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1 **MUSCLE PAIN INDUCED BY HYPERTONIC SALINE IN THE KNEE**
2 **EXTENSORS DECREASES SINGLE-LIMB ISOMETRIC TIME TO TASK**
3 **FAILURE**

4
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56 *Conflict of Interest*

57 The authors declare that they have no conflict of interest.

58

59 *Ethical Approval*

60 The School of Sport and Exercises (University of Kent) Research Ethics Advisory Group
61 (Prop 84_2016_17) approved all procedures and protocols in accordance with the Declaration
62 of Helsinki.

63

64 *Consent to Participate*

65 Written informed consent was gained from the participants prior to participation.

66

67 *Consent for Publication*

68 N/A

69

70 *Availability of Data and Materials*

71 The datasets generated during and/or analysed during the current study are available from the
72 corresponding author on reasonable request.

73

74 *Code Availability*

75 Custom code written in MATLAB R2018a (The MathWorks, Massachusetts, USA).

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Author Contributions

ARM was responsible for the conception of the study. SAS, DM and ARM were responsible for the design of the work. SAS and ARM were responsible for data acquisition. All experiments were performed in the Medway Park Exercise Physiology Laboratory, School of Sport and Exercise Sciences, University of Kent. SAS, DM, SLW, and ARM were responsible for data analysis and interpretation. SAS was responsible for drafting the manuscript. SAS, DM, SLW and ARM were responsible for critically revising and editing intellectual content. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. All authors have read and approved the final version of the manuscript submitted for publication and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

101 **ABSTRACT**

102 **Purpose:** Increased nociceptive activity and the experience of exercise-induced pain (EIP)
103 may contribute to fatigue during endurance exercise. To investigate this, a pain model that
104 produces pain similar to EIP and decouples its' relationship to exercise intensity is required.
105 This study 1) compared the quality of pain caused by a hypertonic saline injection into the
106 vastus lateralis in resting and exercise conditions, and 2) investigated whether this pain
107 contributes to changes in time to task failure. **Methods:** On separate days, eighteen
108 participants completed a time to task failure at 20% maximal voluntary torque (MVT), a
109 resting hypertonic saline intramuscular injection, and in a further three visits a time to task
110 failure at 10% MVT following injection of isotonic saline, hypertonic saline or a control (no
111 injection). **Results:** In a subset of eligible participants (n = 12), the hypertonic saline
112 combined with 10% MVT produced a qualitative experience of pain (assessed by the McGill
113 Pain Questionnaire) that felt similar to EIP. 10% MVT with hypertonic saline significantly
114 elevated pain intensity in the first 20% of the time to task failure and caused a significantly (*P*
115 < 0.05) shorter time to task failure (448 ± 240 s) compared with the isotonic saline ($605 \pm$
116 285 s) and control (514 ± 197 s) conditions. **Conclusion:** These findings demonstrate that
117 hypertonic saline increases the intensity of pain during exercise, which results in a faster
118 occurrence of exercise-induced fatigue. These results provide important evidence supporting
119 pain as a limiting factor in endurance performance.

120

121 **Key words:** Endurance, exercise-induced pain, fatigue, hypertonic saline, isometric,

122 nociception

123

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125

126 **Abbreviations**

ANOVA	Analysis of variance
EIP	Exercise-induced pain
HR	Heart rate
HYP	Hypertonic saline
ISO	Isotonic saline
MPQ	McGill Pain Questionnaire
MVC	Maximal voluntary contraction
MVT	Maximal voluntary torque
PANAS	Positive and Negative Affect Schedule
PRI(T)	Pain rating index (total)
RF	Rectus femoris
RPE	Rating of perceived exertion
SD	Standard deviation
sEMG	Surface electromyography
SRI	Subclass rating index
VAS	Visual analogue scale
VL	Vastus lateralis
VM	Vastus medialis

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133 **INTRODUCTION**

134 Intense and prolonged muscle contractions result in acute pain proportional to the intensity
135 and duration of exercise (Cook et al. 1997). This ‘exercise-induced pain’ (EIP) arises from
136 the sensitisation and activation of ascending group III and IV nociceptive afferents in
137 response to the accumulation of endogenous algesics and increases in noxious and
138 mechanical pressure within the contracting skeletal musculature (O’Connor and Cook 1999).
139 The experience of EIP is often accompanied by fatigue (Pollak et al. 2014), which is defined
140 as an exercise-induced reduction in the capacity to produce muscle force or power (Bigland-
141 Ritchie and Wood 1984). This association has led to the suggestion that EIP may accelerate
142 fatigue development during intense and prolonged exercise (Mauger 2014).

143

144 In support of this notion, the stimulation of muscle nociceptors and increased muscle afferent
145 activity has demonstrated significant reductions in voluntary activation of the elbow flexors
146 (Kennedy et al. 2013) and maximal voluntary force of the knee extensors (Graven-Nielsen et
147 al. 2002). Furthermore, partial blockade of group III and IV muscle afferents at the spinal
148 level results in the attenuation of perceived fatigue, and increases central motor drive (Amann
149 et al. 2009). Based on these findings, it is suggested that the increased activation of group III
150 and IV afferents inhibit central motor drive and the ability to recruit motor units (Amann et
151 al. 2011; Hureau et al. 2019).

152

153 A challenge in studying the fatigue-pain relationship (Mauger 2013; Pollak et al. 2014) is that
154 most experimental pain-induction methods are notably different in their processing and
155 response compared with the transmission and experience of EIP (i.e. differences in the
156 neurological processes that result in the perception of pain, from transduction to perception
157 (Olesen et al. 2012)). For example, ischemic, electrical and thermal pain induction are

158 experimental pain models that are non-specific to the muscle, and can also induce the
159 perception of cutaneous pain (StaaHL and Drewes 2004; Olesen et al. 2012). The additional
160 stimulation of these superficial tissues can produce a subjective pain quality described as
161 “sharp” or “stabbing” as opposed to the “aching” or “cramping” nature of muscle pain
162 (Mense 1993). As such their use may be inappropriate in the investigation of EIP.

163
164 Consequently, an experimental model that induces muscle pain that feels like naturally
165 occurring EIP and allows its contribution to fatigue to be investigated by decoupling EIP
166 from exercise intensity is desirable. The intramuscular injection of hypertonic saline is a well-
167 established and safe experimental method that, under resting conditions, induces standardised
168 and reproducible acute pain often described as ‘aching’ and ‘cramping’ (Graven-Nielsen et
169 al. 1997a, b, c). When injected, this solution activates predominantly group IV afferents with
170 some contribution from myelinated group III nerve fibres (Laursen et al. 1999), which is
171 similar to the nociceptive pathway of EIP (O’Connor and Cook 1999).

