# The influence of nociception on exercise induced fatigue: the analgesic role of acute exercise and long-term training of different exercise modalities

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Last Update: 30th may 2021

## 1. Introduction

### *1.1 Nociception and Health*

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, partially related to the magnitude of the nociceptive signal (1).

Important reductions in nociception are often observed after 8 to 12 weeks of exercise (2); however, as little as 1 session of exercise can induce hypoalgesia (3, 4). This phenomenon is known as exercise-induced hypoalgesia (EIH), a generalized decrease in nociception sensitivity that occurs during exercise and for a short time afterwards (<30 min), relevant in minimizing symptoms in persons with chronic pain (5). Nociception has been quantified in a variety of ways in studies of EIH, with quantitative sensory testing most often used. Quantitative sensory testing describes a series of tests that measure the perceptual responses to systematically applied sensory stimuli (usually pressure, thermal, or electrical) (6). These tests typically involve the quantification of the individual nociceptive threshold and tolerance, which are, respectively, the minimum intensity of a stimulus that is perceived as painful and the maximum intensity to a noxious stimulus that the participant is willing to tolerate (7). These measures are frequently assessed in the immediate post-exercise period (e.g., 0–15 minutes after exercise termination).

The magnitude of EIH appears to vary according to the combination of volume and intensity of exercise (8, 9, 10), the type of noxious stimulus used (3), the method used to quantify nociception (e.g. threshold, tolerance, rating) (3), the site (e.g. over an exercised or non-exercised muscles) (11, 12, 13), and the timing of nociception assessment (e.g. during or following exercise) (14, 15, 16). Analgesic effects are most common seen when noxious mechanical stimuli are used rather than thermal (17) and its effects are more pronounced in local than in remote muscles (12). While aerobic and isometric resistance exercise have been extensively investigated and its analgesic effect are relative unequivocal, dynamic

resistance exercise has been far less studied (18). In addition, to our knowledge, only one study verified the hypoalgesic effect of a 30s Wingate anaerobic exercise (19).

The most commonly proposed mechanism of EIH relies on enhanced descending inhibition by activation of the opioid and cannabinoid systems (5, 18, 20). The contraction of skeletal muscle increases the discharge of mechanosensitive afferents (i.e., A-delta and C fibers) which, in turn, activate central descending opioid analgesic pathways (21, 22). Exercise also increases the release of endogenous cannabinoids (23). The endocannabinoid system is a neuromodulatory system composed of cannabinoid receptors (CB1, CB2), their endogenous ligands, that is, the endocannabinoids (Narachidonylethanolamine [AEA] and 2-arachidonoylglycerol [2-AG]), and proteins responsible for their metabolism (24). The presence of cannabinoid receptors in nociceptive-processing areas of the brain and spinal cord suggests that endocannabinoids contribute to the control of pain through the activation of CB1 receptors (24). The circulating levels of endocannabinoids increase after isometric exercise (23). These opioid and cannabinoids pathways have receptors not only throughout the central nervous systems but also at the periphery and can produce analgesia when stimulated (18). Furthermore, there is evidence of an interplay between the endocannabinoid and opioid systems, such that the activation of one system is mediated by the other system (25).

Exercise-induced changes in the cardiovascular system have also been proposed as a mechanism of EIH (18). Exercise causes an increase in blood pressure which results in the activation of baroreceptors, innervated by vagal afferents, that are synaptically connected to pain modulatory brain areas, associated with blood pressure control, that decrease pain sensibility when activated (26, 27). This network is thought to be composed of interconnected areas including the insular and prefrontal cortices, the amigdala, the hypothalamus, the periaqueductal gray, the nucleous tractus solitarius. However, there's still little evidence to support this (28, 29), once acute increases in blood pressure by exercise could not account for the persistence of EIH after exercise (i.e. 15 min after exercise

cessation blood pressure would have presumably returned to baseline values, or indeed be lower) (18).

The influence of exercise on reducing the sensitivity of the central nervous system has also been explored as a mechanism of EIH (18). There is evidence that acute exercise can reduce temporal summation (30, 31). Very few studies in humans have permitted description of the sites in the central nervous system from which EIH arises. Non-invasive neurophysiological techniques, using functional magnetic resonance imaging (FMRI) and electroencephalographic (EEG) event related potentials (ERP), can provide some insights (18). Ellingson et al., (2016) (32), with FMRI, found that cycling activated brain areas, like the left dorsolateral prefrontal lobe of the anterior insula, that are involved in descending pain pathways inhibition, decreasing pain sensitivity. More recently, Zheng et al. (2021) (33) and Wu et al. (2022) (34), using heat-evoked brain responses, provided evidence that cycling at 70% of heart rate reserve for 20 min and elbow flexor isometric contraction at 40% MVC for 3min, respectively, induced the reduction of N2-P2 peak-to-peak amplitudes (time domain) as well as enhanced alpha oscillation power (frequency domain) in both exercised and non-exercised limbs (corroborated by pressure and heat pain thresholds increases in local and distal muscles). The N2-P2 potentials mainly originates from the anterior and medial cingulate gyrus and reflect the cognitive and emotional processing of pain perception; it´s amplitude reveals subject´s pain perception. Alpha oscillation power reflects the activation of the resting state sensory-motor neural network and reflects the central descending inhibitory function. Therefore, attending the global EIH effect, the authors concluded that it could be related not only to the modulation of nociceptive information transmission via spinal gate mechanism but also rely on a top-down descending pain inhibitory mechanism.

