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Title: Acute Quadriceps Muscle Pain Reduces Isometric Endurance Performance of the Contralateral Limb

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1 Acute Quadriceps Muscle Pain Reduces Isometric Endurance Performance of the
2 Contralateral Limb

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25 **What is the central question of this study?**

26 The purpose of this study is to establish the effect and mechanisms of exclusively non-
27 local, experimental muscle pain on endurance performance and the development of
28 neuromuscular fatigue during exercise.

29 **What is the main finding and its importance?**

30 Elevated muscle pain from the intramuscular injection of hypertonic saline in the knee
31 extensors reduced endurance performance of the contralateral limb. This is due to the
32 elevated sensory neural feedback from the painful limbs which causes the faster
33 attainment of the sensory tolerance limit.

34 **Abstract**

35 *Intro:* Non-local muscle pain may impair neuromuscular fatigue and endurance
36 performance, but the mechanisms are unknown. This study examined the effects of
37 muscle pain on neuromuscular performance of the contralateral limb. *Methods:* On
38 separate visits, nine participants completed an isometric time to task failure (TTF) of the
39 right knee extensors after intramuscular injection of isotonic saline (CTRL) or hypertonic
40 saline (HYP) into the left vastus lateralis. Measures of neuromuscular fatigue were taken
41 before, during and after the TTF using transcranial magnetic stimulation (TMS) and
42 peripheral nerve stimulation. *Results:* Mean pain intensity was greater in the left leg in
43 HYP (3.3 ± 1.9) compared to CTRL (0.4 ± 0.7) ($P < 0.001$) which reduced TTF by 9.8%

in HYP (4.54 ± 0.56 min) compared to CTRL (5.07 ± 0.77 min) ($P = 0.005$). Maximum voluntary force was not different between conditions (all $P > 0.05$). Voluntary activation was lower at minute 3 of the TTF in HYP compared to CTRL ($P = 0.016$). No difference was identified between conditions for doublet amplitude (all $P < 0.05$). Furthermore, no difference in TMS responses between conditions was observed. *Conclusion:* Non-local pain impairs endurance performance of the contralateral limb. This impairment in performance is likely due to the faster attainment of the sensory tolerance limit from a greater amount of sensory feedback originating from the non-exercising, but painful, left leg.

Introduction

Pain can be defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja *et al.*, 2020). During intense exercise, muscle pain is present and is caused by an accumulation noxious biochemicals such as inorganic phosphate, lactate and hydrogen ions (H^+) (Mense, 2009) along with an increase in intramuscular pressure (O'Connor & Cook, 1999). This sensation is referred to as exercise-induced pain (EIP), which increases as a function of exercise intensity (Cook *et al.*, 1997) and time (Smith *et al.*, 2020).

During intense exercise fatigue develops, which can be defined as a transient reduction in the maximal force generating capacity of the muscle that is reversible by rest (Gandevia, 2001). The development of EIP is often paralleled by exercise-induced fatigue (Pollak *et al.*, 2014). Therefore, the two may be interrelated and consequently EIP may in part be responsible for the development of fatigue (Mauger, 2013) and be determinantal to endurance performance (Mauger *et al.*, 2010; Astokorki & Mauger, 2017; Stevens *et al.*, 2018). Support for this notion comes from previous work has found that muscle pain reduces the maximal force generating capacity of the muscle (Graven-Nielsen *et al.*, 1997, 2002; Khan *et al.*, 2011) and impairs endurance performance (Ciubotariu *et al.*, 2004; Smith *et al.*, 2020). This effect appears to be driven by an exacerbation of central fatigue (i.e., effect spinal and supraspinal areas) (Le Pera *et al.*, 2001; Graven-Nielsen *et al.*, 2002; Khan *et al.*, 2011), with little or no contributing effect on the peripheral apparatus of the muscle (peripheral fatigue) (Farina *et al.*, 2005). As the fatigue related effects of muscle pain appear to be centrally mediated, it is possible that non-local muscle pain may

also cause neuromuscular fatigue of a non-painful exercising limb, and subsequently reduce endurance performance (Hureau *et al.*, 2018).

The effect of non-local pain on fatigue and endurance performance has recently been explored by Aboodarda and colleagues (2020). When ischaemia was induced on the left leg to gradually increase muscle pain, a 21% reduction in cycling time to task failure (TTF) was seen in the right leg. Similarly, when the cycling exercise was preceded by a fatiguing protocol (i.e., starting the exercise with a high but diminishing level of non-local pain), TTF was reduced by 43%. Whilst no differences in voluntary activation or lengthening of the transcranial magnetic stimulation (TMS) silent period (reflecting corticospinal inhibition) were seen compared to no prior fatigue, task failure occurred once maximal pain intensities were achieved which was coupled with a lesser end-exercise neuromuscular fatigue compared to no prior fatigue which suggests a faster attainment of the 'sensory tolerance limit' (Hureau *et al.*, 2018). Additionally, reductions in voluntary activation of non-local muscles have been found following a fatiguing protocol and subsequent maintenance of group III/IV afferent firing (and pain) through ischemia (Kennedy *et al.*, 2013, 2014, 2015; Finn *et al.*, 2020). Therefore, it is unclear how muscle pain may act at a non-local level to impact neuromuscular fatigue and endurance performance.

The use of intramuscular injections of hypertonic saline have previously been used to cause acute muscle pain (Graven-Nielsen *et al.*, 2002; Khan *et al.*, 2011; Smith *et al.*, 2020, 2021). For the purpose of exploring the relationship between pain and fatigue, this technique may be advantageous to ischaemia as ischaemia traps blood in the occluded limb and lowers O₂ availability. This can reduce endurance performance independently of any pain mediated central inhibition. Only one study by Deschamps and colleagues (Deschamps *et al.*, 2014) investigated the role of muscle pain induced by hypertonic saline on performance of the contralateral limb. Interestingly in this study, hopping performance was reduced in the non-painful limb, although the mechanisms causing this were not clear.