172
173 However, while hypertonic saline is established for inducing muscle pain, there has been
174 limited comparison with the experience of EIP and minimal application to explore the
175 fatigue-pain relationship. Indeed, in this field hypertonic saline is most widely used to
176 investigate putative pain-induced changes to motor control (Hodges and Tucker 2011),
177 maximal voluntary contraction (Graven-Nielsen et al. 2002), and high intensity, short
178 duration exercise performance (Graven-Nielsen et al. 1997d) rather than its impact on
179 exercise-induced fatigue. In addition, the exercise intensities, durations, and muscle groups
180 used in these studies have limited relevance to exercise conditions where the impact of EIP
181 on fatigue is most prominent (i.e. prolonged duration (> 2 min), exhaustive exercise in large,

182 primary muscle groups involved in locomotive exercise) (Cook et al. 1997; Abbiss and
183 Laursen 2008).

184

185 Therefore, the aims of this study were to: (i) compare the qualitative experience (based on the
186 total and subclass scores from the McGill Pain Questionnaire) of naturally occurring EIP to
187 the pain elicited from an intramuscular injection of hypertonic saline into a locomotor
188 muscle; and (ii) identify the effects of the muscle pain elicited by this method on the
189 performance time of an endurance exercise task. We tested the hypothesis that the addition of
190 an intramuscular injection of 5.8% hypertonic saline into the vastus lateralis (VL) to low
191 intensity exercise: (i) produces a similar quality of pain (as defined by the McGill Pain
192 Questionnaire) compared to naturally occurring EIP caused by a higher exercise intensity;
193 and (ii) results in a shorter time to task failure compared to placebo and control conditions.

194

195 **METHODS**

196 *Ethical approval*

197 The School of Sport and Exercises (University of Kent) Research Ethics Advisory Group
198 (Prop 84_2016_17) approved all procedures and protocols in accordance with the Declaration
199 of Helsinki. Written informed consent was gained from the participants prior to participation.

200

201 *Participants*

202 Eighteen healthy and recreationally active participants (11 male, 7 female; mean \pm SD: age,
203 24.5 ± 4.0 years; height 1.76 ± 0.1 m; body mass 73.9 ± 13.4 kg; physical activity 5.5 ± 2.3
204 $\text{h} \cdot \text{w}^{-1}$) volunteered to participate in the present study. The sample size was estimated based on
205 the effect size reported in a similar exercise and pain study (Deschamps et al. 2014) to satisfy
206 statistical power at 80%. All participants attended each visit in a similar psychological state

207 as assessed by the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988),
208 which was completed at the start of each visit.

209

210 Participants with existing knee pain, cardiorespiratory disease, neurological disorders, blood
211 borne viruses (e.g. hepatitis B/C and HIV), sore deep tissues, phobia to needles and any
212 allergy were excluded from the study. Participants consuming supplements or medications
213 that alter pain perception during the course of the study were also excluded. Before each visit,
214 participants were instructed to refrain from vigorous exercise (24 h) and abstain from the
215 consumption of alcohol (48 h), analgesics (6 h) and caffeine (8 h).

216

217 ***Experimental procedures***

218 Participants attended the laboratory on five occasions, with each visit separated by 2-7 days.
219 In the initial visit, anthropometric measures were recorded, and participants were familiarized
220 with all measures relating to the experimental protocol, including a practice of knee extensor
221 maximal voluntary contractions (MVCs). Five minutes after MVCs, participants performed
222 an isometric time to task failure (TTF) at 20% maximal voluntary torque (MVT). In visit 2,
223 participants received a single injection of hypertonic saline (Rest HYP), whilst seated at rest
224 (*see intramuscular injection procedure*). Upon the completion of the injection, participants
225 were asked to continuously rate muscle pain intensity, with the visit concluding once the
226 participant had returned to the state of 'no pain'. In a further three visits (visits 3-5),
227 participants performed a TTF at 10% MVT in three conditions in the presence of: no
228 injection (10% MVT, Control), isotonic saline (10% MVT + ISO, Placebo) and hypertonic
229 saline (10% MVT + HYP). In the 10% MVT + ISO and 10% MVT + HYP visits, an
230 intramuscular injection was administered prior to the TTF, with the task commencing within

231 3 s of needle removal. Conditions were performed in a single-blind, randomised and counter-
232 balanced order.

233

234 *Time to task failure (TTF) protocol*

235 All visits were performed seated on an isokinetic dynamometer (Cybex HUMAC Norm
236 isokinetic dynamometer; CSMi, Soughton, MA, USA), set up for the right leg with knee
237 angle at 75° flexion (0° = full extension of the knee), and a hip angle at 90°. At the start of
238 each visit, participants completed a 5 min self-paced, submaximal warm-up on a cycle
239 ergometer (Wattbike Ltd, Nottingham, UK) followed by 3×3s MVCs separated by 90 s rest.
240 The highest torque produced across the three MVCs was defined as the MVT. The TTF
241 commenced 5 min after the MVCs, with the participants directed to maintain a submaximal
242 isometric contraction of the knee extensors. The participants received visual feedback of the
243 target torque on a computer screen but were unaware of the overall time elapsed. The task
244 was limited to a maximum of 20 min, or was terminated when the torque fell below the target
245 for more than 3 s. Within 3 s of task cessation participants performed a final MVC.

246

247 *Intramuscular injection procedure*

248 A single bolus of 1.0 mL 5.8% hypertonic saline was injected in the VL (middle third of the
249 lateral aspect of the thigh) of the right leg to induce acute muscle pain. Injection of a single
250 bolus of 1.0 mL 0.9% isotonic saline was implemented as a placebo. The injection was
251 performed manually in a 20 s window (10 s infusion period) using a 3 mL Luer-Lok syringe
252 connected to a 25 G × 38 mm SurGuard2 disposable stainless needle (Terumo, Japan).

253

254 *Perceptual measurements*

255 At the start of each visit, participants were asked to rate (on a visual analogue scale) how
256 much pain they expected to experience (anchored to the non-injury pain experienced during
257 exercise) (0 = “no pain” to 10 = “worst possible pain”) and their confidence to cope with the
258 expected level of pain (0 = “not confident at all” to 10 = “completely confident”). This
259 provides a measure of pain-specific self-efficacy which is believed to a predictor of pain
260 tolerance and endurance (Motl et al. 2007; Schmitz et al. 2013). Two characteristics of pain
261 were evaluated: intensity and quality. During all visits, pain intensity was continuously
262 scored on a moment-to-moment basis using an electronic visual analogue scale (VAS)
263 ranging from 0 (“no pain”) to 10 (“extremely intense pain”) (Cook et al. 1997) and anchored
264 to previous experiences of naturally occurring EIP (Astokorki and Mauger 2017). The device
265 automatically sampled and recorded the reported pain intensity every 5 s, which allowed for
266 values such as VAS onset (the time-point at which the stimulus is first perceived to be greater
267 than “no pain”) peak pain intensity (VAS peak), time to maximal intensity (from the
268 commencement of sampling), mean pain intensity (the mean VAS from the commencement
269 of sampling until task failure), duration of pain (from VAS onset until the state of “no pain”),
270 and VAS area (area under VAS curve) to be calculated.

