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Citation: Ansdell, Paul, Brownstein, Callum, Škarabot, Jakob, Hicks, Kirsty, Howatson, Glyn, Thomas, Kevin, Hunter, Sandra and Goodall, Stuart (2019) Sex differences in fatigability and recovery relative to the intensity-duration relationship. *The Journal of Physiology*, 597 (23). pp. 5577-5595. ISSN 0022-3751

Published by: Wiley-Blackwell

URL: <https://doi.org/10.1113/jp278699> <<https://doi.org/10.1113/jp278699>>

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The Journal of Physiology

<https://jp.msubmit.net>

**JP-RP-2019-278699**

**Title:** Sex differences in fatigability and recovery relative to the intensity-duration relationship

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**Author Conflict:** No competing interests declared

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**Running Title:** Sex differences in fatigability and recovery

**Dual Publication:** No

**Funding:** N/A: Paul Ansdell, Callum George Brownstein, Jakob Škarabot, Kirsty Marie Hicks, Glyn Howatson, Kevin Thomas, Sandra K. Hunter, Stuart Goodall, N/A

1 **Sex differences in fatigability and recovery relative to the intensity-**  
2 **duration relationship**

3  
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17  
18 Key Words: Exercise, fatigue, neuromuscular, NIRS, recovery, sex

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### ABSTRACT

33

34 Females are less fatigable than males during isometric exercise at intensities relative to maximal  
35 voluntary contraction (MVC), however whether a sex difference in fatigability exists when exercise is  
36 prescribed relative to a critical intensity is unknown. This study established the intensity-duration  
37 relationship, and compared fatigability and recovery between sexes following intermittent isometric  
38 contractions normalised to critical intensity. Twenty participants (10 females) completed four  
39 intermittent isometric knee extension trials to task failure to determine critical intensity and the  
40 curvature constant ( $W'$ ), followed by fatiguing tasks at +10 and -10% relative to critical intensity.  
41 Neuromuscular assessments were completed at baseline and for 45 minutes post-exercise. Non-  
42 invasive neurostimulation, near-infrared spectroscopy, and non-invasive haemodynamic monitoring  
43 were used to elucidate the physiological mechanisms responsible for sex differences. Females  
44 demonstrated a greater critical intensity relative to MVC than males ( $25\pm 3$  vs.  $21\pm 2\%$  MVC,  $P=0.003$ ),  
45 with no sex difference for  $W'$  ( $18,206\pm 6,331$  vs.  $18,756\pm 5,762$  N.s<sup>-1</sup>,  $P=0.850$ ). Time to task-failure was  
46 greater for females ( $62.37\pm 17.25$  vs.  $30.43\pm 12.75$  min,  $P<0.001$ ) during the +10% trial, and contractile  
47 function recovered faster post-exercise ( $P=0.034$ ). During the -10% trial females experienced less  
48 contractile dysfunction ( $P=0.011$ ). Throughout both tasks, females demonstrated greater increases in  
49 oxyhaemoglobin ( $P\leq 0.044$ ) and an attenuated exercise pressor reflex. These data show that a sex  
50 difference in fatigability exists even when exercise is matched for critical intensity. We propose that  
51 greater oxygen availability during exercise permits females to sustain a higher relative intensity than  
52 males, and is an explanatory factor for the sex difference in fatigability during intermittent, isometric  
53 contractions.

54

## KEY POINTS

- 55 • Females demonstrate greater fatigue-resistance than males during contractions at intensities  
56 relative to maximum force. However, previous studies haven't accounted for the influence of  
57 metabolic thresholds on fatigability.  
58
- 59 • This study is the first to test whether sex differences in fatigability exist when exercise intensity is  
60 normalised relative to a metabolic threshold: the critical intensity derived from assessment of the  
61 intensity-duration relationship during intermittent, isometric knee extensor contractions.  
62
- 63 • We show that critical intensity in females occurred at a higher percentage of maximum force  
64 compared to males. Furthermore, females demonstrated greater fatigue resistance at exercise  
65 intensities above and below this metabolic threshold.  
66
- 67 • Our data suggests that the sex difference was mediated by a greater capacity for females to  
68 maintain oxygenation of the knee-extensors during exercise.  
69
- 70 • These data highlight the importance of accounting for metabolic thresholds when comparing  
71 fatigability between sexes, whilst emphasising the notion that male data is not generalisable to  
72 female populations.

74 Insight into the metabolic demands of a fatiguing task and the mechanisms responsible for the  
75 attainment of task failure can be gained by determining the intensity-duration relationship, which is  
76 well described in males (Poole *et al.*, 1988; Deckerle *et al.*, 2003; Jones *et al.*, 2008; Vanhatalo *et al.*,  
77 2010). The duration that exercise can be maintained is progressively reduced as the intensity of the  
78 contraction increases, and the relationship becomes hyperbolic once a metabolic threshold, hereafter  
79 termed the critical intensity, has been exceeded (Jones *et al.*, 2010; Poole *et al.*, 2016). This  
80 phenomenon has been frequently reported during dynamic tasks (e.g. cycling and knee extension,  
81 Jones *et al.*, 2008; Vanhatalo *et al.*, 2010), and a similar relationship exists for intermittent, isometric  
82 tasks (Burnley, 2009; Burnley *et al.*, 2012). The critical intensity, the asymptote of the hyperbolic curve,  
83 represents the maximal sustainable work rate at which energy supply can be provided and sustained  
84 from oxidative metabolism (Poole *et al.*, 2016; Burnley & Jones, 2018). Below the critical intensity,  
85 substrate-level phosphorylation and the production of intramuscular metabolites are maintained at a  
86 steady state (Jones *et al.*, 2008; Black *et al.*, 2016). Exercise performed at intensities above the critical  
87 intensity requires ATP to be resynthesized from anaerobic metabolism, leading to a progressive loss  
88 of intramuscular homeostasis and a shorter time to task failure (Jones *et al.*, 2008; Vanhatalo *et al.*,  
89 2010; Schäfer *et al.*, 2019). Exercise above the threshold is associated with progressive intramuscular  
90 perturbations that can accelerate fatigability 4-5 times faster (Burnley *et al.*, 2012; Thomas *et al.*,  
91 2016). However, this has been described primarily in young males (Burnley, 2009; Burnley *et al.*, 2012).  
92 One study included both sexes, but did not conduct a sex comparison of the intensity-duration  
93 relationship (Pethick *et al.*, 2016). Whether the critical intensity differs between males and females  
94 for tasks where the sex difference in fatigability is commonly reported (e.g. intermittent isometric  
95 contractions, Hunter *et al.*, 2004; Ansdell *et al.*, 2018) is unknown, and could provide a physiological  
96 mechanism to explain these previous findings.