Peripheral nerve stimulation (PNS) and transcranial magnetic stimulation (TMS) make it possible to investigate the neuromuscular function (NMF) of an individual in response to muscle pain. Using these techniques in combination with hypertonic saline induced

acute muscle pain, it is possible to investigate the non-local effect of muscle pain on neuromuscular fatigue and endurance performance. Therefore, the purpose of this study was to induce acute muscle pain in the left quadriceps and simultaneously assess endurance and neuromuscular fatigue in the contralateral quadriceps. It was hypothesised that acute muscle pain would reduce endurance performance in the contralateral limb, and this would be accompanied by a decrement in the maximal force generating capacity as well as reductions in voluntary activation.

Methods

Participants

Twelve healthy individuals (3 female) with a mean $\pm$ SD age of 26 ± 5 y, height: 176 ± 9 cm, and body mass: 74.18 ± 13.0 kg participated in the study. All participants were free of lower limb injuries from the past three months, were not taking medication for the treatment of pain or had no pain related conditions. Participants were also screened for any contraindications to TMS (Rossi *et al.*, 2011). Before testing commenced, all participants provided written informed consent and the study was approved by the University of Kent SSES research ethics advisory group (Prop 19_2019_20) and conducted in accordance with the declaration of Helsinki.

Experimental Protocol

Participants visited the laboratory on four separate occasions separated by at least 48 h between visits 1 and 2 and at least 72 h between visits 3 and 4 (injections visits). Participants attended the laboratory at a similar time of day (± 2 h) having avoided strenuous lower body activity 48 h, caffeine 4 h, alcohol 24 h and analgesics 6 h prior to testing. In the first visit, participants were familiarised with measures of NMF (see neuromuscular function testing), questionnaires, perceptual measures, the isometric time to task failure (TTF) exercise and the intramuscular injection of hypertonic saline (if they had not received one before, $n = 6$). Visit two comprised of a second familiarisation of the isometric exercise task where the intensity (% maximum voluntary contraction) was adjusted from the first visit if the TTF was lower than four minutes or greater than six minutes. This was to ensure that task failure coincided with the typical pain duration from

the intramuscular injection of hypertonic saline (Smith *et al.*, 2020). Visits 3 and 4 comprised of the two experimental visits (see figure 1). Participants initially completed the positive and negative affect schedule (PANAS) and pain expectation/pain coping confidence. They then underwent baseline measures of NMF involving PNS and TMS during isometric contractions of the right knee extensors. Participants then waited ten minutes before receiving an intramuscular injection of 1 mL of isotonic saline (0.9%) or hypertonic saline (5.85%) in the muscle belly of the left vastus lateralis (VL). The isotonic saline condition served as a non-painful injection matched control (CTRL) while the hypertonic saline caused acute muscle pain (HYP). Immediately after the injection, participants began the submaximal isometric TTF protocol with the right leg whilst intermittent measures of PNS and TMS were performed. Measures of pain intensity and rating of perceived exertion (RPE) were recorded until task failure, where post-exercise measures of NMF were performed along with the Situation Specific Pain Catastrophizing Scale (SPCS).

Figure 1. Schematic of the experimental protocol. RPE = rating of perceived effort, LL = left leg, RL = right leg. VL = vastus lateralis, VM = vastus medialis, RF = rectus femoris, NMF = neuromuscular function

Equipment and Procedures

Hypertonic Saline Injection. A bolus of 1 mL hypertonic saline (5.85% NaCl) was injected into the middle third of the muscle bell of the left VL to induce muscle pain. The site was cleaned with an alcohol swab and then the saline was manually infused using a 3 mL Luer-Lok syringe (BD, New Jersey, USA) connected to a 1.5 inch 25-gauge

hypodermic needle (SurGuard2, Terumo, Japan) over a 20 s window (five second pause after the insertion, a 10 s infusion period, followed by 5 s pause before needle withdrawal). An identical injection protocol was performed with the isotonic saline (CTRL condition).

Endurance Task. The endurance task was a single limb isometric contraction of the right knee extensors until task failure, which was defined as the inability to maintain the target force for three consecutive seconds despite verbal encouragement to return to the target. The intensity of the endurance task was individually prescribed to attain task failure in 4-6 minutes (mean 19% maximum voluntary force (MVF), range 14 – 25% MVF). At the end of each minute of the endurance task, participants were instructed to relax and to prepare to perform a 3 s MVC with a superimposed doublet. They were instructed to relax for a further 3 s after the MVC while a resting potentiated doublet was delivered. At 30 s and 90 s five TMS pulses were delivered (~3 s between stimulations). A schematic of the experimental protocol can be seen in figure 1.

Neuromuscular Function Testing. Baseline measures of NMF were completed after a warmup of ten contractions at 50% of the participants' perceived maximum (3 s on, 3 s off). Four MVCs were then performed (3-5 s duration, 2 min rest between attempts). The latter two MVCs had a superimposed and resting potentiated doublet delivered during and < 5s after the MVC. TMS pulses were delivered in a batch of 10 submaximal contractions at the same target force of the subsequent endurance task. One additional contraction was performed at the end of this batch with a single superimposed electrical stimulation. Within 10 s of cessation of the endurance task, another MVC with a superimposed doublet was performed along with 5 submaximal contractions with TMS and one contraction with a single electrical stimulation.

Mechanical Recordings. Participants were strapped into a custom-built isometric chair with a hip and knee angle of 90° (0° being full extension). Straps secured the participant around the torso to prevent any extraneous movement and a non-compliant strap was secured 2 cm above the malleoli which was connected to a transducer to measure isometric force of the knee extensors. The transducer was connected to a signal amplifier (DA100c, Biopac Systems Inc, California, USA) and data acquisition module (MP150, Biopac Systems Inc, California, USA) and sampled at a frequency of 1.25 KHz in

compatible software (Acqknowledge 5.0, Biopac Systems, California, USA). Force traces providing instantaneous feedback were displayed on a computer screen in view of the participant.