271

272 The quality of pain was established by the long-form McGill Pain Questionnaire (MPQ)
273 (Melzack 1975) which contains a total of 20 categories of adjectives describing four major
274 subclasses of pain experience (sensory, affective, evaluative and miscellaneous). Each
275 category contains between two to six similar adjectives arranged in ascending order of
276 implied pain intensity and are assigned rank value based on this order (e.g. the descriptor
277 associated with the least pain within the category is assigned a value of 1). Participants were
278 permitted to select a maximum of one word per category (should any of the descriptors
279 apply). The descriptors chosen by the participants were subsequently summed to calculate

280 scores for each subclass (Subclass Rating Index) and the total score of all subclasses (Total
281 Pain Rating Index), with the overall quality of pain expressed by descriptors chosen by more
282 than one-third of participants. The MPQ was completed after the post-TTF MVC in each
283 visit, and the return to “no pain” in the Rest HYP visit.

284

285 During all of the TTF trials at 10% MVT (visits 3-5), participants also reported Rating of
286 perceived exertion (RPE), defined as the effort to drive the limb (Pageaux et al. 2015), using
287 the 15-point Borg (6-20) scale (Borg 1998) every 30 s. Rating of Fatigue, the perceived
288 inability of the muscle to produce torque, was recorded every 30 s for the first min, and every
289 60 s thereafter using the 11-point Rating of Fatigue (ROF) scale (Micklewright et al. 2017).

290

291 *Physiological Measurements*

292 During the TTFs at 10% MVT (visits 3-5) heart rate (HR) was recorded every 30 s using a
293 Polar FT1 HR monitor paired with a coded T34 transmitter (Polar, Polar Electro, Kempele,
294 Finland), and muscle electrical activity was continuously recorded using surface
295 electromyography (sEMG). sEMG was acquired with square surface electrodes (Ag/AgCl, 32
296 × 32 mm; Nessler Medizintechnik, Innsbruck, Austria) mounted in a bipolar set-up on skin
297 which was shaven and cleansed with an alcohol swab. Electrodes were placed over the
298 muscle belly of the VL, rectus femoris (RF) and vastus medialis (VM) in the direction of the
299 muscle fibres, with a reference electrode placed on the patella. The electrical signal was
300 sampled at 2000 Hz (Biopac MP150, Biopac Systems Inc., California, USA) and acquired in
301 Spike2 software (Version 7; Cambridge Electronic Design).

302

303 The sEMG data was analysed using custom code written in MATLAB R2018a (The
304 MathWorks, Massachusetts, USA). To create a linear envelope representation of the data, the

305 raw sEMG signals were rectified by taking the absolute values, and two-pass zero-lag filtered
306 using a fourth-order low-pass Butterworth filter with a cut-off frequency of 5 Hz. To analyse
307 changes over time, the signals were divided into 10 s epochs. The mean sEMG amplitude for
308 the VL, RF and VM over each 10 s epoch was extracted and normalised to the maximum
309 sEMG amplitude of the prior MVCs (% MVC).

310

311 *Statistical analysis*

312 All data are presented in the form of mean \pm standard deviation (SD). Prior to statistical
313 analysis, all data were checked for the assumptions associated with a paired samples t-test, a
314 one-way ANOVA and a repeated measures ANOVA as appropriate. Data that did not satisfy
315 the Shapiro-Wilk test of normality ($P < 0.05$) were logarithmically transformed. The
316 Bonferroni post-hoc correction was applied where appropriate. Cohen's d and partial eta
317 square (η_p^2) values are reported as measures of effect size (Cohen, 1988).

318

319 Due to between subject variability in TTF, an 'individual iso-time' approach as outlined by
320 Nicolò and colleagues (Nicolò et al. 2019) was applied to compare perceptual (pain intensity,
321 RPE, ROF) and physiological (HR, sEMG) variables. The 'shortest' TTF for each participant
322 was used to identify four (RPE, ROF, HR) and ten (pain intensity and sEMG) time-points in
323 which the three conditions were segmented. This approach maintains a majority of the time-
324 series data (i.e. allows for the inclusion of all repeated recordings such as pain, RPE and ROF
325 to be included) and provides a consistent number of data points to allow comparison between
326 participants for all stated variables across the varying TTF times.

327

328 A two-way ANOVA with Treatment factor with 3 fixed levels (10% MVT, 10% MVT + ISO,
329 10% MVT + HYP) and a repeated measures Time factor with 10 time-points was used to test

330 the effect of condition and time on pain intensity and sEMG during the TTF. Two-way
331 ANOVAs with a Treatment factor with 3 fixed levels (10% MVT, 10% MVT + ISO,
332 Experimental) and a repeated measures Time factor with 4 time points were used for
333 measures of RPE, ROF and HR recorded during the TTF. When an interaction effect was
334 observed, post-hoc paired sample t-tests were implemented to evaluate differences between
335 conditions. Statistical significance was accepted at an alpha level of $P < 0.05$ except where a
336 Bonferroni correction was applied (adjusted, $P < 0.0042$). All statistics were performed using
337 SPSS Statistics v24.0 (SPSS, IBM, New York, USA).

338

339 **RESULTS**

340 As the TTF task was limited to a maximum of 20 min, participants that met this cut-off in any
341 condition did not reach task failure or ‘exhaustion’, which does not provide a true indication
342 of endurance performance. To account for this, these participants ($n = 6$) were subsequently
343 removed from the data set, and analysis was performed on the subset of participants ($n = 12$).

344

345 *Comparison of pain intensity and quality*

346 Mean TTF at 20% MVT was 193 ± 50 s. As shown in Table 1, paired samples t-test revealed
347 a significant difference in VAS scores between pain intensity during 20% MVT TTF and
348 experimental muscle pain from Rest HYP ($P < 0.05$). The 20% MVT task induced a
349 significantly greater mean VAS, equivalent to between “somewhat strong” and “strong” pain
350 intensity ($t_{11} = 5.3$, $P < 0.001$, $CI_{.95}$ 1.1, 2.6, $d = 1.8$), which peaked after a longer period of
351 time ($t_{11} = 5.6$, $P < 0.001$, $CI_{.95}$ 64, 147, $d = 1.7$) and lasted for a shorter duration ($t_{11} = -3.9$, P
352 $= 0.002$, $CI_{.95}$ -175, -49, $d = 1.7$) than the experimental muscle pain experienced in Rest HYP.

353

354 Differences in VAS scores were also reported between 20% TTF and the TTFs at 10% MVT
355 ($P < 0.05$). The VAS onset was significantly slower in 10% MVT ($t_{11} = -5.0$, $P < 0.001$, $CI_{.95}$
356 $-44, -17$, $d = 1.0$) and 10% MVT + ISO ($t_{11} = -2.3$, $P = 0.043$, $CI_{.95}$ $-33, -1$, $d = 0.7$), with a
357 quicker onset in 10% MVT + HYP ($t_{11} = 2.2$, $P = 0.0047$, $CI_{.95}$ $0.2, 29$, $d = 0.9$). A greater
358 VAS mean, equivalent to between “strong” and “very strong” pain was observed in the 10%
359 MVT + HYP condition compared to 20% MVT ($t_{11} = -2.8$, $P = 0.017$, $CI_{.95}$ $-2.6, -0.3$, $d =$
360 1.1) and 10% MVT ($t_{11} = -2.3$, $P = 0.044$, $CI_{.95}$ $-1.97, -0.03$, $d = 0.6$).