97 A range of reported physiological differences between males and females would suggest the critical  
98 intensity could differ between sexes for intermittent isometric tasks. Females are reported to be less  
99 fatigable than males across a range of exercise tasks and muscle groups, for contractions performed  
100 at the same intensity relative to maximal strength (Hunter, 2009, 2016a). The sex difference in  
101 fatigability is dependent upon the intensity and contraction modality of the task (Yoon *et al.*, 2007;  
102 Russ *et al.*, 2008; Hunter, 2016a, 2016b). During intermittent isometric contractions, females  
103 demonstrate greater fatigue-resistance compared to males, even when matched for maximal  
104 strength. The magnitude of the sex difference in fatigability might also be magnified at lower  
105 contraction intensities (Hunter *et al.*, 2004; Ansdell *et al.*, 2018), but it remains unclear whether the  
106 relationship between contraction intensity and task duration (time to task failure, i.e. fatigability)

107 differs between males and females, and whether the underlying mechanisms of fatigue differ. A  
108 crucial determinant of the intensity-duration relationship is oxygen delivery to the skeletal muscle,  
109 with positive correlations between critical intensity and the fraction of inspired oxygen (Vanhatalo *et al.*,  
110 *et al.*, 2010; Dekerle *et al.*, 2012). Critical power (during cycling exercise), for example, is positively  
111 correlated with type I fibre proportion and muscle capillarity of the knee extensor muscles (Vanhatalo  
112 *et al.*, 2016; Mitchell *et al.*, 2018). Typically, females have a greater proportion of type I muscle fibres  
113 (Simoneau & Boucard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006), which are less fatigable than  
114 type 2 fibres (Schiaffino & Reggiani, 2011). Females also exhibit greater capillarisation per unit of  
115 *vastus lateralis* muscle (Roepstorff *et al.*, 2006), and an augmented vasodilatory response of the  
116 femoral artery during exercise (Parker *et al.*, 2007). Furthermore, females exhibit greater skeletal  
117 muscle oxygenation and less deoxygenation during upper and lower limb exercise than males when  
118 assessed with near infrared spectroscopy; NIRS (Mantooth *et al.*, 2018; Marshall *et al.*, 2019). Whether  
119 these physiological sex differences could influence the critical intensity of the intensity –duration  
120 relationship for intermittent isometric contraction task is unknown.

121 Finally, recovery of exercise is also influenced by the aforementioned properties of skeletal muscle  
122 and could therefore differ between males and females; however, the extent of possible sex  
123 differences and the involved mechanisms of neuromuscular recovery are not understood. Limited  
124 evidence exists examining the sex difference of recovery for short durations after exercise (10-20  
125 minutes), showing that force producing capacity of female knee extensors recovers more rapidly than  
126 males (Senefeld *et al.*, 2018). Greater capillary density of the exercising muscle(s) can increase the  
127 rate of recovery from fatigue (Tesch & Wright, 1983; Casey *et al.*, 1996), possibly due to an increased  
128 rate of metabolite clearance and ATP/phosphocreatine re-synthesis post-exercise (Casey *et al.*, 1996;  
129 McDonough *et al.*, 2004), or a reversal in disruptions to calcium handling (Fitts & Balog, 1996). The  
130 latter has been shown to differ between sexes during exercise (Harmer *et al.*, 2014). There is a paucity  
131 of data relating to sex differences in recovery, and of the neural and contractile mechanisms involved  
132 following fatiguing exercise.

133 The present study had three primary aims: 1) to compare the relative torque (% MVC) at which critical  
134 intensity is achieved within the intensity-duration relationship for intermittent, isometric tasks in  
135 males and females; 2) determine the mechanisms that contribute to fatigability during intermittent  
136 isometric tasks at intensities of torque above and below the critical intensity in males and females;  
137 and 3) compare the rate of recovery following fatiguing exercise and the underpinning neuromuscular  
138 mechanisms. We *hypothesised* that: 1) due to greater oxygen availability within the muscle, females  
139 would demonstrate a higher critical intensity than men when expressed relative to MVC. 2) There

140 would be no sex difference in the time to task failure when the tasks were compared at the same  
141 metabolic intensity of contraction, relative to critical intensity. 3) Recovery from fatiguing exercise  
142 would be more rapid in females than males due to the properties of contractile elements of the  
143 muscle. To understand the mechanisms of fatigability and recovery both above and below the critical  
144 intensity in males and females, we used motor nerve and cortical stimulation to delineate the  
145 contractile and neural responses to exercise, as well as NIRS to determine the oxygenation of the  
146 muscles.

147

148

## METHODS

### 149 *Ethical Approval*

150 The study received institutional ethical approval from the Northumbria University Health and Life  
151 Sciences Research Ethics Committee (submission reference: 2434) and was conducted according to all  
152 aspects of the Declaration of Helsinki, apart from registration in a database. Participants provided  
153 written, informed consent to volunteer for the study.

154

### 155 *Participants*

156 Using the effect size for the sex difference in exercise tolerance at 50% MVC from Ansdell *et al.* (2017),  
157 a power calculation (alpha = 0.05, power 0.80) determined that a sample size of 16 participants was  
158 required. Therefore, to maximise statistical power, ten males (mean  $\pm$  SD age: 26  $\pm$  5 years, height:  
159 178  $\pm$  8 cm, mass: 83.4  $\pm$  14.4 kg) and ten females (age: 24  $\pm$  2 years, height: 168  $\pm$  9 cm, mass 68.5  $\pm$   
160 7.7 kg) were recruited to take part in the study. The females that volunteered were all using  
161 monophasic oral contraceptive pills (>6 months), and were tested in the 21-day consumption period  
162 of the pill cycle in order to negate the effects of endogenous hormones on neuromuscular function  
163 and fatigability (Ansdell *et al.*, 2019). Participants arrived at the laboratory rested and hydrated, with  
164 strenuous physical activity avoided for 48 hours, and caffeine and alcohol prohibited for 24 hours.

165

### 166 *Experimental Design*

167 All participants visited the laboratory seven times, completing a familiarisation visit, four constant load  
168 trials to estimate critical intensity, then trials 10% above and below critical torque (see *Experimental*  
169 *Protocol*). Testing took place over a three to five week period, with a minimum of 48 h between visits  
170 to permit full recovery of fatigue (Carroll *et al.*, 2016). The time of day for each testing session was  
171 replicated ( $\pm$  1 h) to account for diurnal variations in maximal force generating capacity and  
172 corticospinal excitability (Tamm *et al.*, 2009).