Additionally, several studies in healthy individuals have found a positive association between EIH and the magnitude of conditioned pain modulation (CPM) (i.e. the paradigm of pain inhibits pain) (35) which is known for evaluating the efficiency of descending pain pathways inhibition, involving both serotonergic and opioidergic mechanisms (5).

However, Ellingson et al. 2014 (36) showed that CPM is only a minor contributor to EIH due to differences in temporal and spatial manifestations. In fact, a significant CPM effect was observed only during evoked pain procedures (i.e. pressure), whereas EIH occurred following exercise. Also, during CPM a significantly larger effect on pressure thresholds was found at the remote sites compared to local sites, whereas EIH was significantly larger in the exercising body part compared to remote sites (13). Therefore, local and segmental mechanisms appear to play important roles for EIH.

Thus, the influence of exercise on reducing peripheral nociceptive sensitivity has also been explored as a mechanism of EIH (18). Preliminary data has provided indirect evidence that the hypoalgesic effect induced by exercise could be possible related to the modulation of the signal transmission via A-delta and C fibers. Using electroencephalographic evoked potentials, Jones et al. (2016) (11) verify that somatosensory-evoked potentials (SEPs) to noxious electrical stimuli delivered to the index finger were unchanged after elbow flexion isometric exercise at 40% MVC for 3 min, whereas laser-evoked potentials (LEPs) to noxious heat delivered to hand dorsum decreased after exercise. This result suggests that the peripheral nociceptor might be involved in EIH in humans, because laser heat stimuli activate peripheral afferents and electrical stimuli bypass the receptors by activating the axons of the nociceptive afferents (11). This was further corroborated by Jones et al. (2017) (37) where by blocking blood flow to an unexercised limb during cycling exercise, resulting in unchanged pressure pain threshold afterwards, provided evidence that factors that are released into the blood during exercise (opioids, cannabinoids, catecholamines), which interact with specific receptors, are able to inhibit the nociceptive signal and therefore modify input before reaching the central nervous system. Nevertheless, the effect of acute exercise on LEPs amplitude attenuation observed by Jones et al., (2016) (11) was not statistical different from the passive rest control condition. The approximately 230ms onset latency of LEP was only compatible with conductive velocity of fast myelinated A delta fibers. The augmented pressure pain threshold verified after exercise resulted from an assessment obtained over a period of

seconds eliciting a sensation of gradually increasing pain, only compatible with the activity of non-myelinated C fibers, which were not directly measured by EEG. Thus, if exercise has greater effects on C compared to A delta fibers further investigation should be addressed on the desensitization role of both afferent fibers post exercise.

On the other hand, the effects of long-term training on nociception have been poorly investigated (18). To our knowledge, only three studies examined nociceptive modulation after exercise training in healthy individuals. Jones et al. (2014) (38) observed increases in ischemic pain tolerance in non-exercising region (upper limb), but not in pain threshold, after cycling training for 30 minutes at 75% of $VO_{2max}$ 3 times/week for 6 weeks. Recently, in an attempt to establish intensity-dependent effects, O'Leary et al. (2017) (39) observed that 3 times/week for 6 weeks of high intensity cycling interval training but not volume-matched moderate intensity continuous training increased ischemic pain tolerance in the upper limb. On the opposite, Hakansson et al. (2018) (40), in pain-free overweight individuals, only observed augmented pressure pain threshold over the exercised muscles after 6 weeks of moderate-intensity continuous cycling training and not after volume-matched high intensity training.

It is important to mention that none of the above studies tried to establish mechanisms underneath training-induced analgesic effects. Some reports indicate that exercise training decreases cardiovascular sympathetic modulation mainly via central command (41). However, it has also been proposed that training modulates the exercise pressor reflex at cardiovascular level by attenuating the peripheric discharge of group III and IV muscle afferents in response to noxious, metabolic and mechanical stimuli, and thus regulating the sensation of nociception. Sinoway et al. (1996) (42) reported that 4 weeks of forearm training reduce sympathetic responses and mean arterial pressure during rhythmic voluntary handgrip. In addition, there is compelling evidence that endurance-trained individuals have a lower exercise pressor response compared to untrained people (43, 44). Thus, the available data indicate some level of adaptation within the exercise pressor reflex gain following low-resistance high-repetition muscle training, and this is in accordance with

the findings of Somers et al. (1992) (45).

*1.2 Analgesia and Performance*

The mechanisms underlying neural changes in response to specific experimental interventions can be investigated by measuring reflex responses, namely the H reflex and V wave (46). Despite both responses are under the influence of common neural mechanisms, H-reflex is more sensitive to changes in the intrinsic motoneuron properties and presynaptic inhibition (46, 47, 48), while the V-wave is more sensitive to modifications in supraspinal input to the motoneuron pool (49, 50). In addition, they are task and training dependent (51, 52, 53). Longitudinal studies have revealed positive changes in H-reflex after endurance (52) and Wingate-based training (53), which reflects improvements in spinal excitability. Resistance training has been shown to increase V-wave amplitude (51, 52) which is representative of increased supra-spinal neural drive.