361

362 The VAS in all three conditions performed at 10% MVT peaked after a longer period of time
363 (10% MVT; $t_{11} = -6.5$, $P < 0.001$, $CI_{.95}$ $-344, -170$, $d = 2.0$, 10% MVT + ISO; $t_{11} = -4.9$, $P <$
364 0.001 , $CI_{.95}$ $-484, -185$, $d = 1.7$, 10% MVT + HYP; $t_{11} = -3.5$, $P = 0.005$, $CI_{.95}$ $-321, -74$, $d =$
365 1.2) and lasted longer in duration (10% MVT; $t_{11} = -6.3$, $P < 0.001$, $CI_{.95}$ $-394, -189$, $d = 2.2$,
366 10% MVT + ISO; $t_{11} = -5.6$, $P < 0.001$, $CI_{.95}$ $-538, -234$, $d = 2.0$, 10% MVT + HYP; $t_{11} = -$
367 4.2 , $P = 0.001$, $CI_{.95}$ $-411, -130$, $d = 1.6$) than the 20% MVT condition. This contributed to a
368 greater VAS area (10% MVT; $t_{11} = -5.4$, $P < 0.001$, $CI_{.95}$ $-2551, -1077$, $d = 1.9$, 10% MVT +
369 ISO; $t_{11} = -5.9$, $P < 0.001$, $CI_{.95}$ $-3233, -1466$, $d = 2.2$, 10% MVT + HYP; $t_{11} = -4.4$, $P =$
370 0.001 , $CI_{.95}$ $-2754, -929$, $d = 1.7$) in the 10% MVT conditions compared to 20% MVT.

371

372 Overall, as shown in Table 2, the dimensional quality of pain experienced during 20% MVT
373 was similar to Rest HYP for the sensory ($P = 0.123$) and miscellaneous ($P = 0.189$)
374 dimensions, but not for the affective ($P = 0.008$) and evaluative ($P = 0.007$) subclasses. The
375 20% MVT task produced a greater mean Total Pain Index of 30 ± 11 ($t_{11} = 2.9$, $P = 0.016$,
376 $CI_{.95}$ $2, 18$, $d = 0.7$) than Rest HYP (20 ± 9), and, as shown in Table 2, and was defined by
377 descriptives representing all dimensions in the MPQ. However, the 10% MVT + HYP
378 condition, with a mean total pain index of 29 ± 14 , produced a similar overall subjective

379 quality of pain to 20% MVT ($t_{11} = 0.3$, $P = 0.743$, $CI_{.95} -6, 8$, $d = 0.1$). Paired samples t-test
 380 revealed no significant difference in Subclass Rating Index between 10% MVT + HYP and
 381 10% MVT (Sensory; $P = 0.704$, Affective: $P = 0.429$, Evaluative; $P = 0.878$; Miscellaneous,
 382 $P = 0.410$) as well as 10% MVT + HYP and 20% MVT (Sensory; $P = 0.941$, Affective: $P =$
 383 0.394 , Evaluative; $P = 0.504$; Miscellaneous, $P = 0.810$) for all classifications (Table 2).

384

385

386 **Table 1.** Summary VAS scores from 20% MVT, Rest HYP, 10% MVT, 10% MVT + ISO,
 387 10% MVT + HYP TTF.

	20% MVT	Rest HYP	10% MVT	10% MVT + ISO	10% MVT + HYP
VAS onset (s)	25 ± 22	7 ± 16	55 ± 36**	42 ± 29*	10 ± 9*
VAS mean	4.8 ± 1.0	3.0 ± 1.0**	5.3 ± 1.4	5.5 ± 1.2	6.3 ± 1.7*†
VAS peak	9.7 ± 0.7	5.8 ± 2.1**	9.5 ± 0.8	9.0 ± 1.5	9.2 ± 1.6
VAS time to peak (s)	181 ± 51	75 ± 31**	438 ± 171**	516 ± 282**	379 ± 229*
VAS duration (s)	168 ± 42	281 ± 84*	459 ± 185**	555 ± 270**	438 ± 241*
VAS area	899 ± 315	869 ± 386	2713 ± 1282**	3248 ± 1493**	2740 ± 1521*

388

389 Values are means ± SD. *Significantly different vs 20% MVT ($P < 0.05$). **Significantly
 390 different vs 20% MVT ($P < 0.001$). †Significantly different vs 10% MVT ($P < 0.05$)

391

392

393

394 *Time to task failure (TTF)*

395 An ANOVA revealed a significant difference between conditions ($F_{2,22} = 6.7$, $P = 0.005$, η_p^2
 396 $= 0.378$) with 10% MVT + HYP causing a significantly ($t_{11} = 3.4$, $P = 0.006$, $CI_{.95} 55, 257$, d
 397 $= 0.6$) shorter TTF (448 ± 240 s) compared to both 10% MVT + ISO (605 ± 285 s), and 10%
 398 MVT (514 ± 197 s) ($t_{11} = 2.3$, $P = 0.040$, $CI_{.95} 4, 127$, $d = 0.3$) (Fig. 1a.). No significant

399 differences were observed between 10% MVT and 10% MVT + ISO ($t_{11} = -1.8$, $P = 0.104$,
400 $CI_{.95} -204$, 22 $d = 0.4$).