173

174 *Experimental Protocol*

175 *Visit 1: Familiarisation.* Participants were sat in the isometric dynamometer with hip and knee angles  
176 at 90°. This set up was replicated for all visits. Electrical nerve stimulation threshold was determined,  
177 followed by TMS hotspot, active motor threshold (aMT) and voluntary activation (VA) stimulator  
178 intensity determination (described below). Following this, a baseline neuromuscular function  
179 assessment was performed. After five minutes of passive rest, participants performed the fatiguing  
180 task at 60% MVC. An MVC and electrical stimulation was performed, each minute throughout the  
181 fatiguing task. Immediately following the fatiguing task, participants performed a 'post-exercise'  
182 neuromuscular assessment.

183

184 *Visits 2-5: Critical Intensity Estimation Trials.* To establish critical intensity, participants performed four  
185 trials to task failure. These involved intermittent isometric knee-extensor contractions at submaximal  
186 intensities between 40-80% MVC. The first trial was set at 60% MVC, based on the pre-exercise MVC  
187 in the first trial. The following three estimation trials were set at intensities that elicit task failure  
188 between 2 and 15 minutes in a randomised order (Burnley, 2009; Burnley *et al.*, 2012). Participants  
189 were instructed to match a target force displayed using a visual guideline on a computer screen ~1 m  
190 in front of them, and were blinded to the time elapsed in each trial. The contraction regime for all  
191 trials involved 3 s contractions interspersed with 2 s rest, with an MVC and electrical stimulation  
192 performed at the end of each minute. This contraction duty cycle has previously displayed sex  
193 differences independent of strength, and therefore occlusion differences between males and females  
194 (Ansdell *et al.*, 2017; Hunter *et al.*, 2006). Task failure was deemed as a failure to meet the target force  
195 three consecutive times despite strong verbal encouragement. Participants were informed each time  
196 they failed to reach the target force. Before the submaximal task, participants performed five 3 s MVCs  
197 separated by 30 s, with electrical stimulation during and 2 s after the final three contractions.  
198 Immediately following task failure this was repeated with three MVCs and superimposed electrical  
199 stimulations.

200

201 *Visits 6 and 7: Critical Intensity Trials.* The supra (+10%) and sub (-10%) critical intensity trials began  
202 with electrical nerve stimulation and TMS thresholds being determined. Baseline near infrared  
203 spectroscopy (NIRS) values were recorded once participants were sat in the dynamometer in the same  
204 position as the fatiguing task. NIRS data was captured for the entirety of the trials, and was used to  
205 measure changes in muscle oxygenation during the fatiguing task. Cardiac output ( $\dot{Q}$ ), heart rate (HR),  
206 and mean arterial pressure (MAP) were measured throughout the trial via a fingertip arterial pressure  
207 cuff (Finometer Midi, Finapres Medical System, Arnhem, The Netherlands). Participants completed a

208 standardised isometric warm up (Gruet et al., 2014), before a baseline neuromuscular function  
209 assessment. After five minutes of passive rest participants completed an intermittent isometric  
210 fatiguing task to failure at an intensity relative to their critical intensity (+10 or -10%). An MVC with  
211 electrical stimulation during and ~2 s following was performed and delivered at the end of each  
212 minute of the task to assess neuromuscular function (see below). The -10% trial was terminated after  
213 45 minutes, as this intensity contraction could theoretically be maintained indefinitely without task  
214 failure (Burnley et al., 2012). Therefore, male and female fatigability was compared after an identical  
215 'dose' of exercise. The intensity for the first critical intensity trial was randomised and  
216 counterbalanced. Upon task failure or termination, a post-test neuromuscular function assessment  
217 (see below) was immediately performed, then repeated at 15, 30 and 45 minutes post-exercise.

218

### 219 *Intensity-Duration Relationship*

220 Critical intensity and curvature constant ( $W'$ ) were estimated from the force-impulse relationship of  
221 the four submaximal trials. A linear regression between force impulse at task failure from the four  
222 submaximal trials against time to task failure was plotted to determine the characteristics of the  
223 relationship. The slope of the regression determined critical intensity, and the y-intercept determined  
224  $W'$  (Burnley et al., 2009, 2012). Critical intensity was expressed in Newtons, and as %MVC to account  
225 for sex differences in absolute force production.

226

### 227 *Measurements*

#### 228 Neuromuscular Function

229 Participants completed five isometric knee-extensor MVCs separated by 30 s, with electrical nerve  
230 stimulation delivered during and after the final three contractions to quantify voluntary activation  
231 ( $VA_{MNS}$ ) and quadriceps potentiated twitch force ( $Q_{tw.pot}$ ). In the final two visits (critical intensity trials)  
232 voluntary activation was also assessed with TMS ( $VA_{TMS}$ ) using two sets of five contractions (100, 87.5,  
233 75, 62.5, and 50% MVC, Dekerle *et al.*, 2019); single pulse TMS was delivered during each contraction.  
234 Finally, short interval cortical inhibition (SICI) and corticospinal excitability ( $MEP/M_{max}$ ) were assessed  
235 during a 10% MVC contraction.

236

237

238

#### 239 Force and EMG

240 Participants were seated on a custom-built chair, with force (N) measured using a calibrated load cell  
241 (MuscleLab force sensor 300, Ergotest technology, Norway). The load cell was attached to the

242 participant's dominant right leg, superior to the ankle malleoli, using a cuff. The load cell height was  
243 adjusted to ensure a direct line with the applied force for each participant. Participants were sat  
244 upright with knee and hip angles kept at 90° flexion. Electromyography (EMG) of the knee extensors  
245 was recorded from the *rectus femoris* (RF), with antagonist knee flexor activity recorded from the long  
246 head of the *biceps femoris* (BF). Skin was shaved and cleaned, surface electrodes (Ag/AgCl; Kendall  
247 H87PG/F, Covidien, Mansfield, MA, USA) were then placed 2 cm apart over the muscle belly, according  
248 to SENIAM guidelines (Hermens *et al.*, 2000), with a reference electrode placed over the patella. EMG  
249 electrodes recorded signals during maximal and submaximal contractions and were quantified as root-  
250 mean-square amplitude (rmsEMG). Compound muscle action potentials (maximal M-wave) following  
251 motor nerve stimulation, and MEPs elicited by TMS were also recorded. Surface electrode signals were  
252 amplified ( $\times 1,000$ ; 1902, Cambridge Electronic Design, Cambridge), band-pass filtered (20-2,000 Hz),  
253 digitized (4 kHz, micro 1401, Cambridge Electronic Design) and acquired for off-line analysis (Spike2  
254 version 7.01, Cambridge Electronic Design).