Recent studies suggest that fatigue development may be related, at least in part, with nociception (54), and it may play a role in exercise tolerance (1). During exercise, ascending group III and IV muscle afferents discharge in response to noxious, metabolic and mechanical stimuli within the contracting skeletal muscles to regulate nociception, muscle activation and peripheral fatigue development (55, 56). As exercise-induced nociception is present in nearly all forms of time-dependent competition, the requirement for understanding its influence on performance and how athletes respond to its presence is imperative (1). Thus, the ability of an athlete to tolerate exercise-induced nociception is a critical factor in successful sports performance (54). If neuromuscular signals are consciously interpreted as nociceptive, leading to adjustments in pace or intensity, then individuals with heightened pain tolerance may be able to generate higher levels of muscle power (1). If nociception acts as a moderator of “allowed” exercise intensity, then the mechanisms that affect the level of pain tolerance may theoretically enhance performance by enabling an athlete to exercise beyond the normal protective threshold (eliciting a greater metabolic stress) (57).

The most common methods to examine the effects of group III and IV afferents is to maintain ischemia after a fatiguing contraction or trough intramuscular injection of a hypertonic saline solution (58). Recently, Norbury et al. (2021) (59) observed that induced muscle pain by hypertonic saline solution injection reduces endurance exercise performance through the exacerbation of neuromuscular fatigue, which origins in inhibitory feedback from group III/IV nociceptors, reducing central motor output. Muscle afferents adjust the motoneuronal responses induced by the stimulation at the cervicomedullary level (corticospinal pathway) during exhaustive contraction. Such motoneuronal activation might be affected rather by changes in the corticospinal excitability, altering the effectiveness of the motor unit recruitment as indicated by the amplitude of cervicomedullary motor evoked potentials (CMEPs) or thoracic motor evoked potentials (TMEPs) measured through transcranial magnetic stimulation (TMS) (60). For the upper-limbs, Martin et al., (2006) (61) verify that after 2-min MVC of the homonymous or antagonists muscles the decrease of the CMEPs on elbow extensors was maintained post contraction by holding the muscle ischemic with a cuff inflated, and this was attributed to the maintained discharge of group III and IV afferents. Despite this, the same effect was not observed for the elbow flexors muscles (62, 61). For the lower-limbs, surprisingly, Kennedy et al., (2016) (63) showed that maintaining fire of group III and IV afferents with ischemia post 2 min MVC of the homonymous or antagonists' muscles had no effect on knee extensors TMEPs. Unlike CMPEs or TMEPs pathway, H-reflex, apart from motoneuronal excitability, is also influenced and allows to assess pre and pos synaptic inhibition mechanisms (60). It was observed that H-reflex amplitude diminishes during sustained submaximal contractions at 25% and 50% MVC of the abductor pollicis brevis muscle (64). Also, H-reflex amplitude evoked in the wrist flexor muscles is reduced by metabosensitive group III and IV afferents activation, trough hypertonic saline solution injection, suggesting that the spinal motor reflex activity seems to be uniformly affected by inputs from these afferents (65). Not only the evidence is somewhat conflicting regarding the TMS research, it is important to note that in the H-reflex above-mentioned studies (64, 65), the effects of induced muscle fatigue

or pain were explored without constructing full recruitment curves individually at each time point. Instead, H reflex was obtained at single current intensities compatible with a given %Mmax. Interpretations based on this approach are seriously limited, because it does not allow determining whether the pre-selected current intensity induces a response falling in the ascending part of the recruitment curve, relevant for motoneuronal sensibility assessment.

Furthermore, to our knowledge, research that reported increases in nociception tolerance after long-term training also observed improvements in performance at post-training time point (i.e. VO2max) (38). Unfortunately, the relationship between improved exercise performance and EIH was not explored. Moreover, since training modulates the amplitudes of evoked H and V-wave potentials (51, 52, 53), it is important to examine whether the interaction between EHI and enhanced exercise performance originates at the spinal or supraspinal level. Ultimately, the training-induced analgesic effect might enhance H-reflex and V-wave excitability, leading to an improvement in motor performance.

### *1.3 Purpose*

Since pre and pos synaptic inhibition of group Ia afferents can both be affected by metaboreflex activation (66), explaining H-reflex inhibition under these conditions, further research is needed to explore whether analgesic effects of post-acute and chronic exercise are partially dependent on lower H-reflex inhibition by group III and IV muscle afferents desensitization. In addition, it is important to note that the available research examining the interaction between exercise (acute and chronic) and nociception did not compare the effect of different exercise modalities. Since discharge rate of III and IV afferents is highly dependent on specific task-metabolic requirements (67), it is relevant to determine if exercise modality differentially modulates the possible analgesic response via metaboreflex desensitization (acute and chronic desensitization).

Therefore, with this project, we aim at exploring the influence of experimentally induced metaboreflex activation (group III and IV afferents) (maintained submaximal

