous system activity on post-exercise flow-mediated dilatation in humans. *The Journal of physiology* 593: 5145-5156, 2015.
78. **Cui J, Blaha C, Moradkhan R, Gray KS, and Sinoway LI**. Muscle sympathetic nerve activity responses to dynamic passive muscle stretch in humans. *The Journal of Physiology* 576: 625-634, 2006.
79. **Venturelli M, Cè E, Limonta E, Bisconti AV, Devoto M, Rampichini S, and Esposito F**. Central and peripheral responses to static and dynamic stretch of skeletal muscle: mechano- and metaboreflex implications. *J Appl Physiol (1985)* 122: 112-120, 2017.
80. **Farinatti PTV, Brandão C, Soares PPS, and Duarte AFA**. Acute Effects of Stretching Exercise on the Heart Rate Variability in Subjects With Low Flexibility Levels. *The Journal of Strength & Conditioning Research* 25: 2011.
81. **Shaffer F, McCraty R, and Zerr CL**. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology* 5: 2014.
82. **Hody S, Croisier J-L, Bury T, Rogister B, and Leprince P**. Eccentric Muscle Contractions: Risks and Benefits. *Frontiers in Physiology* 10: 2019.
83. **Harrison NA, Cooper E, Voon V, Miles K, and Critchley HD**. Central autonomic network mediates cardiovascular responses to acute inflammation: relevance to increased cardiovascular risk in depression? *Brain, behavior, and immunity* 31: 189-196, 2013.

84. **Billman G**. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Frontiers in Physiology* 4: 2013.
85. **Hotta N, and Ishida K**. Mechanism and implications of hyperpnea exaggeration at the onset of exercise in mechanical hyperalgesia after eccentric exercise. *The Journal of Physical Fitness and Sports Medicine* 7: 161-170, 2018.
86. **Cook DB, VanRiper S, Dougherty RJ, Lindheimer JB, Falvo MJ, Chen Y, Lin J-MS, and Unger ER**. Cardiopulmonary, metabolic, and perceptual responses during exercise in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): A Multi-site Clinical Assessment of ME/CFS (MCAM) sub-study. *PloS one* 17: e0265315, 2022.
87. **Amann M, Proctor LT, Sebranek JJ, Eldridge MW, Pegelow DF, and Dempsey JA**. Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *Journal of applied physiology (Bethesda, Md : 1985)* 105: 1714-1724, 2008.
88. **Smith SA, Mitchell JH, Naseem RH, and Garry MG**. Mechanoreflex mediates the exaggerated exercise pressor reflex in heart failure. *Circulation* 112: 2293-2300, 2005.
89. **Peçanha T, Meireles K, Pinto AJ, Rezende DAN, Iraha AY, Sales ARK, Bonfiglioli KR, de Sá-Pinto AL, Lima FR, Irigoyen MC, Gualano B, and Roschel H**. Sympathetic Overactivity and Increased Cardiovascular Responses to Muscle Metaboreflex Activation in Post-menopausal Women with Rheumatoid Arthritis. *The FASEB Journal* 33: 696.613-696.613, 2019.

Figures legend

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

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

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

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

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 $^{\S\dagger *}$
Heart Rate (bpm)	68 ± 3	60 ± 2	81 ± 4 §†	99 ± 4 $^{\S\dagger *}$
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$^{\S\dagger *}$
RMSSD (ms)	52 ± 21	38 ± 25	41 ± 18	31 ± 16§
LF/HF (ms^2)	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
RBC (10^{12}cell*L^{-1})	5.04 ± 2.7	4.7 ± 2.4$^{\S *}$	4.98 ± 1.9	4.7 ± 1.8$^{\S *}$
WBC (10^{9}cell*L^{-1})	6.8 ± 2.2	6.8 ± 2.4	6.8 ± 2.6	6.8 ± 2.5
PLT (10^{9}cell*L^{-1})	2.8 ± 0.2	2.8 ± 0.4	2.4 ± 0.6	2.5 ± 0.5
LYMPH (10^{9}cell*L^{-1})	2.8 ± 0.1	2.8 ± 0.4	2.4 ± 0.3	2.5 ± 0.5
MONO (10^{9}cell*L^{-1})	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
NEU (10^{9}cell*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 = 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 ± 250	1322 ±377	545 ± 228	1113 ± 542	423 ± 202	956 ± 327	390 ± 146§	745 ± 192§
LVC (ml/min/mmHg)	8.6 ± 3.6	15 ± 5.1	6.1 ± 3.3	11.8 ± 6.6	4.4 ± 1.8	9.7 ± 3.4	2.7 ± 1.1§	6.9 ± 1.7§
MAP (mmhg)	-0.2 ± 0.1	0.1 ± 0.1	-0.2 ± 0.1	0.1 ± 0.2	-0.2 ± 0.1	0.1 ± 0.1	-0.2 ± 0.1	0.1 ± 0.1
Heart Rate (bpm)	2.0 ± 0.7	1.0 ± 0.3	2.1 ± 0.5	1.0 ± 0.4	1.9 ±1.2	0.9 ± 0.6	1.8 ±1.2	0.9 ± 0.6
Cardiac Output (l/min)	0.2 ± 0.1	0.19 ± 0.04	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1
Stroke Volume (ml)	0.6 ± 0.2	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	0.3 ± 0.2