255

#### 256 Transcranial Magnetic Stimulation

257 Single and paired pulse magnetic stimuli of 1 ms duration were delivered over the contralateral motor  
258 cortex (postero-anterior intracranial current flow) with a concave double cone coil (110 mm diameter,  
259 maximum output 1.4 Tesla) powered by a BiStim unit and two Magstim 200<sup>2</sup> stimulators (The Magstim  
260 Company, Whitland, UK). Optimal stimulation location was located by stimulating at ~50% maximum  
261 stimulator output during a 10% MVC contraction. The position eliciting the greatest MEP amplitude in  
262 the RF, and a concurrent small MEP in the antagonist BF muscle, was marked with indelible ink to  
263 ensure consistent placement within trials. Stimulator output for aMT was defined as the lowest  
264 stimulus intensity required to elicit a MEP of at least 0.2 mV in three out five stimulation in the RF  
265 during a 10% MVC contraction. Mean aMT was not different between males and females ( $39 \pm 7$  vs.  
266  $43 \pm 10\%$ ,  $P = 0.379$ ), or between visits ( $41 \pm 9$  vs.  $41 \pm 9\%$ ,  $P = 0.423$ ). The conditioning pulses were  
267 delivered at 70% aMT, 2 ms prior to a test stimulus at 120% aMT during a 10% MVC contraction  
268 (Brownstein *et al.*, 2018). Ten unconditioned and ten conditioned stimuli were delivered and the  
269 resultant motor evoked potential (MEP) amplitudes were averaged and presented as a normalised  
270 value ((conditioned MEP/unconditioned MEP) $\times 100$ ) as an index of SICI. The average amplitude of the  
271 unconditioned pulse normalised to maximal M-wave was used as an index of corticospinal excitability.  
272 Stimulator output for VA<sub>TMS</sub> was determined as the greatest mean SIT elicited by two pulses delivered  
273 during a ~6 s contraction at 50% MVC, as TMS intensity was increased in a step-wise (i.e. 5%  
274 increments) fashion from 50% maximal stimulator output (Thomas *et al.*, 2016; Brownstein *et al.*,  
275 2017). Each contraction was separated by 30 s rest. Mean stimulator intensity was not different

276 between males and females ( $63 \pm 6\%$  vs.  $66 \pm 11\%$ ,  $P = 0.462$ ) or between visits ( $66 \pm 10\%$  vs.  $63 \pm 7\%$ ,  
277  $P = 0.218$ ). The intensities used activated a large proportion of the motoneuron pool for the RF that  
278 was not different between trials at baseline ( $53 \pm 13\%$   $M_{MAX}$  vs.  $53 \pm 16\%$   $M_{MAX}$ ,  $P = 0.920$ ). The TMS  
279 pulse also avoided substantial activation of the antagonist (*biceps femoris*) with small incidental MEPs  
280 recorded at baseline ( $0.68 \pm 0.52$  mV vs.  $0.70 \pm 0.1$  mV,  $P = 0.902$ ).

281

#### 282 Motor Nerve Stimulation

283 Single electrical stimuli (200  $\mu$ s duration) were delivered to the femoral nerve via 32 mm-diameter  
284 surface electrodes (CF3200; Nidd Valley Medical, North Yorkshire, UK) using a constant-current  
285 stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode was placed high  
286 in the femoral triangle over the nerve, and the anode positioned midway between the greater  
287 trochanter and iliac crest. The cathode was repositioned until the largest knee extensor twitch  
288 amplitude ( $Q_{tw}$ ) and maximal RF M-wave ( $M_{MAX}$ ) was elicited at rest. Stimulations began at 20 mA, and  
289 increased by 20 mA until a plateau in  $Q_{tw}$  and M-wave amplitude occurred. This value was then be  
290 increased by 30% to ensure supramaximal stimulations during the protocol. Mean stimulus intensity  
291 was not different between sexes ( $276 \pm 142$  vs.  $190 \pm 75$  mA,  $P = 0.057$ ) or between visits ( $241 \pm 104$   
292 vs.  $229 \pm 107$  mA,  $P = 0.492$ ).

293

#### 294 Near Infrared Spectroscopy

295 A multi-distance, continuous-wave, single channel NIRS (NIRO-200NX, Hamamatsu, Hamamatsu City,  
296 Japan) evaluated changes in *vastus lateralis* muscle oxy- ( $HbO_2$ ), and deoxy- (HHb) haemoglobin  
297 concentrations [ $\mu$ M], as well as tissue oxygenation index ( $TOI = HbO_2 \div [HbO_2 + HHb] \times 100$ ), sampled  
298 at a rate of 1 Hz. The light-emitting probe comprised of diodes operating at three wavelengths (735,  
299 810, and 850 nm). The probe was placed on the *vastus lateralis*, 20 cm above the fibular head lateral  
300 side of the patella (Keane *et al.*, 2018). Optodes were held in place by an elasticised, tensor bandage  
301 and covered by an opaque, dark material to avoid motion and ambient light influences. Pre-exercise,  
302 participants remained seated and avoided muscle contraction for five minutes to establish baseline  
303 muscle oxygenation, with the final 30 s used as the pre-exercise value. During the fatiguing tasks, the  
304 30 s window around 25, 50, 75% of the task, as well as the final 30 s of the task (100%), were expressed  
305 as changes from baseline ( $\Delta\%$ ).

306

#### 307 Haemodynamic Monitoring

308 Mean arterial blood pressure and heart rate were measured continuously throughout the final two  
309 testing visits using finger arterial pressure pulse wave analysis (Finometer Midi, Finapres Medical

310 System, Arnhem, The Netherlands). This system was also used to estimate  $\dot{Q}$  using the Modelflow  
311 equation (Wesseling *et al.*, 1993). An appropriately sized cuff was placed between the distal proximal  
312 inter-phalangeal joint of the middle finger. To minimise the effect of arm and hand movement during  
313 the trials, arm position was maintained stationary throughout the trial. To account for hydrostatic  
314 pressure differences between the level of the hand and heart, a height correction unit was used. The  
315 Finapres was activated prior to the exercise tasks to allow calibration via the Physiological function within  
316 the BeatScope software. This technique has previously been validated and shown to be reliable at rest  
317 and in exercise conditions (Parati *et al.*, 1989; Waldron *et al.*, 2017). Signals were linearly interpolated  
318 and resampled at 1 Hz (Faisal *et al.*, 2009), then a 5 s rolling average was used to smooth the data  
319 (Beltrame *et al.*, 2017), before 30 s time intervals were taken pre-exercise, 25, 50, 75 and 100% of  
320 time to task failure. Pre-exercise, participants remained seated for five minutes to establish baseline  
321 values, with the final 30 s used as pre-exercise values.