isometric contraction followed by blood-flow occlusion) Vs. a control condition (no isometric contraction nor blood flow occlusion), on H-reflex recruitment curve and V wave excitability (aim #1). Since the activation of group III/IV afferents can inhibit the corticospinal pathway (61) we hypothesize that the normalized maximal amplitude and the slope of the ascending limb of the H-recruitment curve will decrease with metaboreflex activation. Additionally, we intend to examine the acute impact of different exercise modalities (resistance, supramaximal and endurance) on nociception sensibility (by the application of a cold tolerance test and by determining pressure pain threshold) (aim #2). This will allow us to investigate the role of different acute exercise paradigms on possible metaboreflex desensitization in its relationship with altered neuromuscular responses at post-exercise time point (attenuated inhibition of H-reflex and V-wave amplitude during metaboreflex activation). Also, once past research has shown that muscle metaboreflex activation reduces the sensitivity of the baroreflex (68), we will measure arterial pressure (AP) and heart rate variability (HRV) during cold pain tolerance testing. Despite all exercise modalities could enhance reducing pain sensibility after exercise, we hypothesize that resistance and supramaximal exercise stimuli will enhance nociceptive tolerance via metaboreflex desensitization compared to that seen with endurance exercise (attenuated increase of AP and sympathetic component of HRV during cold pressor test). Finally, we intend to explore the impact of long-term resistance, supramaximal and endurance training on nociceptive sensibility, using the same tests of aim #2 (aim #3). We hypothesize that chronic EIH will be of greater magnitude after supramaximal and resistance exercise training vs. endurance training. In addition, we hypothesize that decreased nociception will be related with attenuated inhibition of H-reflex and V-wave amplitude and attenuated increase of AP and sympathetic component of HRV during metaboreflex activation after training and this will result in increased exercise performance via heightened H-reflex and V-wave excitability.

## 2. Research Plan and Methods

### 2.1 Aim #1

*2.1.1 Participants*

We will recruit a total of 16 participants ((G Power 3.0.10 software) to achieve 80% of correctly reject the null hypothesis, based on Duchateau et al., (2002) (64)). Specific inclusion and exclusion criteria are discussed in subsection 2.4.1

*2.1.2 Study design*

To study the influence of experimentally induced metaboreflex activation on H-reflex and V-wave excitability, participants will be tested on 2 different conditions (with and without maintained submaximal isometric contraction followed by blood flow occlusion, using a randomized, crossover approach). The experimental design is illustrated in figure 1.

### 2.2 Aim #2

*2.2.1 Participants*

The intervention study will enroll 24 participants ((G Power 3.0.10 software) to achieve 80% of correctly rejecting the null hypothesis, based on Jones et al., (2016) (11)). Eight participants will be randomly allocated to each exercise modality (i.e. resistance, supramaximal and endurance). Specific inclusion and exclusion criteria are discussed in subsection 2.4.1.

*2.2.2 Study design*

In a randomized and crossover trial, on 2 different days, participants will be tested before and after performing acute bouts of different exercise modalities or

passive rest. Participants will be tested for: (1) pressure pain threshold, (2) cold pain tolerance with concomitant measures of AP and HRV, (3) the influence of metaboreflex activation (maintained submaximal isometric contraction followed by blood flow occlusion) on H-reflex and V-wave excitability. For those enrolled in endurance and resistance exercise modalities, participants will visit the laboratory on a previous day for $VO_{2max}$ and maximal repetition assessments for rigorous exercise prescription on experimental day. The experimental design is illustrated in figure 2.

*2.2.3 Exercise protocols*

Participants allocated to resistance exercise will perform 3 sets of 2 bilateral leg exercises (leg press and seated calf raise). The load intensity will be set at 8-12 maximal repetitions. Participants allocated to endurance exercise will perform 30 min on the speed compatible with 75% of $VO_{2max}$. Participants allocated to supramaximal exercise will perform 2 bouts of 30 s of supramaximal cycling exercise, using a relative load equivalent to 7.5% of the individual body mass (MonarkErgomedic 894E®). Both bouts of supramaximal exercise will be interspersed with 4 min of active recovery.

**2.3 Aim #3**

*2.3.1 Participants*

We will recruit a total of 30 participants. Sample size is overestimated in order to allow us to accommodate an attrition rate of ~ 20% during follow up, once participants will only be eligible for final testing if they attain at least 85% of the training sessions, with the goal of allocating at least 8 participants to each of the three training groups (G Power 3.0.10 software; to achieve 80% of correctly reject the null hypothesis, based on Jones et al. 2014 (38)). Specific inclusion and exclusion criteria are discussed in

subsection 2.4.1

*2.3.2 Study design*

Each intervention group will perform 6 weeks of exercise training (resistance, supramaximal and endurance training). Participants allocated to each training group will be evaluated before and after training. On one day participants will be tested for pressure pain threshold and cold pain tolerance with concomitant measures of AP and HRV. The influence of metaboreflex activation (maintained submaximal isometric contraction followed by blood flow occlusion) on H-reflex and V-wave parameters will also be measured. On another day, participants will be evaluated for resting AP and HRV, neuromuscular adaptations and performance. The experimental design is illustrated in figure 3.

*2.3.3 Training protocols*

All participants allocated to each training group will exercise 3 days/week at the same time of the day, with 24 h of interval between sessions. Those enrolled in resistance training will perform 3 sets of 2 bilateral leg exercises (leg press and seated calf raise). In the first week, the load intensity will be set at 15-18 maximal repetitions. From then on, the load intensity will be performed over 8–12 maximal repetitions.

The supramaximal training regimen will consist of 30 s of supramaximal cycling exercise (MonarkErgomedic 894E®), interspersed with 4 min of active recovery, using a relative load equivalent to 7.5% of the individual body mass. During the first four weeks of training, participants will complete two 30s all-out sprints per session. From then on, volume will be increased to four 30s all-out sprints per session.