Electromyography (EMG). Bipolar surface EMG was used to record muscle activity of the VL, vastus medialis (VM), rectus femoris (RF) and biceps femoris (BF) with 37.5 mm x 37.5 mm Ag/AgCl electrodes (Whitesensor 4831Q, Ambu Ltd, Denmark) at an inter electrode distance of 37.5 mm. The electrodes were placed on the muscle belly proximal to the knee and parallel with the fibres of the muscle for the VL and VM. The RF was placed on approximately the centre of the muscle belly while the BF was placed on the muscle belly 50% of the distance between the ischial tuberosity and the lateral epicondyle of the tibia. Each site was shaved, abraded and cleaned to reduce impedance and the electrode locations were marked for replication in subsequent visits. All EMG data was recorded continuously at a frequency of 2.5 KHz and amplified (gain 1000 for VL, 2000 for VM and BF) with a signal amplifier (EMG2-R, Biopac Systems, California, USA and EMG100c, Biopac Systems, California, USA) before being band pass filtered (10-500 Hz) and recorded onto compatible software (Acqknowledge v5.0, Biopac Systems, California, USA).

Peripheral Nerve Stimulation. An electrical stimulator (DS7r, Digitimer, Hertfordshire, UK) (maximum voltage = 400 v) capable of delivering a single square wave pulse was used for PNS. The anode was an adhesive electrode (100 mm × 50 mm; Phoenix Healthcare Products Ltd, Nottingham, UK) which was secured to the gluteal fold. Initially the cathode was a motor point stimulation pen (Motor Point Pen; Compex; DJO Global, Guildford, UK) which was placed within the femoral triangle to innervate the quadriceps femoris muscles. The motor point pen was used to identify the precise location within the femoral triangle which evoked the largest twitch force and compound muscle action potential (M-wave) peak to peak amplitude. A 32x32 mm electrode was placed over this site (Nessler Medizintechnik, Innsbruck, Austria) for all subsequent stimulations. To determine the intensity required to achieve supramaximal stimulation, 20 mA stepwise increments in stimulation intensity were delivered from 100 mA until a plateau in twitch force and M-Wave amplitude was observed. An additional 30% was added to ensure supramaximal stimulation (M_{max}). This intensity was used throughout the rest of the respective trial. Doublets were delivered as 100 Hz paired stimuli (1 ms pulse duration)

for the assessment of central and peripheral fatigue (see below data analysis) whereas single stimuli (2 ms pulse duration) were delivered for the normalisation of motor evoked potentials.

Transcranial Magnetic Stimulation. Single pulse transcranial magnetic stimulation was delivered with a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Carmarthenshire, UK) via a double cone coil (110 mm diameter) delivering a posterior-anterior current which was placed over the motor cortex to assess cortical excitability of the right quadriceps muscle. Initially the participant's vertex was marked as the midpoint between the nasal-inion and the tragus. The coil was placed 2 cm laterally to this position. Stimulations were superimposed over a submaximal contraction (same as target force for subsequent exercise; ~20% MVF) of the knee extensors at 50% of maximal stimulator output until the hotspot was found, which was defined as the location which provided the greatest peak to peak EMG amplitude of the motor evoked potential (MEP_{AMP}) in the VL while minimising the MEP_{AMP} of the BF. This location was marked on a skin-tight hat the participant was wearing. The participant also wore a cervical collar to prevent excessive movement of the head. Subsequent TMS pulses were separated by approximately 3 s which were superimposed over the submaximal knee extensor contraction. Stepwise increments in the stimulator intensity of 5% were used until a plateau in the average of the five MEPs was reached (< 5% increase). Each batch of TMS pulses (10 at baseline, 5 during exercise) were accompanied by the delivery of a single PNS to acquire MEP/M_{max} ratio.

Perceptual Measures. To assess the magnitude of pain, the pain perception scale was used (Cook *et al.*, 1997) and participants rated their muscle pain for each leg every 30 s. The scale ranged from 0 which corresponded to 'no pain at all' to 10 which corresponded to 'Extremely intense pain (almost unbearable)'. Participants were instructed to anchor the upper pain ratings to the worst EIP they had previously experienced. Rating of perceived effort (RPE) was recorded on the 6-20 point scale (Borg, 1982) to avoid participants conflating pain and effort ratings. Instructions were also given to participants to exclusively rate their effort based on the 'effort to drive the limb' with a rating of 20 anchored to the level of drive given during the MVC performed prior (Pageaux, 2016).

Questionnaires. The PANAS (Watson *et al.*, 1988) was given to participants prior to each experimental visit. Also, the expectation of pain and the confidence to cope with pain was recorded on a 0 (no pain/not confident at all) to 10 scale (worst possible pain/completely confident). Post exercise, the SPCS (Edwards *et al.*, 2006) was administered.

Data Analysis

Baseline NMF was calculated as the mean of the raw value, or just the raw value where one measurement was taken. MVF and doublet amplitude were calculated as the peak instantaneous force. Voluntary activation (VA) was calculated as: $VA = 100 - \frac{\text{superimposed doublet force}}{\text{peak force}} \times 100$ (Strojnik & Komi, 1998). The mean of the five MEP peak to peak amplitudes was normalised to the proximal M-Wave peak to peak amplitude in order to calculate the MEP/M_{max} ratio. The TMS silent period (SP) was calculated as the point from the stimulus artefact to the resumption of the EMG signal by the same investigator. The root mean square (RMS) of the electromyogram was calculated offline (Acqknowledge V5.0; Biopac systems Inc, California, USA) using a 100 ms time constant. MVC RMS amplitude was calculated as the 250 ms either side of peak force, whereas for the submaximal contraction amplitude the mean amplitude of 20 s at the start of each minute and 20s before task failure was used.