322

### 323 *Data Analysis*

324 Voluntary activation using motor nerve stimulation was determined using the twitch interpolation  
325 method (Merton 1954) by comparing the amplitude of the superimposed twitch (SIT) with the  
326 amplitude of the potentiated resting twitch ( $Q_{tw.pot}$ ) using the following formula:  $VA_{MNS} (\%) = (1 - [SIT$   
327  $\div Q_{tw.pot}]) \times 100$ . Voluntary activation using TMS was assessed during two sets of contractions at 100,  
328 87.5, 75, 62.5 and 50% MVC (Dekerle *et al.* 2019). Single pulse TMS was delivered during each  
329 contraction, and the linear regression between SIT amplitude and contraction intensity was  
330 extrapolated to the y intercept to obtain an estimated resting twitch (ERT; Todd *et al.* 2003). In order  
331 to achieve significant linearity ( $P < 0.05$ ), a total of five out of 850 SITs across all trials were excluded  
332 (0.6%), which led to five regressions containing 9 data points rather than 10 (1 pre-exercise, 4 post-  
333 exercise). As a result, mean  $r^2$  values for ERTs were linear throughout the study ( $0.93 \pm 0.06$ ). The SIT  
334 during 100% MVC was compared with the ERT using the following formula:  $VA_{TMS} (\%) = (1 - [SIT \div ERT])$   
335  $\times 100$ . Short interval intracortical inhibition was quantified as the percentage ratio between the  
336 amplitude of conditioned MEPs to the amplitude of unconditioned MEPs. Corticospinal excitability  
337 was determined by expressing the mean MEP amplitude during the 10% MVC as a percentage of  $M_{MAX}$ .  
338 The root-mean-square of EMG activity (rmsEMG) was recorded during the preceding 100 ms before  
339 each stimulation, and the middle 500 ms epoch of each 3 s contraction during the fatiguing task.  
340 rmsEMG was then expressed as a percentage of  $M_{max}$ . The NIRS ( $O_2Hb$ ,  $HHb$ ,  $TOI$ , and  $cHb$ ) and  
341 Finapres (HR,  $\dot{Q}$ , MAP) data were expressed as a percentage of baseline, and the 30 s epochs  
342 throughout exercise are presented as  $\Delta\%$ .

343

344 Despite a linear relationship between TTF and work done in the estimation trials ( $r^2 = 0.98$ ), and a  
345 physiologically normal value for the critical intensity (22.7% MVC), one female participant  
346 demonstrated no signs of fatigability during the supra-critical intensity trial (i.e. MVC did not  
347 decrease), thus the trial was terminated after 90 minutes, and the participant was excluded from  
348 further analyses. Similarly, one male was excluded due to the intensity-duration relationship residing  
349  $>3$  SDs from the mean value for males (critical intensity = 31.3% MVC,  $W' = 2005 \text{ N}\cdot\text{s}^{-1}$ ), likely caused  
350 by premature task failure in the higher intensity estimation trial(s).

351

### 352 *Statistical Analysis*

353 Data are presented as mean  $\pm$  SD within the text and figures. Normal Gaussian distribution of data  
354 was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data was  
355 logarithmically transformed. This occurred for  $\text{rmsEMG}/M_{\text{max}}$  during the fatiguing tasks, therefore,  
356 statistical tests were run on the transformed data, but in text and figures the non-transformed data is  
357 presented. The alpha for all statistical tests was set at  $P < 0.05$ .

358

359 For variables assessed pre-, during, and post- exercise (MVC,  $VA_{\text{MNS}}$ ,  $Q_{\text{tw.pot}}$ ,  $\text{rmsEMG}$ ,  $O_2\text{Hb}$ , HHb, TOI,  
360 HR, CO, and MAP) a two-way (2 $\times$ 5) repeated measures ANOVA was used to assess differences  
361 between sex (male vs. female) and over time (Pre, 25, 50, 75% TTF, and Post). For variables that were  
362 assessed pre and post-exercise (ERT,  $VA_{\text{TMS}}$ ,  $M_{\text{MAX}}$ ,  $\text{MEP}/M_{\text{max}}$ , SICI) a two-way 2 $\times$ 2 repeated measures  
363 ANOVA was used to assess differences between sex (male vs. female) and over time (Pre vs. Post). For  
364 variables that were assessed during the recovery period (MVC,  $VA_{\text{MNS}}$ ,  $Q_{\text{tw.pot}}$ , ERT,  $VA_{\text{TMS}}$ ,  $M_{\text{MAX}}$ ,  
365  $\text{MEP}/M$ , SICI) a two way (2 $\times$ 4) repeated measures ANOVA was used to assess difference between sex  
366 (male vs. female) and over time (Post, and 15, 30 and 45 min post-exercise). If significant main or  
367 interaction effects were observed, these were followed up by *post-hoc* Bonferroni-corrected pairwise  
368 comparisons.

## RESULTS

369

### 370 *Intensity-Duration Relationship*

371 The trials to estimate the intensity-duration relationship ranged from 1.6 – 16.0 minutes in duration  
372 (Table 1). In order to match the TTFs between sexes, the trial intensities were required to be greater  
373 in females than the males (mean difference of 10-11% MVC for the four trials, all  $P < 0.001$ ).  
374 Furthermore, the relationship between TTF and impulse across the four trials was linear ( $r^2$  range: 0.89  
375 – 1.00) for all participants (Figure 1A).

376

377 \*Figure 1 here\*

378

379 Maximal voluntary contraction was greater in males compared to females ( $708 \pm 119$  N vs.  $458 \pm 59$   
380 N,  $P < 0.001$ ); however, absolute critical intensity was not significantly different ( $143 \pm 26$  N vs.  $123 \pm$   
381  $26$  N,  $P = 0.109$ ). When normalised to MVC, females had a greater critical intensity compared to  
382 males ( $24.7 \pm 2.5$  vs.  $20.8 \pm 2.3\%$  MVC,  $P = 0.003$ , Figure 1B), however, there was no difference in  $W'$   
383 ( $18,206 \pm 6,331$  vs.  $18,765 \pm 5,762$  N.s<sup>-1</sup>,  $P = 0.850$ , Figure 1C).

384

385 Males and females demonstrated a consistent decline in MVC,  $Q_{tw.pot}$ , and  $VA_{MNS}$  across the four  
386 estimation trials (Figure 2, Trial  $\times$  Time interactions  $P \geq 0.144$ ), confirming that all trials took place in  
387 the same exercise intensity domain.

388

389 \*Figure 2 here\*

390

### 391 *Supra (+10%) Critical Intensity Trials*

#### 392 *Fatigability*

393 Compared to males, females had a greater time to task failure for the intermittent isometric  
394 contraction tasks performed at 110% of critical intensity ( $3,742 \pm 1,035$  vs.  $1,826 \pm 765$  s,  $P < 0.001$ ,  
395 Figure 3).