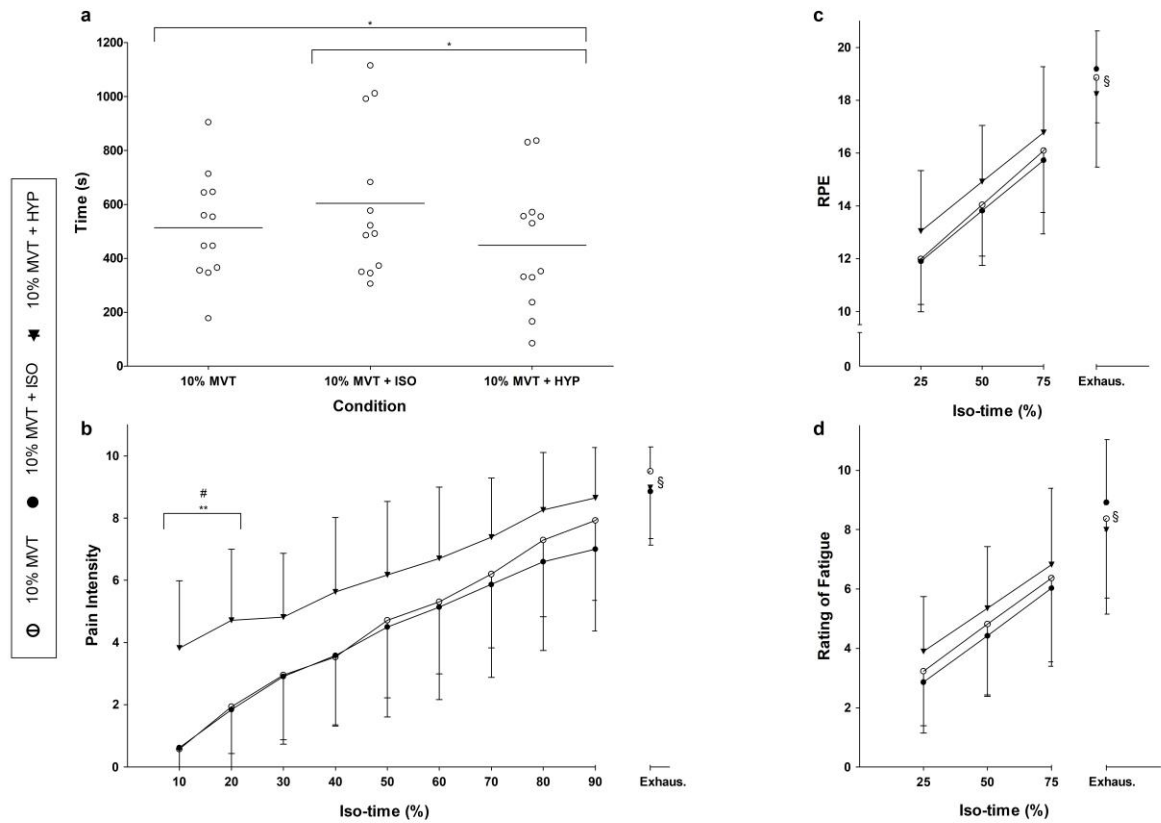
401

402 Paired samples t-tests showed that post-TTF MVT significantly decreased in 10% MVT (pre
403 = 304 ± 56 N.m, post = 191 ± 62 N.m), 10% MVT + ISO (pre = 300 ± 62 N.m, post = $197 \pm$
404 64 N.m) and 10% MVT + HYP (pre = 308 ± 65 N.m, post = 187 ± 66 N.m) in comparison to
405 pre-TTF MVT ($P < 0.001$). No significant difference was observed between conditions for
406 absolute decrement in MVT ($F_{2,22} = 1.0$, $P = 0.379$, $\eta_p^2 = 0.204$). An ANOVA also
407 demonstrated no significant difference between conditions for positive affect ($F_{2,22} = 1.8$, $P =$
408 0.189 , $\eta_p^2 = 0.141$), and negative affect ($F_{2,22} = 1.4$, $P = 0.263$, $\eta_p^2 = 0.114$) recorded prior to
409 the TTF.

410

411

412



413

414 **Fig. 1** Performance and perceptual differences between conditions. TTF differences

415 between conditions (a), and pain intensity (b) and RPE (c) and ROF (d) over iso-time between conditions during

416 the TTF. *Significant difference between conditions ($P < 0.05$). **Significant difference between 10% MVT +

417 HYP and 10% MVT ($P \leq 0.001$). #Significant difference between 10% MVT + HYP and 10% MVT + ISO ($P <$

418 0.001). §Significant main effect of iso-time.

419

420 *Pain intensity*

421 An ANOVA revealed a significant difference in pain expectations between conditions ($F_{2,22} =$

422 9.6, $P = 0.001$, $\eta_p^2 = 0.467$), but not in confidence to cope with the expected pain ($F_{2,22} = 2.3$,

423 $P = 0.125$, $\eta_p^2 = 0.172$). Subsequent pairwise comparisons found greater expectations of pain

424 in 10% MVT + ISO (7.2 ± 1.9) ($t_{11} = -3.8$, $P = 0.003$, $CI_{95} -2, -1$, $d = 0.7$) and 10% MVT +

425 HYP (7.5 ± 1.3) ($t_{11} = -4.5$, $P = 0.001$, $CI_{95} -2, -1$, $d = 1.0$) compared to 10% MVT (6.0 ± 1.6)

426 with no significant difference between 10% MVT + ISO and 10% MVT + HYP ($t_{11} = -0.7$, P

427 $= 0.518$, $CI_{95} -1, 1$, $d = 0.2$).

428

429 The 3×10 (condition \times iso-time) repeated measures ANOVA highlighted a significant effect
430 of condition ($F_{2,22} = 6.5, P = 0.006, \eta_p^2 = 0.372$) and iso-time ($F_{2,8,31.2} = 82.2, P < 0.001, \eta_p^2$
431 $= 0.882$) for perceived pain during the TTF (Fig. 1b.). A significant interaction effect for pain
432 over iso-time between conditions during the TTF was observed ($F_{3,9,42.4} = 3.4, P = 0.018, \eta_p^2$
433 $= 0.236$). Follow up targeted paired-sample t-tests with a Bonferroni correction revealed a
434 significantly greater VAS pain intensity at 10% iso-time (43 ± 21 s) in 10% MVT + HYP
435 compared to both 10% MVT ($t_{11} = -6.4, P < 0.001, CI_{.95} -43.7, -21.3, d = 1.9$) and 10% MVT
436 + ISO ($t_{11} = -5.8, P < 0.001, CI_{.95} -44.2, -19.9, d = 1.9$) and at 20% iso-time (86 ± 42 s) in
437 contrast with 10% MVT ($t_{11} = -4.3, P = 0.001, CI_{.95} -42.1, -13.4, d = 1.3$) and 10% MVT +
438 ISO ($t_{11} = -6.3, P < 0.001, CI_{.95} -38.9, -18.6, d = 1.5$).

439

440 *Perceptual measurements*

441 The 3×4 (condition \times iso-time) repeated measures ANOVA revealed no significant main
442 effect of condition for ROF or RPE ($P > 0.05$). Both ROF and RPE had a significant effect of
443 iso-time (ROF; $F_{1,4,15.9} = 104.1, P < 0.001, \eta_p^2 = 0.904$, RPE; $F_{1,5,16.3} = 87.8, P < 0.001, \eta_p^2 =$
444 0.889), and an interaction effect (ROF; $F_{2,1,23.4} = 6.9, P = 0.004, \eta_p^2 = 0.387$, RPE; $F_{2,8,31.1} =$
445 $4.6, P = 0.010, \eta_p^2 = 0.296$) (Fig. 1c. and Fig. 1d.). Follow-up paired samples t-tests with a
446 Bonferroni correction ($P > 0.004$) revealed no significant differences at any iso-time point
447 between conditions for both ROF and RPE.