Statistical Analysis

All data are presented as mean $\pm$ SD or as mean and interquartile range when not normally distributed. Data was analysed in JAMOV 1.6.7. (The Jamovi Project, 2020). Data was initially checked for assumptions with the Shapiro-Wilk test and the Mauchly test. If these assumptions were violated, data was analysed with a non-parametric test or Greenhouse-Geiser corrected, respectively. A paired samples t-test was used to compare TTF between CTRL and HYP. A 2 x 5 repeated measures analysis of variance (condition x time) was used to analyse neuromuscular variables at baseline, minutes one to three, and task failure. A 2 x 4 repeated measure ANOVA (condition x time) was used to analyse TMS data. A 2 x 8 repeated measures ANOVA (condition x time) was used to analyse pain data. If an effect was observed, followed-up post-hoc tests were performed to determine differences between conditions at different time points and were Bonferroni-Holm corrected where appropriate (Holm, 1979). Paired samples t-tests were used for differences in TTF, mean

and peak pain and the Δ MVF, Δ VAL and Δ Doublet/time, which were Bonferroni corrected. 95% confidence intervals, Cohen's d effect sizes (ES) (Cohen, 1992) where 0.2, 0.5 and 0.8 represent small, medium and large effect sizes respectively and partial eta squared (η_p^2) were reported where appropriate and 0.01, 0.06 and 0.14 reflect small, medium and large effect sizes respectively.

Results

Due to a low pain response in some individuals with the hypertonic saline, participants who did not rate a peak pain of at least 4/10 in the left leg were excluded to. An intensity of 4/10 was selected as this corresponds to the anchor point of 'somewhat strong' on the pain scale which was deemed to be lowest intensity which would display a measurable difference between conditions.

This subsequently left $n = 9$ (mean $\pm$ SD age: 27 ± 5 y, height: 178 ± 10 cm, body mass: 76.8 ± 12.9 kg, 2 females). All participants arrived at the lab in a similar psychological state (PANAS positive affect ($P = 0.563$), negative affect ($P = 0.102$)). Participants also had similar levels of pain expectation (Wilcoxon $P = 0.396$) and pain coping confidence ($P = 0.466$).

Time to Task Failure.

TTF was 9.8% shorter in HYP (4.54 ± 0.56 min) compared to CTRL (5.07 ± 0.77 min) (mean difference = 0.53 min, 95% CI [0.21, 0.84 min], $t_8 = 3.847$, $P = 0.005$, $d_z = 1.28$).

Pain Intensity

Left Leg (Non-exercised Leg). There was a condition x time interaction for left leg pain ($F_{2,507, 20,054} = 13.932$, $P < 0.001$, $\eta_p^2 = 0.635$). Left leg pain did not change over time in CTRL (all $P > 0.05$), whereas in HYP, pain was greater than CTRL at all timepoints (all $P < 0.05$) except at 210 s ($P = 0.098$) and at task failure ($P > 0.999$) (see figure 2b). Mean pain was greater in HYP (3.3 ± 1.9) than in CTRL (0.4 ± 0.7) (mean difference = 2.8, 95% CI [1.9, 3.8], $t_8 = 6.719$, $P < 0.001$, $d_z = 2.24$). Peak pain was also greater in HYP (5.6 ± 1.1) than CTRL (0.9 ± 1.0) (mean difference = 4.5, 95% CI [3.7, 5.3], $t_8 = 12.388$, $P < 0.001$, $d_z = 4.13$).

Right Leg (Exercised Leg). No condition x time interaction was observed for right leg pain ($F_{7,56} = 1.842$, $P = 0.097$, $\eta_p^2 = 0.187$) or main effect of condition ($F_{1,8} = 1.407$, $P > 0.999$, $\eta_p^2 < 0.001$). There was a main effect of time ($F_{2,209,17.674} = 53.909$, $P < 0.001$, $\eta_p^2 = 0.871$). In CTRL, pain increased from 30 s to 120 s ($P = 0.005$) then from 120 s to 180 s ($P = 0.010$) and from 180 s to task failure ($P < 0.001$). In HYP pain increased from 30 s to 120 s ($P < 0.001$) then 120 s to 180 s ($P < 0.001$) and from 180 s to task failure ($P < 0.001$) (figure 2c). There was no difference between mean pain for the right leg (CTRL = 5.3 ± 0.8 , HYP = 5.1 ± 1.0) ($P = 0.109$) or peak pain (CTRL = 9.0 [9 – 10], HYP = 9.0 [8.5 – 10] (Wilcoxon $P = 0.586$).

There was no difference in the sum of pain catastrophizing scores post-TTF ($P = 0.733$).

Figure 2. A. TTF of the endurance task. * denotes significantly different from CTRL ($P = 0.005$). Data presented as mean and individual data. B. Left leg pain during the

endurance task. ** denotes significantly different from CTRL ($P < 0.001$). C. Right leg pain during the endurance task.