396

397 \*Figure 3 here\*

398

399 Throughout the +10% task and at task failure MVC,  $Q_{tw.pot}$ ,  $VA_{MNS}$ ,  $VA_{TMS}$ , and  $MEP/M_{max}$  all decreased  
400 (all time effects  $P < 0.001$ , Figures 4 and 5), whilst  $rmsEMG/M_{max}$  increased ( $P < 0.001$ , Table 2).  
401 However, SICI ( $P = 0.232$ ) and  $M_{max}$  ( $P = 0.109$ ) did not change. When comparing the changes between  
402 sexes, MVC ( $F_{2,2,34.5} = 4.36$ ,  $P = 0.017$ ,  $\eta_p^2 = 0.214$ ), and  $Q_{tw.pot}$  ( $F_{4,64} = 2.52$ ,  $P = 0.049$ ,  $\eta_p^2 = 0.136$ )

403 decreased more in males compared with the females (Figure 4, panel A & B), whilst the rmsEMG/ $M_{\max}$   
404 increased more in the males than the females ( $F_{2,2,34,5} = 7.33$ ,  $P = 0.002$ ,  $\eta_p^2 = 0.314$ ). However,  $VA_{MNS}$ ,  
405  $VA_{TMS}$ , MEP/ $M_{\max}$ , and SIC1 were not different between the sexes ( $P \geq 0.062$ ).

406

407 \*Figure 4 here\*

408

409 Recovery

410 In the 45 minute recovery period, MVC,  $Q_{tw.pot}$ ,  $VA_{MNS}$ ,  $VA_{TMS}$ , and MEP/ $M_{\max}$  all demonstrated a return  
411 towards baseline (recovery effects all  $P < 0.001$ , Figures 4 and 5). Females however, demonstrated a  
412 faster recovery for  $Q_{tw.pot}$  ( $F_{3,48} = 3.13$ ,  $P = 0.034$ ,  $\eta_p^2 = 0.164$ ), and  $VA_{TMS}$  ( $F_{1.8,25.4} = 3.63$ ,  $P = 0.045$ ,  $\eta_p^2$   
413  $= 0.206$ ), with no difference in recovery for MVC,  $VA_{MNS}$ , or MEP/M ( $P \geq 0.096$ ).

414

415 \*Figure 5 here\*

416

417 Oxygenation and Haemodynamics

418 Muscle oxygenation was altered during the +10% fatiguing task (Figure 6), with  $O_2Hb$  ( $F_{1.4,22.5} = 7.00$ ,  $P$   
419  $= 0.009$ ,  $\eta_p^2 = 0.304$ ), HHb ( $F_{1.4,22.5} = 11.53$ ,  $P = 0.003$ ,  $\eta_p^2 = 0.419$ ), and TOI ( $F_{1.1,18.3} = 7.12$ ,  $P = 0.004$ ,  $\eta_p^2$   
420  $= 0.393$ ) all demonstrating changes from baseline. Females demonstrated a lesser increase in HHb  
421 ( $F_{1.4,22.5} = 8.96$ ,  $P = 0.007$ ,  $\eta_p^2 = 0.359$ ), and decrease in TOI ( $F_{1.2,18.3} = 7.12$ ,  $P = 0.013$ ,  $\eta_p^2 = 0.308$ ) than  
422 males (Figure 6B and C). For  $O_2Hb$ , females demonstrated an increase from baseline, whilst males  
423 decreased ( $F_{1.4,22.5} = 8.05$ ,  $P = 0.005$ ,  $\eta_p^2 = 0.335$ , Figure 6A).

424

425 \*Figure 6 here\*

426

427 The +10% fatiguing task induced changes in cardiovascular function (Table 2) with HR ( $F_{4,64} = 47.39$ ,  $P$   
428  $< 0.001$ ,  $\eta_p^2 = 0.748$ ),  $\dot{Q}$  ( $F_{4,64} = 19.70$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.552$ ), and MAP ( $F_{4,64} = 12.24$ ,  $P < 0.001$ ,  $\eta_p^2 =$   
429  $0.433$ ) all increasing. Females demonstrated a lesser increase in HR ( $F_{4,64} = 8.99$ ,  $P < 0.001$ ,  $\eta_p^2 =$   
430  $0.360$ ) and  $\dot{Q}$  ( $F_{4,64} = 4.02$ ,  $P = 0.006$ ,  $\eta_p^2 = 0.201$ ), but not MAP ( $P = 0.175$ ).

431

432 *Sub (-10%) Critical Intensity Trials*

433 Fatigability

434 All participants successfully completed the 45 minutes of exercise below critical intensity and did not  
435 reach task failure. MVC,  $Q_{tw.pot}$ ,  $M_{\max}$ ,  $VA_{MNS}$ , and  $VA_{TMS}$  all decreased (time effects:  $P \leq 0.016$ )  
436 throughout the intermittent isometric task, whereas rmsEMG/ $M_{\max}$  ( $P = 0.020$ ), and MEP/M ( $P =$

437 0.017) increased. Short interval intracortical inhibition did not change ( $P = 0.061$ ). Of these variables,  
438 a sex  $\times$  time interaction was demonstrated for  $Q_{tw.pot}$  ( $F_{1.97,31.49} = 5.31, P = 0.011, \eta_p^2 = 0.249$ ) indicating  
439 a lesser decrease over the course of the intermittent isometric task. Post-hoc differences are displayed  
440 in Figure 7 and Table 2.

441

#### 442 Recovery

443 In the 45 minute recovery period the MVC,  $Q_{tw.pot}$ ,  $VA_{MNS}$ , and  $VA_{TMS}$  increased (recovery effects:  $P \leq$   
444 0.032). Conversely,  $M_{max}$  ( $P = 0.267$ ), MEP/M ( $P = 0.080$ ), and SICI ( $P = 0.085$ ) demonstrated no  
445 recovery effects. Of the variables demonstrating recovery effects,  $VA_{TMS}$  demonstrated a sex  $\times$  time  
446 interaction ( $F_{1.45,20.26} = 4.57, P = 0.033, \eta_p^2 = 0.246$ ), indicating a faster recovery in females compared  
447 with males. No other variables (MVC,  $Q_{tw.pot}$ , and  $VA_{MNS}$ ) demonstrated this sex by time interaction ( $P$   
448  $\geq 0.069$ ).