Endurance training will be performed on a motorized treadmill (Jaeger®,

Laufergotest, Germany). During the first week of training, relative intensity will be set at 65-75% $VO_{2max}$ and each training session will last 30–40 min. From then on, the duration of the training sessions will be increased to 40–50 min and the treadmill speed will be set to elicit 75–85% $VO_{2max}$

*2.3.4 Exercise Performance*

*2.3.4.1 Resistance training group*

Both before as well as after training, participants will be evaluated for maximal and submaximal knee extension strength. Measurements will be taken in the seated position, with the participants maintaining their knee flexed at 70º (69). MVC will be determined based on the highest peak torque obtained in 3 trials, interspersed by 60 s of passive recovery. Then, participants will be asked to maintain 40% of MVC until failure. Visual feedback from the dynamometer software will be provided. Task termination will occur whenever the participants exhibit strength decrements ≥ 10% for more than 10 s.

*2.3.4.2 Endurance training group*

Exercise performance will be determined based on the maximal distance completed by each participant in response to a time-trial run. For the time-trial run, both before and after training, participants will be asked to endure a submaximal treadmill running for as long as possible at the speed compatible with 50% of the distance between the 1st ventilatory threshold and $VO_{2max}$ (Δ50 speed).

Before each time trial, on a different day, participants will complete a treadmill ramp protocol to volitional exhaustion. This test will be used with two specific purposes: 1) to determine participants $VO_{2max}$, 1st ventilatory threshold, and the Δ50

speed; 2) to prescribe the treadmill running speeds for the training period.

*2.3.4.3 Supramaximal training group*

Both before as well as after training, participants will perform the maximal number of 30 s maximal all-out cycling trials (70), interspersed by a 4-minute of active recovery period, until mean power value decreases to 50% of the first supramaximal bout.

## 2.4 Common methodology

*2.4.1 Participants (aim #1, #2 and #3)*

Recreationally active men aged 18-30 years will be recruited. Women will not be included once responses to experimental pain have been demonstrated to vary according to the menstrual cycle phase (71). Participants will be recruited from the local communities and from the Faculty surroundings via word-of-mouth for this study. Exclusion criteria will include known metabolic, cardiovascular, respiratory, orthopedic disease and taking medication for the treatment of pain or having any pain related conditions. Each participant will complete a health- screening as well as a physical activity questionnaire and baseline measures (body mass, height and blood pressure). Finally, participants will be provided with verbal information about the requirements and demands of the study and required to give written, informed consent to the experimental procedures, which was approved by the Faculty of Human Kinetics ethics committee (CEFMH Nº33/2021) and according with the Declaration of Helsinki. Participants will be told to avoid strenuous physical activity 24 hr, caffeine ingestion 4 hr and analgesics intake 6 hr prior to each testing session (59).

*2.4.2 MVC (aim #1, #2 and #3)*

All neuromuscular assessments will be made on the participants' dominant lower limb (plantar-flexors), using a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical System 3). All testing sessions will be conducted between 07:00 and 11:00 hours, to avoid daily variations in muscle strength related to human circadian rhythms. Measurements will be taken in the seated position, with the participants maintaining their hips and knee flexed at 120° and ankle at 110° of plantar flexion (70). The flexed position at the knee joint will be used to reduce the mechanical contribution of the gastrocnemii to the plantar-flexor torque. Participants will be asked not to alter their posture and to focus on the task. MVC and rate of torque development (RTD) will be determined for plantar flexor muscles, based on three isometric contractions, lasting 5 s each. A 60 s interval of rest will be respected between trials. Participants will be instructed to exert their maximum force as fast and hard as possible with verbal encouragement and visual feedback from the dynamometer software being provided. The highest peak torque value will be defined as the participants MVC value (72, 73).

### *2.4.3 Surface EMG (aim #1, #2 and #3)*

Surface EMG will be recorded continuously from EMG electrodes placed on soleus and anterior tibial muscles (Delsys DE 2.x series EMG sensors). After skin preparation, a 2-slot adhesive interface will be applied on the EMG sensor to firmly stick the sensor to the skin. A conductive reference electrode will be placed around the left ankle of each participant (36). Surface EMG signals will be band-pass filtered (10 – 2000 Hz) and amplified using a Delsys Bagnoli-8 amplifier to a total gain of 1000. Then, a 16-bit analog-to-digital converter (National Instruments, USB-6251) will be used to sample the signal at 10 kHz. EMG data will be recorded in synchrony with the torque signal originating from the Biodex System, using Mr. Kick software (Knud

Larsen, SMI,Aalborg University) (73).

*2.4.4 Percutaneous Electrical Nerve Stimulation (aim #1, #2 and #3)*

A constant-current isolated stimulator (STIMSOLA, Biopac Systems; Inc; CA, US) will be used to stimulate posterior tibial nerve. The cathode electrode (8 mm diameter, Ag-AgCl) will be placed on the popliteal fossa and the anode (5 x 10 Compex, Medical SA) proximal to the patella. Each participant will receive a range of electrical stimuli (1-40 mA) to determine the position eliciting the greatest response with the minimum stimulus intensity. The optimal position will be identified using a handheld cathode ball electrode. Finally, stimulation electrode will be fixed with rigid straps and taping (72, 73).