Maximal Voluntary Force (MVF)

For MVF there was no condition x time interaction ($F_{4,32} = 2.590$, $P = 0.055$, $\eta_p^2 = 0.245$) or main effect of condition ($F_{1,8} = 0.340$, $P = 0.576$, $\eta_p^2 = 0.041$). However, there was a main effect of time ($F_{1.255, 10.038} = 31.420$, $P < 0.001$, $\eta_p^2 = 0.797$). MVF decreased by 34% in CTRL from baseline (685 ± 138 N) to task failure (450 ± 131 N) (mean difference = 235 N, 95% CI [145, 326 N], $t_{32} = 10.330$, $P < 0.001$, $d_z = 1.99$) which was reduced to a similar extent (32%) in HYP (baseline = 681 ± 149 N, task failure = 461 ± 148 N) (mean difference = 220 N, 95% CI [130, 309 N], $t_{32} = 9.563$, $P < 0.001$, $d_z = 1.88$). There was also no difference in the $\Delta MVF/\Delta Time$ (CTRL = $47.6 \text{ N}\cdot\text{min}^{-1}$, HYP = $50.2 \text{ N}\cdot\text{min}^{-1}$) (mean difference = $2.6 \text{ N}\cdot\text{min}^{-1}$, 95% CI [-10.7, 5.5 $\text{N}\cdot\text{min}^{-1}$], $t_8 = 0.740$, $P = 0.480$, $d_z = 0.25$).

Figure 3. Neuromuscular variables during the isometric TTF. A. Maximum voluntary force. B. Voluntary Activation Level. C. Doublet amplitude. D. Cortical Silent Period.

Data presented as mean $\pm$ SD. * denotes significantly different between conditions ($P < 0.05$). # denotes significantly different from baseline ($P < 0.05$).

Voluntary Activation (VA)

No condition x time interaction was observed for VA ($F_{1,956, 15.649} = 1.543$, $P = 0.245$, $\eta_p^2 = 0.162$). However, there was a main effect of condition ($F_{1,8} = 8.014$, $P = 0.022$, $\eta_p^2 = 0.500$) and main effect of time ($F_{1,662, 13.294} = 8.830$, $P = 0.005$, $\eta_p^2 = 0.525$). VA decreased in CTRL from $97.0 \pm 2.1\%$ to $91.8 \pm 7.5\%$ (mean difference = 5.2 %, 95% CI [0.05, 10.3%], $t_{32} = 3.850$, $P = 0.005$, $d_z = 0.776$) and decreased from $96.1 \pm 2.6\%$ to $87.7 \pm 8.9\%$ in HYP (mean difference = 8.3 %, 95% CI [2.4, 14.2%], $t_{32} = 4.618$, $P < 0.001$, $d_z = 1.09$). This decrease was not different between conditions (mean difference = 4.1 %, 95% CI [-1.0, 9.2%], $t_8 = 1.837$, $P = 0.309$, $d_z = 0.61$). No difference was also seen between conditions at minute 1 ($P = 0.306$) or minute 2 ($P = 0.403$). However, there was a greater reduction in VA at minute 3 in HYP compared to CTRL (mean difference = 4.0 %, 95% CI [1.7, 6.3%], $t_8 = 4.006$, $P = 0.016$, $d_z = 1.33$). There was no difference in the $\Delta\text{VAL}/\Delta\text{Time}$ (Wilcoxon $P = 0.123$).

Potentiated Doublet

For potentiated doublet there was no condition x time interaction ($F_{2,178, 17.420} = 2.044$, $P = 0.157$, $\eta_p^2 = 0.204$) or main effect of condition ($F_{1,8} = 0.620$, $P = 0.454$, $\eta_p^2 = 0.072$), but there was a main effect of time ($F_{1,249, 9.994} = 19.070$, $P < 0.001$, $\eta_p^2 = 0.704$). Potentiated doublet decreased by 26 % in CTRL from baseline (340 ± 83 N) to task failure (250 ± 79 N) (mean difference = 89 N, 95% CI [45, 135 N], $t_{32} = 7.398$, $P < 0.001$, $d_z = 1.54$) and decreased to a similar extent in HYP (22 %, baseline = 343 ± 83 N, task failure = 267 ± 88 N) (mean difference = 75 N, 95% CI [36, 114 N], $t_{32} = 7.384$, $P < 0.001$, $d_z = 1.49$). No difference was seen in the $\Delta\text{Doublet}/\Delta\text{Time}$ ($P = 0.633$).

Electromyography

RMS Amplitude for MVCs

Vastus Lateralis. No condition x time interaction was observed ($F_{1,461, 11.690} = 2.046$, $P = 0.178$, $\eta_p^2 = 0.204$). There was also no main effect of condition ($F_{1,8} = 0.505$, $P = 0.497$,

$\eta_p^2 = 0.204$) but there was a main effect of time ($F_{1,8} = 12.140$, $P < 0.001$, $\eta_p^2 = 0.603$). In CTRL, EMG amplitude decreased from minute 1 to minute 2 (mean difference = 16.5 %, 95% CI [7.6, 25.4 %], $t_{24} = 3.853$, $P = 0.004$, $d_z = 1.43$) but did not further decrease (both $P > 0.05$). In HYP EMG amplitude decreased from minute 1 to minute 3 (mean difference = 19.1 %, 95% CI [2.7, 35.6 %], $t_{24} = 3.433$, $P = 0.011$, $d_z = 0.89$) but did not decrease any further ($P = 0.907$).

Vastus Medialis. There was no condition x time interaction ($F_{1,461,11.686} = 0.493$, $P = 0.566$, $\eta_p^2 = 0.058$) or main effect of condition ($F_{1,8} = 0.305$, $P = 0.596$, $\eta_p^2 = 0.037$) but there was a main effect of time ($F_{3,24} = 4.407$, $P = 0.013$, $\eta_p^2 = 0.355$). Follow up post-hoc tests revealed no differences in CTRL (all $P > 0.05$), but EMG amplitude decreased from minute 1 to minute 3 in HYP (mean difference = 17.3 %, 95% CI [1.3, 33.3 %], $t_{24} = 3.797$, $P = 0.016$, $d_z = 0.83$).