449

450

\*Figure 7 here\*

451

#### 452 Oxygenation and Haemodynamics

453 Muscle oxygenation was altered during the intermittent isometric task (Figure 8). Whilst  $O_2Hb$  ( $F_{1.6,26.5}$   
454  $= 10.27, P = 0.001, \eta_p^2 = 0.391$ ) increased, HHb did not change ( $P = 0.945$ ), and TOI decreased ( $F_{1.36,21.71}$   
455  $= 4.98, P = 0.027, \eta_p^2 = 0.237$ ). Of these variables,  $O_2Hb$  demonstrated a sex  $\times$  time interaction ( $F_{1.64,26.25}$   
456  $= 3.77, P = 0.044, \eta_p^2 = 0.191$ ), indicating a greater increase in females compared with males.

457

458

\*Figure 8 here\*

459

460 Heart rate ( $F_{2.6,41.5} = 18.42, P < 0.001, \eta_p^2 = 0.535$ ),  $\dot{Q}$  ( $F_{2.83,45.23} = 4.06, P = 0.014, \eta_p^2 = 0.380$ ) and MAP  
461 ( $F_{1.96,31.37} = 6.72, P = 0.004, \eta_p^2 = 0.296$ ) all increased throughout the intermittent isometric task (Table  
462 2), with a sex  $\times$  time interaction for HR ( $F_{2.59,41.49} = 5.59, P = 0.004, \eta_p^2 = 0.259$ ), indicating a greater  
463 increase in HR in males than females.

464

465

## DISCUSSION

466 The present study aimed to compare the intensity-duration relationship between males and females  
467 during intermittent, isometric knee extensor exercise, and assess whether a sex difference in  
468 fatigability and recovery existed when exercise was normalised to the critical intensity. We found that  
469 females demonstrated a greater relative critical intensity during intermittent isometric knee extensor  
470 exercise compared with males. Contrary to our hypothesis however, females lasted approximately  
471 twice as long than males for an open-ended exercise 10% above this threshold. Following exercise  
472 normalised to 110% and 90% of critical intensity, females demonstrated a lower degree of contractile  
473 impairment, and a faster rate of recovery following the 110% trial. Furthermore, females were able to  
474 better maintain muscle oxygenation during exercise, which provides a plausible explanation for the  
475 observed sex differences in exercise tolerance and fatigability. These data are the first to demonstrate  
476 that when normalised to a maximum, critical intensity is greater for females during intermittent  
477 isometric contractions. This was likely underpinned by a greater ability to maintain muscle  
478 oxygenation, observed via NIRS, and led to a more fatigue-resistant muscle in females.

479

### *Intensity-Duration Relationship*

480  
481 Of the two parameters of the intensity-duration relationship, a sex difference was observed for critical  
482 intensity, but not  $W'$ . Females had a critical threshold  $\sim 4\%$  MVC greater than males, due to a steeper  
483 slope in the TTF-impulse relationship (Figure 1A) and smaller absolute MVC. Critical intensity notes  
484 the maximal sustainable metabolic rate during exercise, at which oxidative energy provision is  
485 sufficient and reaches a steady state (Poole *et al.*, 2016). Increasing the fraction of inspired oxygen  
486 ( $FiO_2$ ) during exercise increases critical intensity (Vanhatalo *et al.*, 2010), whereas decreasing  $FiO_2$   
487 reduces the maximal sustainable intensity (Dekerle *et al.*, 2012). Similarly, complete blood flow  
488 occlusion reduces critical power to less than zero (Broxterman *et al.*, 2015a). Differences in skeletal  
489 muscle properties between males and females could explain the difference in critical intensity. It is  
490 well established that in the *vastus lateralis*, females possess a greater relative proportion of type I  
491 muscle fibres (Simoneau & Bouchard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006) and greater  
492 capillary density (Roepstorff *et al.*, 2006) than males. When combined with a greater vasodilatory  
493 response of the femoral artery to exercise in females (Parker *et al.*, 2007), it is likely that these factors  
494 permit greater delivery of oxygenated blood to the muscle tissues of the knee-extensors, contributing  
495 to an ability to sustain greater rates of oxidative metabolism (i.e. critical intensity) than males. These  
496 observations could explain why females were able to attain a higher relative critical intensity than  
497 males in the present study. Indeed, recent evidence suggests that type I fibre % and muscle  
498 capillarisation are positively correlated with critical power during cycling exercise (Vanhatalo *et al.*,

499 2016; Mitchell *et al.*, 2018). Mitchell *et al.* (2018) suggested that greater capillary supply likely leads  
500 to greater oxygen supply and extraction during exercise. To support this, during both +10% and -10%  
501 trials in the present study, a sex difference was observed for O<sub>2</sub>Hb, with females demonstrating  
502 greater increases from resting values (Figures 5 and 7). Therefore, the present data suggest that  
503 females are able to maintain elevated delivery of oxygen to the knee-extensors, leading to a greater  
504 relative rate of maximal sustainable oxidative metabolism.

505

506 The curvature constant of the intensity-duration relationship ( $W'$ ), was not different between sexes.  
507 Whilst less is known about the origins and determinants of  $W'$  (Poole *et al.*, 2016), evidence suggests  
508 that there is no relationship between it and skeletal muscle properties (Vanhatalo *et al.*, 2016; Mitchell  
509 *et al.*, 2018). More likely,  $W'$  is related to the depletion of intramuscular energy stores (e.g.  
510 phosphocreatine, PCr) and accumulation of metabolites (e.g. Pi, H<sup>+</sup>, ADP; Vanhatalo *et al.*, 2010). This  
511 notion has been suggested to oversimplify such a concept, with the possibility of a different source in  
512  $W'$  between whole-body and single-muscle exercise (Poole *et al.*, 2016). However, in single-muscle  
513 exercise, Broxterman *et al.* (2015b) suggested that  $W'$  might be related to the maximum tolerable  
514 degree of neuromuscular dysfunction. Considering there was no difference in the  $\Delta\%$  in MVC,  $Q_{tw,pot}$ ,  
515 and  $VA_{MNS}$  between males and females at task-failure in the +10% trial (Figure 3A), this notion could  
516 explain why  $W'$  was not different between sexes in the intermittent, isometric model used in the  
517 present study.

518

#### 519 *Fatigability and Recovery above Critical Intensity*

520 Despite normalising exercise to the intensity-duration relationship, which is a key step when modelling  
521 fatigability (Burnley & Jones, 2018), females outlasted males during the open-ended isometric  
522 intermittent contraction task (Figure 3). A similar  $W'$  in males and females would suggest that task  
523 failure should occur in a similar time, as evidence previously suggested task failure occurs once this  
524 work capacity is completely utilised and a 'critical metabolic milieu' is attained (Vanhatalo *et al.*, 2010).  
525 One potential explanation could be the absolute force produced by females was ~40% lower than  
526 males in the present study. This meant for a male and female with identical critical intensity (%MVC)  
527 and  $W'$ , the impulse ( $N \cdot s^{-1}$ ) per contraction was lower in absolute terms during the +10% fatiguing task  
528 for contractions at similar %MVC. This led to a slower rate of  $W'$  utilisation and decrease in indices of  
529 neuromuscular function during the fatiguing task (e.g. MVC,  $Q_{tw,pot}$ ,  $VA_{MNS}$ ), until a constant degree of  
530 post-exercise dysfunction was reached at task failure (Figure 4). In the present dataset it was not  
531 possible to *post hoc* match individual male and female participants for critical intensity,  $W'$  and MVC  
532 within <10% of each other, thus, it is not possible to discount the potential effect of absolute force.