*2.4.5 Standard H-recruitment curves (aim#3)*

Before and after long-term training, standard methods will be used for M-wave and H-reflex recordings (52). These measurements will be taken while each participant maintains a submaximal low intensity contraction of the plantar flexors (10% MVC) with visual feedback from the dynamometer software being provided. The upper current intensity will be determined, firstly, by progressively increasing the current intensity by 5 mA from 0 until there is no further increase in peak torque or in peak-to-peak M-wave amplitudes. At each intensity level, three stimuli will be delivered at 3-seconds intervals. To construct the H-recruitment curves, the upper current intensity (compatible with maximal M-wave) will be divided into 22 segments equally separated on a logarithmic scale, with 16 stimuli/intensity randomly applied to construct the recruitment curves, delivered at 3s intervals.

*2.4.6 V wave recordings, contractile properties and octet contractions (aim #3)*

Before and after long-term training, V waves will be elicited during 5 MVCs, with 1

min of rest between trials, via supramaximal electrical stimulation (150% of the current needed to evoke maximal M wave; 1-ms single square pulse) delivered to the tibial nerve when torque exceeds 90% MVC (52). Contractile muscular properties will be measured from muscle twitches evoked by three single pulses (1-ms square pulse) at 120% of the current needed to evoke maximal M-wave, delivered every 12s (74). Evoked octet contractions (8 pulses at 300Hz), which has been found to elicit the maximum capacity of rate of torque development, will be applied through three supramaximal pulses (at 120% of the current needed to evoke maximal M-wave), delivered every 12s (74).

*2.4.7 H-recruitment curves and V-wave recordings with metaboreflex activation (aim #1, #2 and #3)*

During the blood-flow occlusion trials, to ensure metabolite accumulation participants will previously perform a plantar-flexion contraction during 3 minutes at 40% MVC (aim #1). 25% MVC will be used in aim #2 and #3, in order to avoid any potential hypoalgesic effect to interfere with the analgesic effect of exercise or training protocols. There is evidence that 25% MVC isometric contraction for 5 min doesn't produce elevations in pain threshold (29) at the same time it is a contraction with sufficient intensity to produce nociceptive afferents activation (64). Then, using a rapid inflator system (Hokanson E20 AG101, Bellevue, WA), an adult-size cuff 12 x 124 cm (Hokanson, SC12L, Bellevue, USA) (placed on the upper portion of the thigh) will be inflated to a pressure of 300 mmHg to ensure complete blood-flow occlusion (75). This procedure will preserve the activation of group III/IV afferent fibers for subsequent measurement of H-reflex and V-wave excitability. To construct the H-recruitment curves, the upper current intensity (compatible with maximal M-wave) will be divided

into 22 segments equally separated on a logarithmic scale, with 4 stimuli/intensity, to limit the time under ischemia (~4 min), randomly applied to construct the recruitment curves, delivered at 3s intervals. After 2 min rest, the isometric plantar flexion contraction will be repeated with subsequent thigh circulatory occlusion. V waves will be elicited during 5 MVCs with 15 s of rest between trials (63) to limit the time under ischemia (~2 min), via supramaximal electrical stimulation (150% of the current needed to evoke maximal M wave; 1-ms single square pulse) delivered to the tibial nerve when torque exceeds 90% MVC. In control condition of aim #1 this H-reflex and V-wave collecting procedure will also be used but without sustained isometric contraction followed by blood flow occlusion. In aim #2 it will be assessed 15-30 min post-exercise or rest.

*2.4.7.1 Ischemia pain ratings (aim #1, #2 and #3)*

The participants will be asked to rate the intensity of ischemia pain sensation using a 11-point (0−10) numerical scale (76), every 60s. The left end of the scale (=0) will be anchored with no pain, the middle of the scale will be anchored with moderate pain (=5) and the right end of the scale will be anchored with worst pain possible (=10).

*2.4.8 Pressure pain threshold (aim #2 and #3)*

Pressure pain threshold (PPT) will be assessed on the right rectus femoris (exercising muscle) and biceps brachii (non-exercising muscle). We will use a handheld algometer to perpendicularly deliver pressures to the participants' skin, and the pressure force will be increased at a rate of approximately 1 kg·s−1. The pressure will be stopped when they feel the pressure became painful. This procedure will be repeated three times for each test site. To minimize the impacts of repeated stimuli on the experimental outcomes, a recovery period of 20–30 s will be set between each measurement. The pain

pressure threshold will be recorded as the average of these three measures (34). In aim #2 it will be assessed 0-15 min post-exercise or rest.

*2.4.9 Cold pain tolerance (aim #2 and #3)*

A cold pain tolerance (CPT) test, with the participants comfortably seated while immersing the right foot into a tank 5cm above the ankle joint (13) in cold water at ~2ºC (1º - 4º) (77) will be used to determine participants pain tolerance before and after both acute exercise and long-term training. Water temperature will be adjusted through immersion of ice until achieving the desired temperature. Participants will be instructed to remove their foot from the water at any time when unable to support strong cold nociception sensation. In aim #2 it will be assessed 0-15 min post-exercise or rest (after pressure pain threshold assessment). Once breathing frequency might modify the subjective level of cold pressor pain (77), we will fix ventilatory frequency using a metronome for standardize the duration of the inspiration and expiration phases (2s each).

*2.4.9.1 Cold pain ratings (aim #2 and #3)*

The participants will be asked to mark the intensity of cold pain sensation using a visual analogue scale (VAS) with a horizontal line of 100mm (78), every 30 s.