Rectus Femoris. For EMG of the RF, there was no condition x time interaction ($F_{3,24} = 0.360$, $P = 0.782$, $\eta_p^2 = 0.043$) or main effect of condition ($F_{1,8} = 1.588$, $P = 0.243$, $\eta_p^2 = 0.166$), but there was a main effect of time ($F_{3,24} = 8.168$, $P < 0.001$, $\eta_p^2 = 0.505$). In CTRL, EMG amplitude decreased from minute 1 to task failure (mean difference = 23.1 %, 95% CI [2.3, 44.0 %], $t_{24} = 3.922$, $P = 0.004$, $d_z = 0.85$). In HYP, EMG amplitude decreased from minute 1 to minute 3 (mean difference = 16.5 %, 95% CI [1.0, 32.0 %], $t_{24} = 3.304$, $P = 0.015$, $d_z = 0.82$) and did not further decrease at task failure ($P = 0.727$).

RMS Amplitude During the TTF

Vastus Lateralis. For EMG amplitude of the isometric TTF there was no condition x time interaction ($F_{1,560,12.481} = 1.266$, $P = 0.305$, $\eta_p^2 = 0.137$). However, there was a main effect of condition ($F_{1,8} = 6.624$, $P = 0.033$, $\eta_p^2 = 0.453$) and time ($F_{1,160,9.278} = 7.809$, $P = 0.018$, $\eta_p^2 = 0.494$). EMG_{RMS} amplitude was greater at minute 1 (mean difference = 2.3 %, 95% CI [1.2, 3.5 %], $t_8 = 4.657$, $P = 0.010$, $d_z = 1.55$) and minute 2 (mean difference = 2.1 %, 95% CI [0.6, 3.6 %], $t_8 = 3.261$, $P = 0.048$, $d_z = 1.09$) in HYP than in CTRL but was not different at any other timepoint (all $P > 0.05$). EMG_{RMS} also increased from minute 1 to task failure in CTRL (mean difference = 13.1 %, 95% CI [2.8, 23.4 %], $t_{32} = 4.771$, $P <$

0.001, $d_z = 0.98$) and from minute 1 to task failure in HYP (mean difference = 11.2 %, 95% CI [4.0, 11.2 %], $t_{32} = 4.703$, $P < 0.001$, $d_z = 0.93$).

Vastus Medialis. There was a condition x time interaction for VM EMG amplitude ($F_{4, 32} = 3.223$, $P = 0.025$, $\eta_p^2 = 0.287$). EMG amplitude increased from minute 1 to task failure in both CTRL (mean difference = 11.1 %, 95% CI [3.2, 19.1 %], $t_{32} = 3.771$, $P = 0.017$, $d_z = 1.08$) and HYP (mean difference = 8.8%, 95% CI [1.7, 15.8 %], $t_{32} = 4.439$, $P = 0.003$, $d_z = 0.95$).

Rectus Femoris. For the RF there was a condition x time interaction ($F_{1.292, 10.339} = 5.759$, $P = 0.030$, $\eta_p^2 = 0.419$). EMG amplitude increased from minute 1 to task failure in CTRL (mean difference = 8.8%, 95% CI [1.7, 15.8 %], $t_{32} = 4.439$, $P = 0.003$, $d_z = 0.95$).

Figure 4. Root mean square electromyography Amplitude during the isometric TTF. A. Submaximal amplitude of the vastus lateralis muscle. B. Submaximal amplitude of the vastus medialis muscle. C. Submaximal amplitude of the rectus femoris muscle. D. MVC amplitude of the vastus lateralis muscle. E. MVC amplitude of the vastus medialis muscle. F. MVC amplitude of the rectus femoris muscle. * denotes significantly different between conditions. # denotes significantly different from minute 1.

MEP/M_{max}

There was no condition x time interaction for MEP/M_{max} ($F_{3, 24} = 0.995$, $P = 0.412$, $\eta_p^2 = 0.111$) or main effect of condition ($F_{1, 8} = 0.429$, $P = 0.531$, $\eta_p^2 = 0.051$), but there was a

main effect of time ($F_{3,24} = 7.002$, $P = 0.001$, $\eta_p^2 = 0.467$). In CTRL, MEP/ $M_{\max}$ increased from baseline (0.35 ± 0.10) to 30 s (0.46 ± 0.13) (mean difference = 0.13, 95% CI [0.048, 0.16], $t_{24} = 3.883$, $P = 0.004$, $d_z = 1.45$) and subsequently decreased from 30 s to 90 s (0.32 ± 0.13) (mean difference = 0.13, 95% CI [0.089, 0.18], $t_{24} = 3.153$, $P < 0.001$, $d_z = 2.31$). and then remained unchanged ($P = 0.263$) at task failure (0.37 ± 0.16). In HYP MEP/ $M_{\max}$ only decreased from 30 s (0.42 ± 0.15) to 90s (0.32 ± 0.11) (mean difference = 0.10, 95% CI [0.046, 0.16], $t_{24} = 5.037$, $P = 0.026$, $d_z = 1.38$).

Cortical Silent Period (CSP)

No condition x time interaction was seen for CSP ($F_{3,24} = 2.071$, $P = 0.131$, $\eta_p^2 = 0.206$) or main effect of condition ($F_{1,8} = 2.716$, $P = 0.138$, $\eta_p^2 = 0.253$). However, there was a main effect of time ($F_{1.348, 10.780} = 2.071$, $P = 0.019$, $\eta_p^2 = 0.457$). In CTRL, CSP lengthened from baseline (127 ± 26 ms) to 90 s (158 ± 37 ms) (mean difference = 31 ms, 95% CI [4, 58 ms], $t_{24} = 3.361$, $P = 0.010$, $d_z = 0.89$) but did not change any further ($P > 0.999$). In HYP, CSP lengthened from baseline (121 ± 24 ms) to 90 s (148 ± 39 ms) (mean difference = 27 ms, 95% CI [3, 57 ms], $t_{24} = 3.001$, $P = 0.037$, $d_z = 0.70$) but did not change any further ($P = 0.451$).