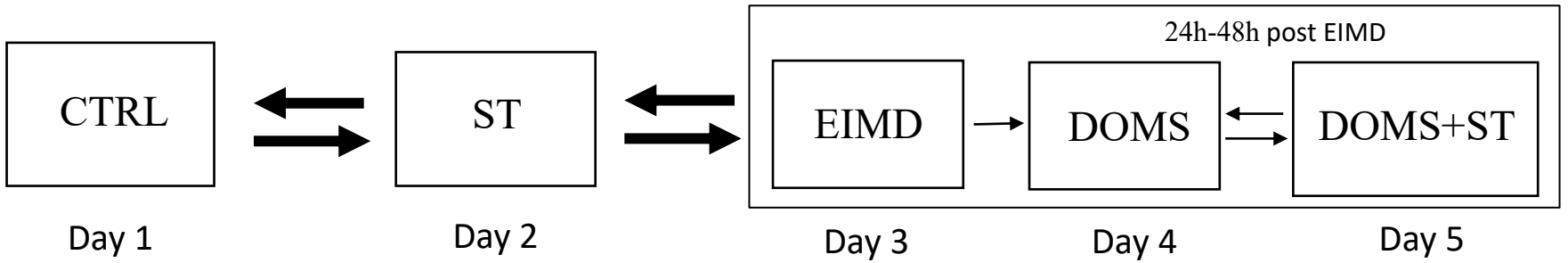
Data are presented as mean ± standard deviation. FBF = Femoral Blood Flow; LVC = Leg Vascular Conductance; MAP = mean arterial pressure; Δpeak = delta peak; AUC = area under the curve; CTRL = Control Condition; DOMS= delayed onset muscle soreness condition; ST= Stretching condition; DOMS + ST = delayed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