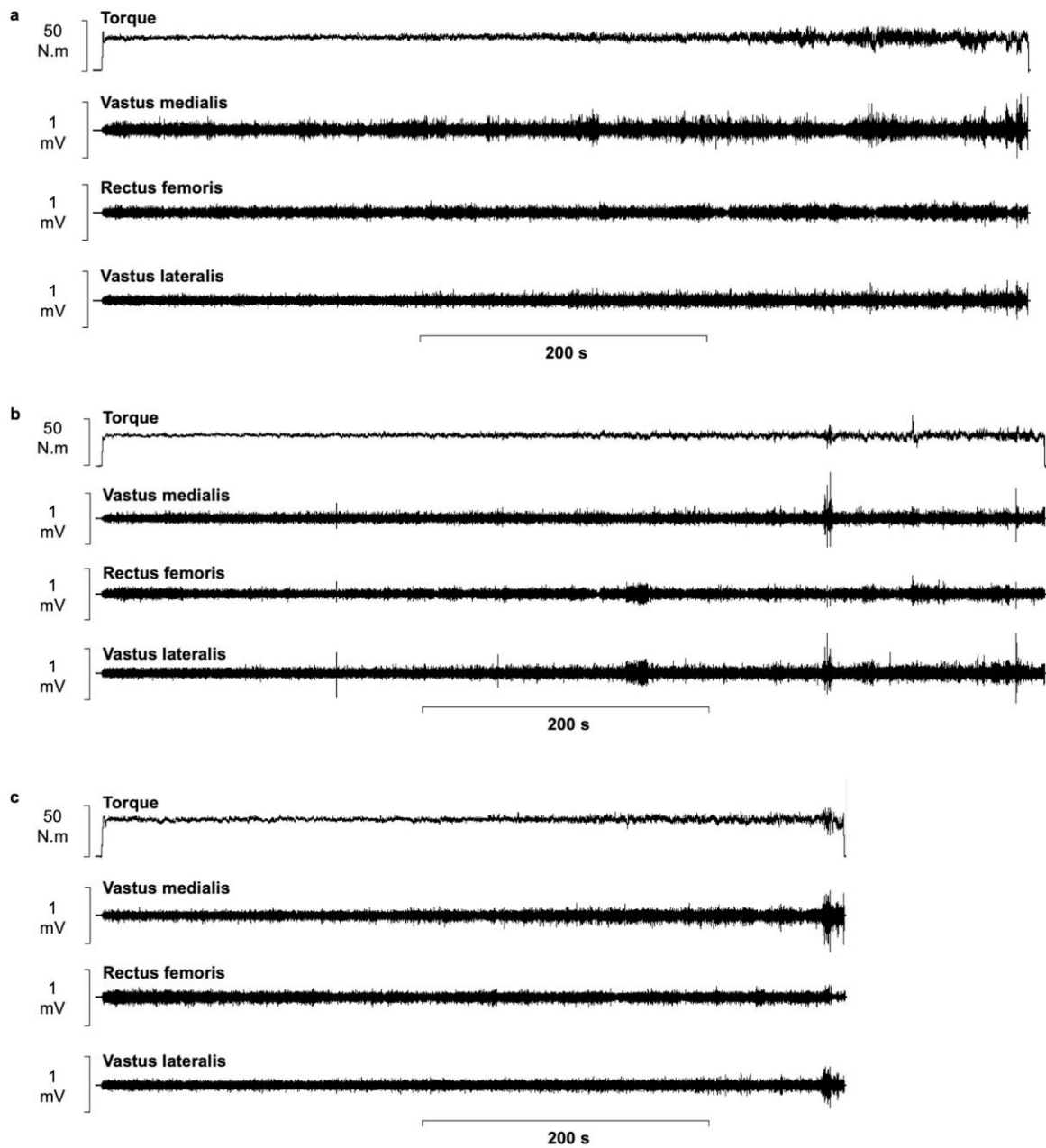
448

449 *Surface electromyography (sEMG)*

450 Due to a loss in sEMG signal, two participants were removed from the dataset and analysis
451 was performed on the remaining participants ($n = 10$). A 3×10 (condition \times iso-time)
452 repeated measures ANOVA demonstrated no significant main effect of condition in either the

453 VL ($F_{2,18} = 1.3$, $P = 0.288$, $\eta_p^2 = 0.129$), VM ($F_{2,18} = 1.9$, $P = 0.174$, $\eta_p^2 = 0.177$) or RF
454 ($F_{2,18} = 0.5$, $P = 0.613$, $\eta_p^2 = 0.053$). A significant effect of iso-time in the activity of the VL
455 ($F_{1.5,13.2} = 19.3$, $P < 0.001$, $\eta_p^2 = 0.682$), VM ($F_{1.8,16.4} = 14.2$, $P < 0.001$, $\eta_p^2 = 0.612$), and RF
456 ($F_{2.0,18.2} = 6.7$, $P = 0.007$, $\eta_p^2 = 0.426$) was reported (Fig. 2). There was no interaction effect
457 observed in the RF ($F_{18,162} = 0.4$, $P = 0.994$, $\eta_p^2 = 0.037$). A significant interaction effect was
458 reported for VL ($F_{18,162} = 3.5$, $P < 0.001$, $\eta_p^2 = 0.278$) and VM ($F_{18,162} = 2.2$, $P = 0.006$, η_p^2
459 $= 0.195$) activity over iso-time between conditions, however subsequent follow-up targeted
460 paired sample t-tests with a Bonferroni correction demonstrated no significant differences
461 (Fig. 3a-3c).