Rating of Perceived Effort (RPE)

For RPE, no condition x time interaction effect was observed ($F_{3,24} = 0.817$, $P = 0.497$, $\eta_p^2 = 0.093$). However, there was a main effect of condition ($F_{1,8} = 9.091$, $P = 0.017$, $\eta_p^2 = 0.532$) and main effect of time ($F_{1.422, 11.373} = 110.848$, $P < 0.001$, $\eta_p^2 = 0.933$). However, post-hoc tests revealed no significant differences at any time point between conditions (all Wilcoxon $P > 0.05$). RPE increased at each time point in both conditions (all $P < 0.001$). The $\Delta RPE/\Delta Time$ was greater in HYP ($3.0 [2.9 - 3.2]$ au.min⁻¹) compared to CTRL ($2.6 [2.4 - 2.9]$ au.min⁻¹) (Wilcoxon $P = 0.013$).

Discussion

The primary novel finding of this study is that acute muscle pain reduces endurance performance in the contralateral limb. This appears to be due the summation of neural inhibitory feedback from muscle pain in the left leg and with rising EIP in the right leg which causes an individual to reach their sensory tolerance limit more rapidly.

Importantly, in comparison to localised muscle pain, non-local pain appears to act differentially to impair endurance performance.

Pain and TTF

The hypertonic saline injection caused a rapid increase in pain of the left leg which peaked by 60 s at ~5.5/10 (strong pain), and then slowly decreased over time likely due to the gradual dissipation of the intramuscular saline. By 210 s, the pain in the left leg was not significantly different between conditions. As expected, in the exercising limb (right leg) there was a gradual increase in pain intensity over time for both conditions, which reached near maximal levels (9/10 pain intensity). There was no difference in the kinetics of perceived pain intensity during the TTF between HYP and CTRL, meaning that the pain in the exercising leg was not potentiated by concurrent pain in the contralateral leg. Therefore, we can exclude an exacerbation of right leg pain being responsible for reducing the TTF. The mechanism likely responsible for the impaired endurance is the faster attainment of the sensory tolerance limit (Hureau *et al.*, 2018), caused by an enhanced level of neural inhibitory feedback due to pain in the left leg in HYP.

Only one other study has investigated the impact of exclusively non-local muscle pain on endurance performance. Aboodarda and colleagues (2020) noted a 21% reduction in TTF with concurrent rising pain compared to the control, almost two-fold greater than in the current study. This may be explained by the gradual increase of muscle pain in the contralateral limb due to the ischemic environment induced in the Aboodarda study, whereas with a hypertonic saline injection muscle pain rapidly increases then slowly decreases (i.e., figure 2.). As a result, there was likely a greater summation of afferent feedback in the latter parts of the exercise in the Aboodarda study which was exerting a greater inhibitory effect.

Neuromuscular Fatigue

Despite a significant pain response in the left leg, the impact on neuromuscular fatigue during the endurance task was limited. No difference was observed in maximum voluntary force between conditions at any time point. This is in contrast to work by Deschamps and colleagues (2014) who found a reduction in maximal hopping performance after a hypertonic saline injection into the contralateral VL. However, it is

difficult to draw direct comparisons between MVCs and maximal hopping efforts as there are neuromuscular differences in task performance (i.e., static vs dynamic contractions and balance/co-ordination). On the other hand, when hypertonic saline was injected into iliotibial tract (targeting non-muscle nociceptors), a notable decrement in the force generating capacity of the contralateral knee extensors and ipsilateral hand grip muscles was observed (Oda *et al.*, 2018). This was however observed with a greater pain intensity than achieved in this study (peak pain 8.5/10 versus 5.6 respectively) Whilst it cannot be ruled out that pain may reduce contralateral muscle strength, the findings of the present study do not suggest that a reduction in maximal force generating capacity (and the consequential increased relative intensity of the endurance task) was responsible for the shorter TTF. This is in contrast with localised muscle pain, which does reduce maximal force generating capacity and then the relative demands of the task (Graven-Nielsen *et al.*, 1997; Smith *et al.*, 2020).

Post-exercise neuromuscular fatigue was not different between CTRL and HYP which is in contrast with several studies which have either induced contralateral pain or fatigue and then performed a subsequent TTF (Amann *et al.*, 2013; Johnson *et al.*, 2015; Aboodarda *et al.*, 2020). Neural inhibitory feedback which can ‘spill over’ from the non-local area is thought to cause an individual to reach their sensory tolerance limit at an accelerated rate (Hureau *et al.*, 2018). A shortened TTF results in less end-exercise neuromuscular fatigue, which is typically seen with an attenuated reduction in maximum voluntary force and peripheral fatigue (assessed by PNS). It is unclear in the current study why despite a reduction in TTF, there was no difference in end-exercise neuromuscular fatigue as there was also no difference in the rate of change in fatigue (i.e., no change in $\Delta\text{MVF}/\Delta\text{Time}$) which suggests that fatigue did not occur more rapidly. Perhaps only the modest reduction in TTF observed in this study (~10%) was insufficient to cause a significant attenuation of end-exercise neuromuscular fatigue, whereas in prior studies reductions in TTF have been much greater (21 – 50% reduction).

Voluntary activation was only different between conditions at minute 3 of the TTF (figure 3). Interestingly, this did not result in a decrease in the maximal force generating capacity of the knee extensors. It is not clear why this occurred, as peripheral fatigue also did not differ at this time point. The reduction in VA at minute 3 likely reflects a combination of neural inhibitory feedback from both limbs acting to constrain voluntary drive to the right

knee extensor. From the minute two to three timepoint, there was still a considerable amount of nociceptive activity in the left leg from the hypertonic saline injection, causing neural inhibitory feedback at the CNS. In the right leg, there would have likely been the stimulation of fatigue sensitive and nociceptive group III/IV afferents which consequently caused inhibition (i.e., reductions in VA and lengthening of TMS SP). In combination, the nociceptive activity of both limbs was sufficient to cause an elevated reduction in central motor output. The reason for this reduction in central motor drive is likely to prevent the attainment of an intolerable level of voluntary activity (Gandevia, 2001; Hureau *et al.*, 2018).