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

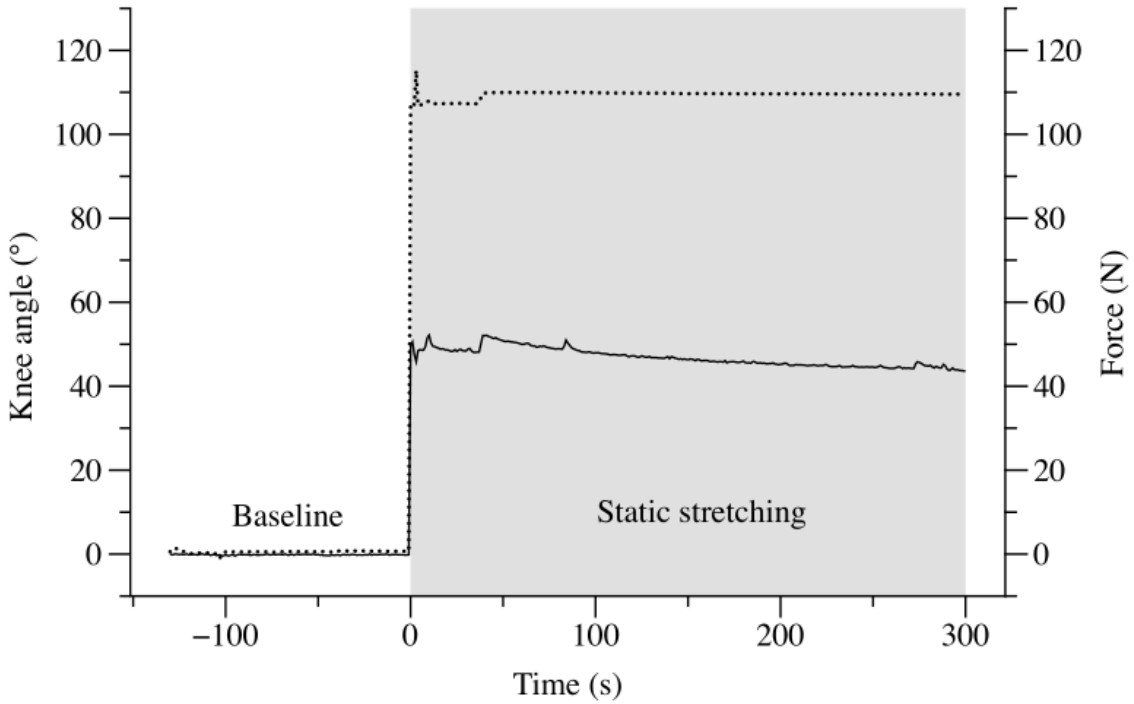
Variable	ΔST	ΔDOMS	ΔDOMS+ ΔST	ΔDOMS+ST	Effect
LVC (ml/min/mmHg)	-1.1 ± 1.3	-0.9 ± 1.0	-2.0 ± 2.2	-1.7 ± 0.9	Additive
FBF (ml/min)	-68 ± 133	-70 ± 92	-138 ± 210	-1.7 ± 0.9	Additive
MAP (mmhg)	13.5 ± 1.8	-3.0 ± 7.4	10.4 ± 8.8	31.8 ± 2.9*	Hyper-additive
Heart Rate (bpm)	15.5 ± 2.4	0.0 ± 3.6	15.5 ± 4.4	25.8 ± 3.6*	Hyper-additive
Cardiac Output (l/min)	-2.17 ± 0.5	-0.3 ± 1.0	-2.4 ± 0.9	-5.0± 0.8*	Hyper-Additive
Stroke Volume (ml)	12.5 ± 8.7	9.1 ± 15.1	21.7 ± 18.3	22.8 ± 9.0	Additive
RMSSD (ms)	-10.3 ± 6.0	-13.6 ± 16.3	-23.8 ± 17.1	-20.9 ± 14.1	Additive
LF/HF (ms^2)	0.8 ± 0.8	0.9 ± 1.5	1.8 ± 1.4	1.2 ± 0.9	Additive
FBF Δpeak (ml/min)	-378 ± 288	-257 ± 263	-636 ± 494	-411 ± 281	Additive
LVC Δpeak (ml/min)	-4.4 ± 3.2	-2.7 ± 3.4	-7.1 ± 5.7	-6.09 ± 3.64	Additive
MAP Δpeak (mmhg)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	Additive
Heart Rate Δpeak (bpm)	0.0 ± 0.5	0.1 ± 0.9	0.1 ± 1.2	0.2 ± 1.0	Additive
Cardiac Output Δpeak (l/min)	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	Additive
Stroke Volume Δpeak (ml)	0.0 ± 0.2	0.0 ± 0.3	0.0 ± 0.4	0.0 ± 0.3	Additive
FBF AUC (ml)	-367 ± 334	-210 ± 493	-578 ± 735	-579 ± 335	Additive
LVC AUC (ml/m)	-5.1 ± 3.6	-3.1 ± 5.4	-8.2 ± 7.7	-7.7 ± 4.1	Additive
MAP AUC (mmHg)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	Additive
Heart Rate AUC (bpm)	0.0 ± 0.5	0.1 ± 0.4	0.0 ± 0.6	0.1 ± 0.5	Additive
Cardiac Output AUC (l)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	Additive
Stroke Volume AUC (ml)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	Additive