*2.4.10 Arterial pressure and heart rate variability (aim #1, #2 and #3)*

AP will be assessed using an automatic monitor (Tango SunTech Medical Morrisville, NC) during thigh blood flow occlusion procedures to confirm the sustained activation of the metaboreflex. In addition, AP will be measured before and after acute exercise and long-term training during cold pain tolerance testing (every minute). Cardiac autonomic regulation, both before and after acute exercise and long-term training during cold pain tolerance testing, will be assessed continuously from a Polar

R-R recorder (Polar RS 800 CX, Finland) trough spectral decomposition of beat-by-beat HRV (79). Resting AP and HRV (continuous recording during 5 min) will also be assessed before and after training.

*2.4.11 Negative risk involved in the experiments and control countermeasures (aim #1, #2 and #3)*

The risk of injury during the course of the study are minimal. Nevertheless, participants will be asked to report any adverse reaction. Additionally, besides nociception, no past reports indicate complications derived from temporary total blood-flow occlusion (75) nor cold-water limb immersion (77). The application of electrical current to the tibial nerve can generate some discomfort as well as the Wingate testing and the training protocols. Participants will be monitored and supervised during each experimental session. No insurance is foreseen for this study.

*2.4.12 Data analysis (aim #1, #2 and #3)*

*Neuromuscular function* The peak-to-peak amplitude of the M and V waves will be computed offline from unrectified EMG epochs. Although H waves will be normalized to the corresponding $M_{max}$, V waves will be normalized to the supramaximal M wave ($M_{sup}$) elicited concomitantly with the V wave ($V/M_{sup}$) (52). V waves with concomitant $M_{sup}$ < 90% of the highest $M_{sup}$ will be excluded from further analyses to ensure stable conditions (73). A general squares model will be fitted to the ascending part of the H-reflex recruitment curve and the following parameters will be then extracted (80): (a) current intensity at H-reflex threshold, (b) current intensity at 50% of $H_{max}$, (c) current intensity at $H_{max}$ and (d) slope of the ascending limb of the recruitment curve at 50% of $H_{max}$. Data analysis will be computed exclusively for recruitment curves with r square values >0.90 (80). The amplitude of background surface EMG will be derived from the

average rectified value (ARV) analyzed over an epoch of 500 ms before stimulation. The ARV values will be normalized to those obtained during the highest MVC (ARV computed from a 500-ms time period centered at peak force) and expressed as a percentage. During the MVCs performed with supramaximal stimulation, EMG ARV will be computed using a 500-ms period before stimulation. Then, these values will be normalized to the corresponding M wave obtained during supramaximal stimulation (52). Analyses of twitch contractions will be based on the average of the 3 supramaximal measurements. We will determine maximal twitch torque amplitude, time to peak twitch torque and half-relaxation time (74). Volitional and evoked RTD will be measured in several time windows (0-50ms, 50-100ms and 100-150ms) from force onset, using the trial with the highest peak RTD value (74).

*Heart rate variability (aim #2 and #3)* Power spectral analysis will be performed based on autoregressive modeling, following data detrending and re-sampling (4Hz) (Kubios HRV Analysis Software for Windows, University of Kuopio, Finland). Two main components will be considered: low frequency (LF) – frequency band from 0.04 to 0.15 Hz; high frequency (HF) – frequency band from 0.15 to 0.4 Hz. The ratio LF/HF will be calculated as an index of sympathovagal balance (79).

*2.4.13 Statistical analysis (aim #1, #2 and #3)*

Normality will be verified through the Shapiro-Wilk test. Univariate ANOVAs will be used to explore differences in the impact of blood-flow occlusion vs. non-blood flow occlusion on each H-reflex and V-wave variable (aim #1). Repeated measures ANOVA will be computed to determine significant main effects for time (pre- vs. post-acute exercise), condition (resistance vs. supramaximal vs. endurance exercise) and time-by-condition interactions on pain tolerance/threshold/ratings, AP, HRV and H-

reflex parameters and V-wave amplitude with metaboreflex activation (aim #2). Repeated measures ANOVA will be computed to determine significant main effects for time (pre- vs. post training), condition (resistance vs. supramaximal vs. endurance training) and time-by-condition interactions on exercise performance, pain tolerance/threshold/ratings, AP, HRV, H-reflex parameters and V-wave amplitude with metaboreflex activation, and neuromuscular adaptations to training (aim #3). Correlations between the impact of exercise or training on pain tolerance/threshold/ratings and performance on H-reflex, V-wave, AP and HRV variables will also be explored. Significance level will be set at $p < 0.05$. All data will be treated using SPSS 25.0 for statistical analysis.

*2.4.14 Final study procedures (aim #1, #2 and #3)*

All data will be compiled in Neuromuscular Research Laboratory private cloud. We will ensure the participants confidentiality by attributing a code number to each person. Only the main researcher and his supervisor will have access to the data, and always through the code numbers. All participants will have access to their results via an individual report.