TMS Responses

The MEP/M_{max} ratio increased at 30 s then decreased at 90 s during the exercise task reflecting an increase in excitability early on in the exercise, before exercise-induced fatigue likely then decreased the excitability of the corticospinal pathway (Finn *et al.*, 2018). However, there was no observable difference in MEP/M_{max} between conditions. This means that the excitability of the cortico-spinal pathway was unaffected by non-local muscle pain. These findings are in agreement with Le Pera and colleagues (Le Pera *et al.*, 2001) who observed no effect of muscle pain on motor evoked potential amplitude of the contralateral hand. The TMS silent period, which is thought to reflect inhibition of the corticospinal pathway (Goodall *et al.*, 2018; Škarabot *et al.*, 2019), increased 90 s into the endurance task but was not further increased with the addition of non-local muscle pain. This is also in alignment with previous work (Aboodarda *et al.*, 2020) where no lengthening of the TMS SP with non-local pain and a shortening with non-local fatigue was observed. Therefore, when pain is non-local, it appears to have no influence on the cortico-spinal pathway during fatiguing exercise. However, this may not be the case for localised muscle pain where corticospinal adjustments may be responsible for changes in motor function (Schabrun & Hodges, 2012). Future work should investigate TMS responses in a paired pulse paradigm to explore short intracortical inhibition (SICI) or intracortical facilitation (ICF).

Electromyographic Responses.

An interesting finding in the present study was that the EMG amplitude during the TTF was greater in HYP than in CTRL for the VL during the first two minutes of exercise.

The EMG recorded during submaximal tasks are thought to provide a crude measure of the neural drive to the muscle (Farina *et al.*, 2010). In this context, pain in the left VL subsequently increased the neural drive to the right VL during the endurance task. An increase in either motor unit firing rate and/or the increase in the number of motor units may explain the increase in EMG amplitude. The reason for such change could be attributed to the centrally mediated inhibition caused by muscle pain (Farina *et al.*, 2004; Liew *et al.*, 2019; Martinez-Valdes *et al.*, 2020) which could necessitate a greater need for central drive to ensure the maintenance of force. As a result, the earlier recruitment of more fatigable higher threshold motor units could lead to the earlier development of fatigue and a shortened TTF.

Sensory Tolerance Limit

The sensory tolerance limit (Hureau *et al.*, 2018) postulates that sensory feedback from locomotor muscles, muscles not directly involved in the exercise and corollary discharge summates until an intolerable level is achieved which causes a decrease in voluntary activation and a subsequent reduction in exercise intensity. This ‘limit’ can be reached from feedback from non-local/exercising areas. With this study, it appears that the additional sensory neural feedback in the non-exercised left leg combined with the rising exercise-induced fatigue and pain within the right leg to cause the individual to reach their sensory tolerance limit sooner, and therefore cause task failure earlier. Indeed, similar findings for exercise with the quadriceps have occurred when additional sensory neural feedback from respiratory muscles (Wüthrich *et al.*, 2013), contralateral quadriceps (Amann *et al.*, 2013; Aboodarda *et al.*, 2020) and upper body muscles (Johnson *et al.*, 2015) and been used. The greater $\Delta\text{RPE}/\Delta\text{Time}$ observed in the current study supports explanation provided from the faster attainment of the sensory tolerance limit as RPE is considered to a surrogate measure of proximity to the sensory tolerance limit. Overall, this study adds to the existing literature to support the hypothesis that sensory neural feedback in the form of muscle pain from non-local areas contribute to a sensory tolerance limit and reduces endurance performance.

Methodological Considerations

The acute nature of the hypertonic saline injection resulted in a decreasing pain intensity within the left leg at the latter stages of the endurance task. Indeed, at the 210 s time point

and the point of task failure, pain VAS scores were not different between conditions. It should be acknowledged that the neuromuscular and endurance performance reducing effects of non-local pain may have been more pronounced if a consistent or increasing level of pain was induced. Therefore, the effects in the present study are likely an underestimation of the role of non-local muscle pain on endurance performance. Muscle ischaemia can achieve this (Aboodarda *et al.*, 2020), however a confounding factor is that blood trapped in the occluded leg may reduce oxygen availability and reduce endurance performance independently.

Two females were participants in this study; however, we did not control for phases of the menstrual cycle or hormonal contraceptives. This could potentially be important as the response to experimental pain has been shown to be influenced by the menstrual cycle phase (Sherman & LeResche, 2006). Specifically, analgesia in the luteal phase may (Vincent *et al.*, 2018) have caused differing levels of pain in response to exercise. Nevertheless, both female participants exhibited a significant pain response to the hypertonic saline similar to that of male participants and in the exercising leg, pain was also similar. Therefore, any influence of the menstrual cycle would have likely been minimal.

We did not record EMG activity in the contralateral (left) leg during the TTF. It is plausible that there could be some muscle activity in the left leg which may have influenced the pain response in the left leg. However, the extent of muscle activity is likely to have been minimal as participants were instructed to keep their leg relaxed from the point of injection. A lack of muscle activity also has been reported in the previous work by Aboodarda and colleagues (2020). Therefore, while we cannot quantify the extent of muscle activity in the left leg during the TTF, it is unlikely to have been great enough to cause any change to the outcomes of the present study.

Conclusion

In summary, muscle pain/nociceptive activity in a contralateral limb causes a significant reduction in endurance performance. This effect is centrally mediated and likely arises from a faster attainment of the sensory tolerance limit due to elevated levels of sensory neural feedback relayed from nociceptors in the painful left leg.

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