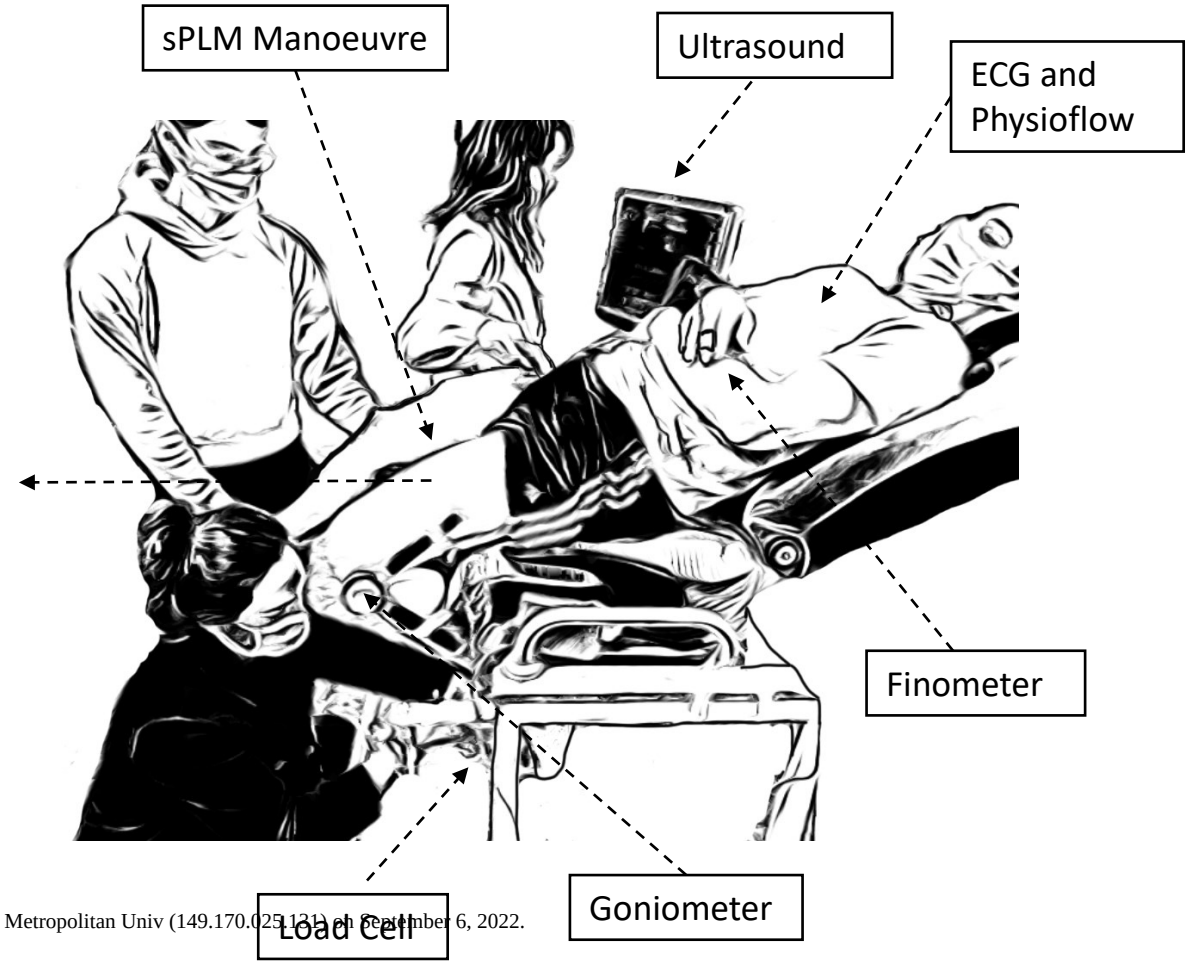
Data are presented as mean ± standard deviation. FBF= Femoral Blood Flow; LVC= Leg Vascular Conductance; bpm= beat per minute; MAP= mean arterial pressure; AUC = area under the curve; Δpeak = delta peak; ΔDOMS= delta between delayed onset muscle soreness and control conditions; ΔST= delta between stretching and control conditions; ΔDOMS +ΔST = delta delayed onset muscle soreness summed with delta stretching; ΔDOMS +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 ΔDOMS+ ΔST.