*2.4.15 Conflicts of Interest*

There are no conflicts of interest to declare

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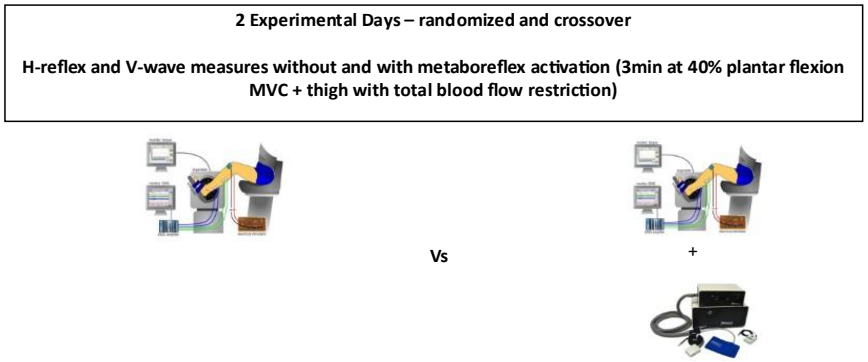


Figure 1. Experimental design of aim #1

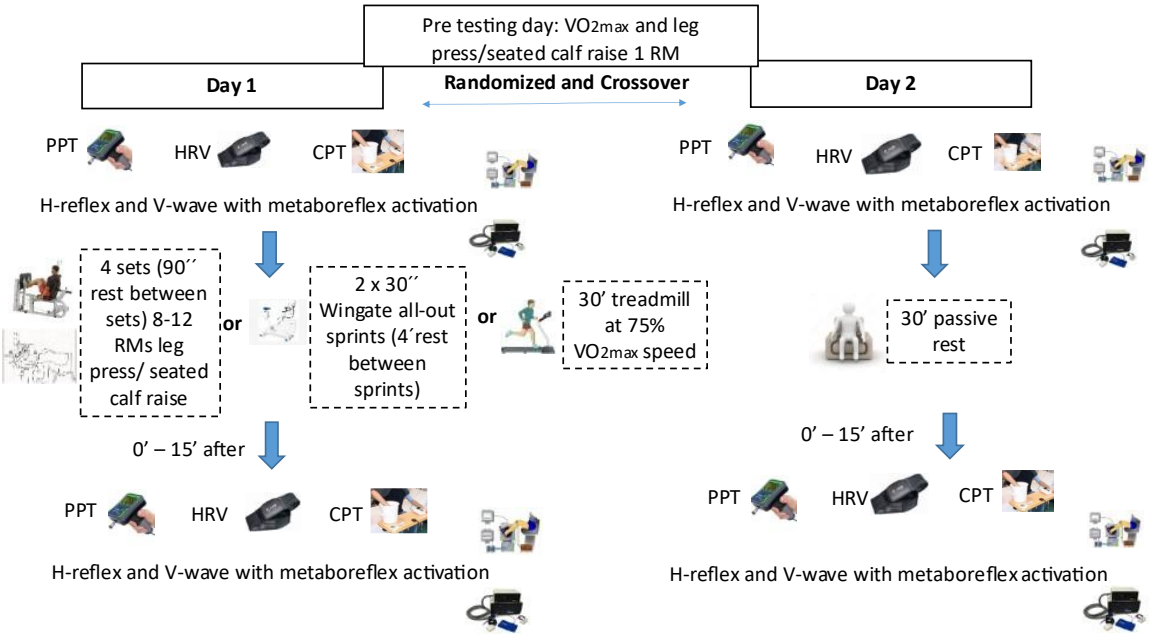


Figure 2. Experimental design of aim #2

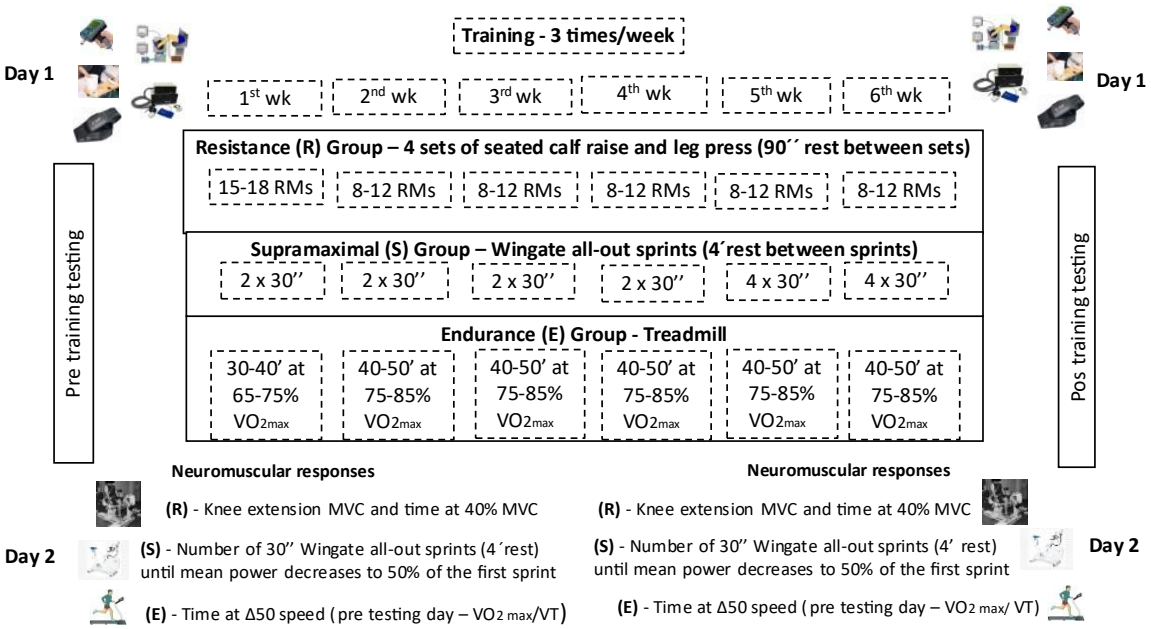


Figure 3. Experimental design of aim #3