462



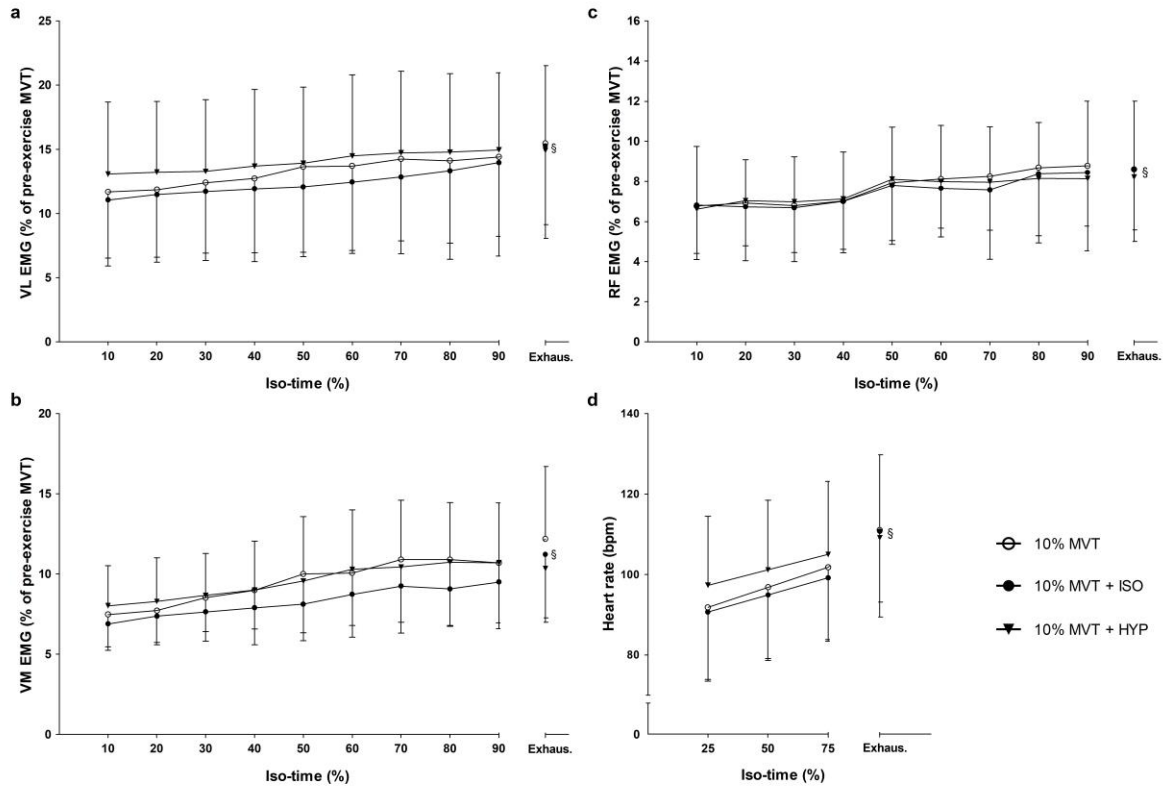
463

464 **Fig. 2** Torque and sEMG data during the TTF of the 10% MVT (a), 10% MVT + ISO (b) and 10% MVT +

465 HYP (c) conditions for a representative participant. The TTF was significantly shortened in the 10% MVT +

466 HYP condition (note the relative time scale).

467



468

469 **Fig. 3** Physiological differences between conditions. EMG of the VL (a), VM (b) and RF (c) over iso-time
 470 between conditions during the TTF. HR differences between conditions over iso-time during the TTF (d).
 471 §Significant main effect of iso-time ($P < 0.05$).

472

473

474 *Heart rate (HR)*

475 The 3×4 (condition \times iso-time) repeated measures ANOVA revealed no significant main
 476 effect of condition ($F_{1.3,14.1} = 0.8$, $P = 0.404$, $\eta_p^2 = 0.071$). There was a significant effect of
 477 iso-time ($F_{1.1,12.3} = 39.6$, $P < 0.001$, $\eta_p^2 = 0.783$), and an interaction effect for HR and iso-time
 478 between conditions during the TTF ($F_{1.7,18.9} = 6.0$, $P = 0.012$, $\eta_p^2 = 0.352$). Subsequent paired
 479 samples t-test with a Bonferroni correction revealed no significant differences between
 480 conditions (Fig. 3d).

481

482

483 **Table 2.** Frequently selected words from the MPQ subclasses

Subclass	20% MVT	REST HYP	10% MVT	10% MVT + ISO	10% MVT + HYP	
Sensory	Throbbing (33%)	Throbbing (50%)	Lacerating (33%)	Throbbing (50%)	Throbbing (58%)	
	Sharp (58%)	Shooting (42%)	Cramping (58%)	Cramping (41%)	Drilling (33%)	
	Cramping (33%)	Sharp (33%)	Pulling (33%)	Burning (50%)	Cramping (67%)	
	Pulling (33%)	Cramping (67%)	Searing (33%)	Aching (67%)	Burning (42%)	
	Hot (33%)	Aching (67%)	Aching (50%)		Aching (50%)	
	Burning (33%)	Tender (33%)			Heavy (33%)	
	Hurting (33%)					
	Aching (58%)					
	SRI	18 ± 6	15 ± 6	18 ± 9	15 ± 6	18 ± 9
	Affective	Exhausting (50%)		Exhausting (75%)	Tiring (33%) Gruelling (33%)	Tiring (42%) Exhausting (42%) Gruelling (33%)
SRI		3 ± 3	1 ± 1*	3 ± 2	2 ± 2	3 ± 2
Evaluative	Intense (50%)	Intense (33%)	Intense (58%)	Intense (67%)	Intense (67%)	
	SRI	4 ± 2	2 ± 2*	3 ± 2	3 ± 1	3 ± 1
Miscellaneous	Radiating (33%) Tight (33%)	Radiating (33%)				
	SRI	5 ± 4	3 ± 3	4 ± 3	5 ± 4	5 ± 4
	PRI(T)	30 ± 11	20 ± 9*	28 ± 12	26 ± 11	29 ± 14

484 The frequently selected words from the MPQ are shown with the percentage of participants
 485 that selected these words. Data on Subclass Rating Index (SRI) and Pain Rating Index (Total)
 486 presented as Mean ± SD. *Significantly different vs 20% MVT (P < 0.05). **Significantly
 487 different vs 20% MVT (P < 0.001). †Significantly different vs 10% MVT (P < 0.05)
 488

489

490 **DISCUSSION**

491 This study confirms that the pain experienced during knee extensor exercise at 10% MVT can
492 be made to feel like that of a higher exercise intensity, through the intramuscular injection of
493 hypertonic saline into the VL. Using this intervention, exercise-induced fatigue occurred
494 more rapidly, with participants reaching task failure earlier when exercising with a greater
495 pain intensity (Fig. 1b). This study therefore provides indicative evidence to support the
496 notion that pain is a significant factor affecting endurance exercise performance.

497

498 *Hypertonic saline combined with light exercise feels like EIP*

499 The novel question the present study strived to determine was whether the addition of
500 hypertonic saline to light intensity exercise at 10% MVT produces an elevated pain intensity
501 which also feels similar to the naturally occurring EIP during a higher exercise intensity (20%
502 MVT). Thus, the first key finding from this study is that when combined with light exercise
503 (10% MVT), the hypertonic saline induced a descriptive quality of pain similar to the EIP
504 from both the 10% and 20% MVT exercise tasks (but with a higher intensity). This is in
505 contrast to the administration of hypertonic saline at rest, where our findings were consistent
506 with the established literature - a moderate to somewhat strong pain, described as cramping,
507 aching, throbbing and intense (Graven-Nielsen et al. 1997a, b, c). Furthermore, in these
508 resting conditions, whilst the sensory and miscellaneous quality of experimental pain was
509 similar to the naturally occurring EIP experienced during the 20% MVT task, there were
510 differences in pain intensity and quality. In particular, the 20% MVT task produced a higher
511 pain intensity that was also described in the affective (e.g. 'exhausting') dimension. This
512 suggests that for hypertonic saline to induce a pain that feels like EIP, it needs to be *combined*
513 with at least light intensity exercise. When this was done, participants experienced an

514 elevated overall intensity of pain (compared to both 10% and 20% MVT) but were unable to
515 distinguish between the experimental muscle pain produced by the hypertonic saline and the
516 EIP from the muscular contraction. The findings of this study therefore provide support for
517 this hypertonic saline model for uncoupling the exercise intensity and EIP relationship (Cook
518 et al. 1997) – i.e. causing a light exercise intensity to *feel* like a harder exercise intensity.