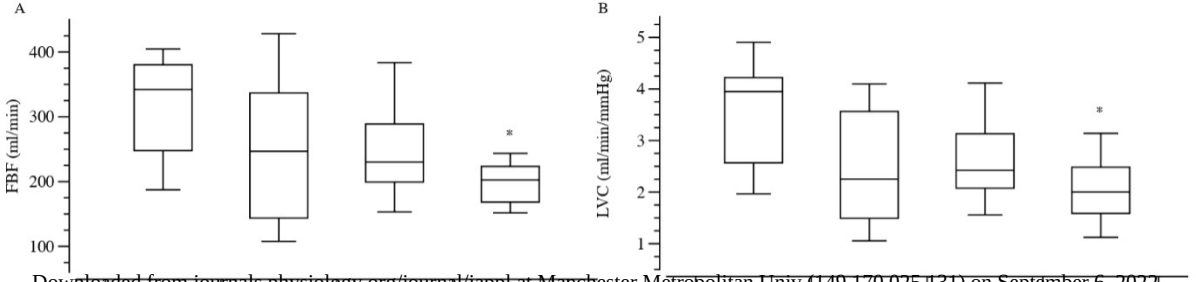
Study Design

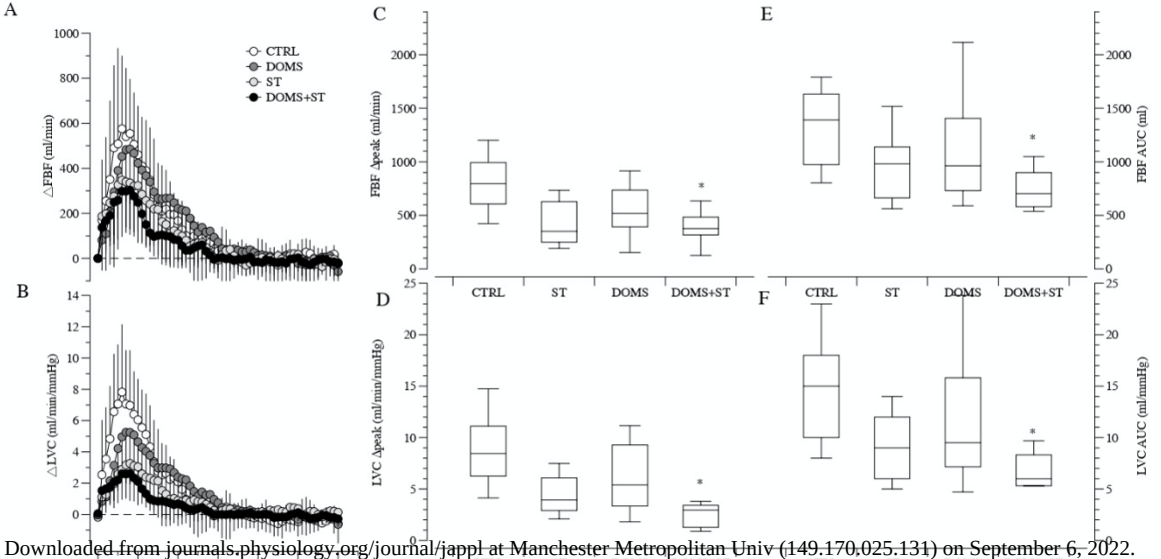


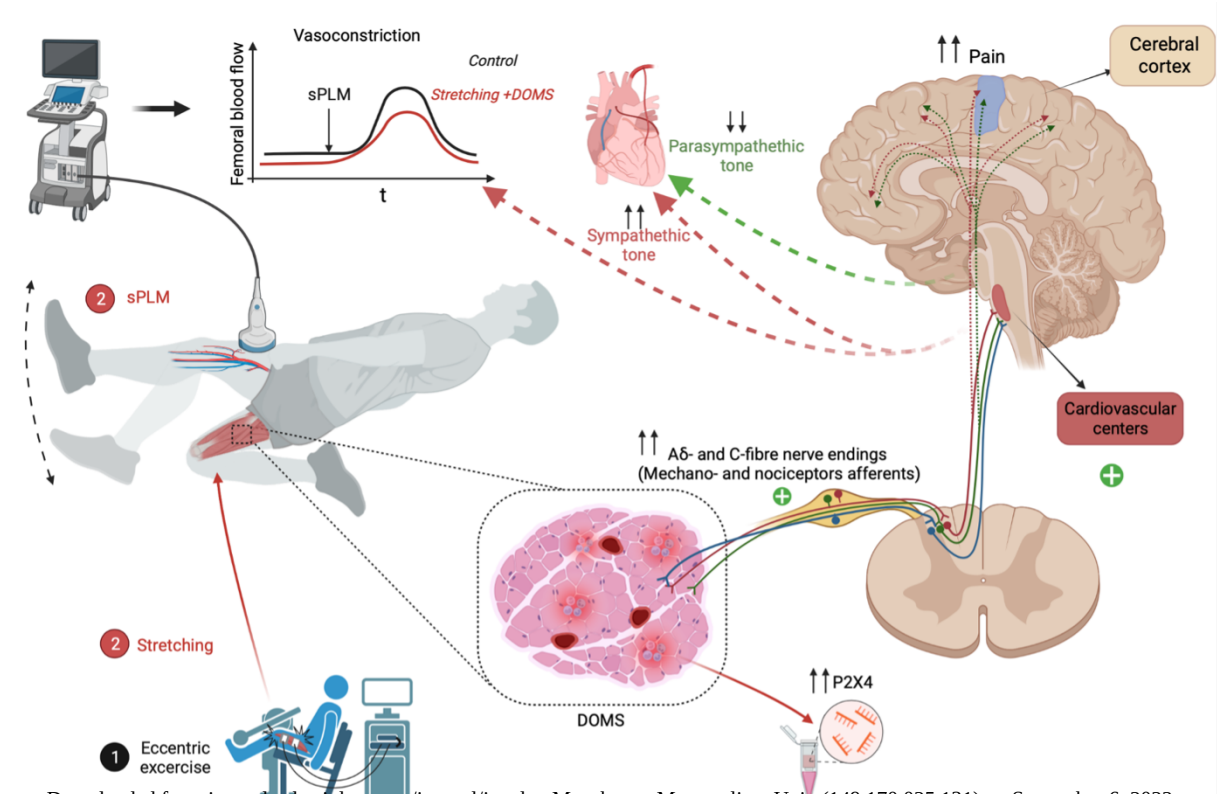
Static Stretching Protocol







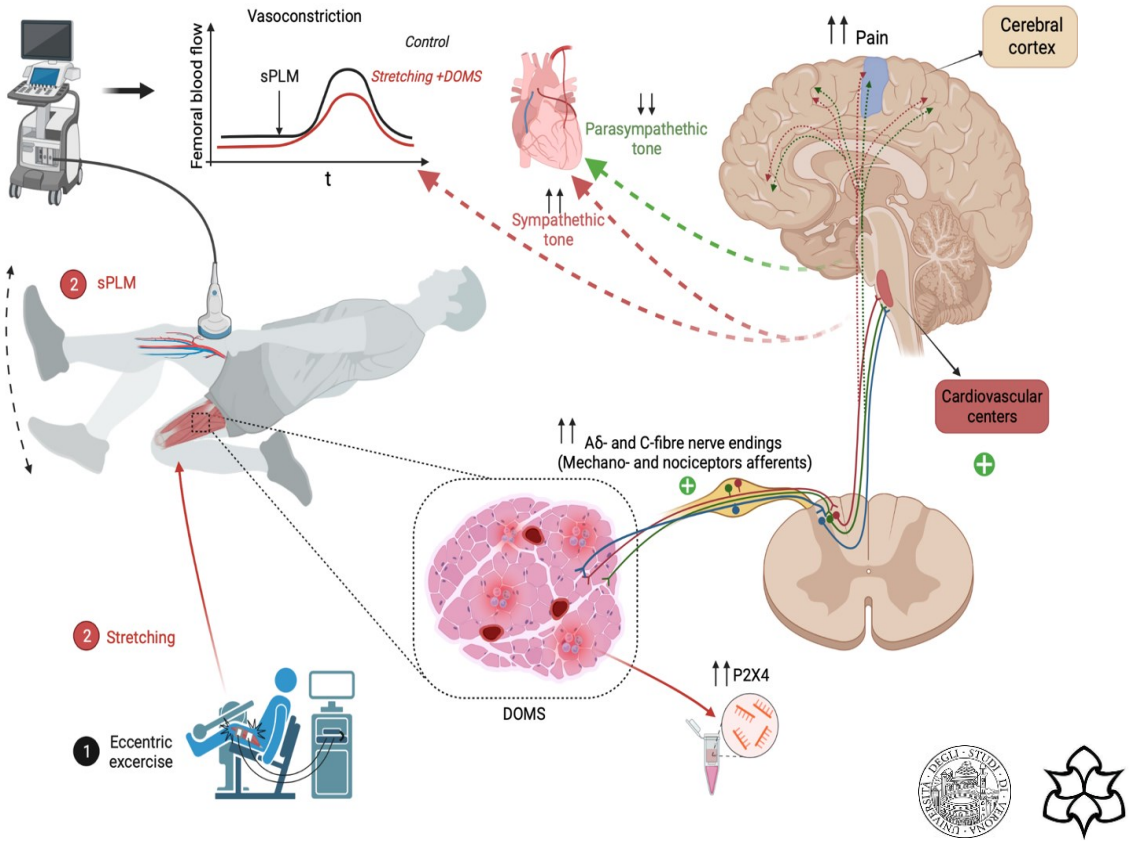




Effects of nociceptive and mechanosensitive afferents sensitization on central and peripheral haemodynamics following exercise-induced muscle damage.

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.