519

520 *Effect of pain on isometric TTF*

521 The present study demonstrates that greater levels of pain in a fresh, undamaged, large
522 locomotor muscle group significantly shortens TTF during an isometric endurance task.
523 Indeed, TTF was significantly shorter in the 10% MVT + HYP condition than both the 10%
524 MVT and 10% MVT + ISO conditions, with an impaired performance of 12 to 26%. As all
525 conditions were performed at the same intensity (10% MVT) and with participants in a
526 similar psychological state, these differences in TTF can be attributed solely to increasing the
527 experience of pain in the 10% MVT + HYP condition, as clearly shown in Figure 1b.

528

529 Previous research that has used hypertonic saline to induce muscle pain have predominantly
530 applied it in smaller muscles or muscle groups (e.g. biceps brachii, tibialis anterior and
531 gastrocnemius (Graven-Nielsen et al. 1997d; Ciubotariu et al. 2004; Khan et al. 2011)) and
532 have not focused on producing a pain experience that feels like EIP. The VL is a large muscle
533 with a key role in the generation of force during basic locomotor tasks (e.g. walking, stair
534 climbing) and contributes to propulsive energy during cycling (Raasch et al. 1997), as well as
535 the stance and swing phase in running (Sasaki and Neptune 2006). Understanding the effects
536 of an increased overall pain experience in this muscle (and surrounding knee extensor group)
537 at a contraction intensity utilised during cycling exercise (Löllgen et al. 1980) therefore
538 provides information that closely translates to exercise performance and a clinical context.

539 Care should however be taken when extrapolating findings to whole-body exercise or
540 dynamic contraction.

541

542 During the impaired TTF performance in the 10% MVT + HYP condition, pain intensity was
543 significantly elevated in the first 20% of the task, with a continued linear increase until task
544 failure. Indeed, the intensity of pain reported in the 10% MVT + HYP condition was elevated
545 by approximately 3.3 at 10% iso-time and 2.8 at 20% iso-time on the VAS scale. The
546 hypertonic saline in the 10% MVT + HYP condition would have increased the activation of
547 the group III and IV nociceptive afferents *in addition* to the rapidly increasing noxious
548 environment arising from the metabolites produced as a result of the exercise task (O'Connor
549 and Cook 1999), which might explain the shorter TTF in the 10% MVT + HYP condition.

550

551 This explanation is in accordance with the “Sensory Tolerance Limit”, where in open-loop
552 exercise tasks (i.e. TTF) the increased inhibitory feedback from Group III and IV afferents
553 contributes to an individual and task-specific threshold, which when reached the exercise is
554 voluntarily terminated (Amann and Dempsey 2008; Amann 2011). With similar values for
555 RPE and ROF between conditions it is likely the elevated pain intensity during the TTF at
556 10% MVT + HYP resulted in this sensory tolerance limit being reached sooner, causing a
557 faster occurrence of task failure compared to the 10% MVT and 10% MVT + ISO conditions
558 (Aboodarda et al. 2020).

559

560 In addition, the increased nociceptive activity (a specific type of afferent feedback) may have
561 limited central motor drive and voluntary activation of the knee extensors (Amann et al.
562 2009, 2011; Aboodarda et al. 2020), a notion which is supported by evidence showing a
563 relationship between group III and IV muscle afferents and neuromuscular fatigue (Amann et

564 al. 2015; Sidhu et al. 2018), In support of this, Henriksen and colleagues (Henriksen et al.
565 2011) reported a reduced capacity of the knee extensors to produce a MVT in the presence of
566 pain. Furthermore, findings from Graven-Nielsen and colleagues (Graven-Nielsen et al.
567 2002) demonstrated that experimental muscle pain (from the hypertonic saline model)
568 reduces MVT despite an unaffected twitch torque, implying that performance decrements
569 were due to mechanisms residing in the central nervous system rather than the peripheral
570 musculature (Graven-Nielsen et al. 2002).

571

572 Rather than a uniform inhibitory/facilitatory effect on agonist and antagonist muscle activity
573 (Pain Adaptation Model, Lund et al. 1991), it is now recognised that pain does not cause
574 uniform inhibition/excitation effects across the motor neurone pool, but instead causes a
575 redistribution of activity within and between muscles (Hodges and Tucker 2011).

576 Accordingly, the decreased performance caused by the overall increased pain experience in
577 the current study could also be explained by a slight change in the direction of knee extensor
578 torque to a more lateral/medial plane (Tucker and Hodges 2010). In this context, the gross
579 feature of the task would remain (i.e. knee extension), but the efficiency of this movement
580 would be compromised. Motor unit recruitment order, or a recruitment of larger units at
581 lower torques, could have also affected the task performance. In an endurance task lasting
582 several minutes, the preferential recruitment of large high threshold motor units (which may
583 include Type II muscle fibres) above low threshold small motor units (Type I muscle fibres)
584 would likely have consequences for the rate at which fatigue occurs (both metabolic and
585 neural), leading to a shorter TTF (Edwards 1981). Whilst not observed in the present study,
586 an increase in sEMG would be indicative of an increased central drive to the muscle and/or
587 an increased recruitment of high threshold motor units (Gerdle et al. 2000), which would be

588 in-line with Hodges and Tucker’s “moving differently in pain” theory (Hodges and Tucker
589 2011).

590

591 *Methodological considerations*

592 The methods used in this study preclude the ability to identify which, or combination of these
593 mechanisms may have contributed to the shorter TTF. Indeed, combinations of peripheral
594 nerve/transcranial stimulation, multiple force transducers, and fine wire electrodes would be
595 required for this. In addition, the sensitivity of the sEMG set-up in the present study did not
596 allow for the detection in non-uniform changes across the motor neurone pool (i.e. any
597 alterations are unlikely to be discovered with bipolar sEMG). As such, an approach that
598 allows for the identification of individual motor units would be more appropriate for the
599 observation of subtle changes in activity within and between the muscles (i.e. high density
600 EMG). Differential responses to pain between male and female participants are also
601 acknowledged, with the present study not accounting for or attempting to control the
602 menstrual cycle of the female participants. Indeed, hormonal changes across the different
603 phases of the menstrual cycle may cause some difference in pain perception to experimental
604 pain (Sherman and LeResche 2006).

605

606 *Conclusion*

607 The injection of hypertonic saline into the VL during a sustained low-intensity isometric
608 contraction provides an overall qualitative experience of pain that feels like naturally
609 occurring EIP induced by a higher intensity exercise. When applied to submaximal exercise,
610 this additional pain caused a shorter TTF compared with a placebo and control condition. It is
611 plausible that the mechanisms responsible for the shorter TTF were related to increased
612 activity of group III and IV nociceptive afferents from the injected muscle. The present study

613 therefore provides important evidence that muscle pain has a direct impact on endurance
614 performance.

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