533 The sex difference in critical intensity and fatigability above critical intensity can therefore explain  
534 previous studies that have normalised to an arbitrary % of MVC and shown a sex difference in  
535 fatigability (e.g. 50% MVC, Hunter *et al.*, 2004; Ansdell *et al.*, 2018). For example, at 50% MVC, males  
536 would be exercising at a greater relative intensity above their threshold, therefore would experience  
537 a faster rate of fatigue (Burnley *et al.*, 2012), but also as a consequence of greater absolute force  
538 production, deplete  $W'$  faster.

539

540 Following the +10% trial, females demonstrated a faster rate of recovery for  $Q_{tw,pot}$  (Figure 3B), which  
541 supports the conclusions of (Senefeld *et al.*, 2018) who demonstrated a similar pattern following a  
542 fixed-duration dynamic fatiguing task. Recovery of contractile function following long-duration  
543 isometric exercise is related predominantly to restoration of intracellular calcium handling/sensitivity,  
544 rather than metabolite clearance (Carroll *et al.*, 2016). Female skeletal muscle demonstrates a 24%  
545 lower maximal rate of  $Ca^{2+}$ ATPase activity (Harmer *et al.*, 2014), which has previously been suggested  
546 to lead to lower calcium-related impairments during exercise, and create a more fatigue-resistant  
547 muscle compared to males (Hunter, 2014). Thus, it could be the case that differences in calcium  
548 handling in female skeletal muscle translated to better post-exercise recovery kinetics. Although  
549 somewhat speculative, calcium handling has been studied *in vitro* to support the sex difference in  
550 fatigability (Harmer *et al.*, 2014), but no similar data in cell models exists to compare recovery of  
551 calcium handling between males and females after exercise. Therefore, calcium-related properties of  
552 skeletal muscle could help to explain why female contractile function recovered quicker in the present  
553 study, but further research to support this proposition is warranted.

554

#### 555 *Fatigability and Recovery below Critical intensity*

556 For the same duration of exercise below critical intensity, both sexes experienced an initial decrease  
557 in MVC and  $Q_{tw,pot}$ , then no further impairment throughout the fatiguing task (Figure 6). Whilst females  
558 experienced a lesser decrease from baseline, the attainment of a constant degree of contractile  
559 dysfunction is consistent with the notion that exercise below the critical intensity reaches a 'steady-  
560 state' of metabolic adjustment (Burnley & Jones, 2018). A similar study in males (Burnley *et al.*, 2012)  
561 speculated that the origins of contractile dysfunction below threshold might be related to the effects  
562 glycogen depletion had on calcium transients in skeletal muscle (Ørtenblad *et al.*, 2013). During whole-  
563 body exercise, females oxidise relatively more fat than carbohydrate compared to males (Roepstorff  
564 *et al.*, 2002, 2006); when combined with the more fatigue-resistant calcium properties in female  
565 muscles (Harmer *et al.*, 2014), this could explain why the post-exercise  $\Delta\%$  in  $Q_{tw,pot}$  was less in females  
566 (Figure 7C). Similar to the +10% trial, females were better able to maintain oxygen availability within

567 the working muscles (Figure 7A), however, this is not thought to be a limiting factor to exercise  
568 performance below critical intensity (Poole *et al.*, 2016), as oxidative metabolism is not at maximal  
569 rates. Post-exercise, MVC,  $Q_{tw,pot}$  and VA all demonstrated returns towards baseline, however, male  
570  $Q_{tw,pot}$  was still reduced 45 minutes post-exercise. If muscle glycogen-related factors are the cause of  
571 this contractile impairment below threshold, the continued impairment at 45 minutes would be  
572 expected, as complete re-synthesis can take >2 hours following single-limb exercise (Pascoe *et al.*,  
573 1993).

574

#### 575 *Further Considerations*

576 The responses to corticospinal stimulation (MEP/ $M_{max}$ ) showed divergent effects when comparing  
577 pre-post exercise changes above and below critical intensity. Following the +10% trial, a depression  
578 was observed (Figure 4E), whereas following the -10% trial, a facilitatory effect occurred (Figure 6E).  
579 During whole-body exercise, fatigue induced at high-intensities is suggested to activate group III/IV  
580 afferent neurons, causing inhibition of spinal motoneurons (Weavil *et al.*, 2016) and increasing  
581 GABAergic inhibition within the motor cortex (Sidhu *et al.*, 2018). These adjustments are suggested to  
582 reduce the capacity of the central nervous system to activate the working muscles during exercise  
583 (Sidhu *et al.*, 2017); this could explain why the reduction in corticospinal excitability was only observed  
584 above critical intensity, when decreases in measures of VA were also demonstrated. The present study  
585 assessed the activity of group III/IV neurons indirectly through the monitoring of the metaboreflex,  
586 and demonstrated an augmented response above threshold (Table 2). Interestingly, females had a  
587 lesser increase in HR and  $\dot{Q}$  during the +10% trial, which could explain the slower rate of central  
588 nervous system dysfunction (Figure 4C). On the contrary, moderate intensity exercise increases  
589 corticospinal excitability (Lulic *et al.*, 2017). This effect occurs at lower intensities without the  
590 development of fatigue or the attainment of task failure; such an effect was observed in the present  
591 study, where facilitatory effects were only evident during exercise below critical intensity, alongside  
592 minor decrements in VA. The critical intensity might therefore provide an integrative neuromuscular  
593 threshold at which facilitatory neuroplasticity is attainable after exercise beneath. Future research  
594 should investigate the effects of exercise intensity on both MEPs and spinal evoked potentials in the  
595 lower limbs (e.g. Škarabot *et al.*, 2019) to discern the aetiology of exercise-induced neuroplasticity.

596

597 The present study utilised an intermittent, isometric model of exercise (Burnley, 2009; Burnley *et al.*,  
598 2012) to compare the intensity-duration relationship between sexes. Whilst the principles of the  
599 model remain the same across different exercise modalities (Jones *et al.*, 2010), it is well established  
600 that the determinants of exercise tolerance differ between single-limb and whole-body exercise

601 (Hureau *et al.*, 2018; Thomas *et al.*, 2018). Indeed Poole *et al.* (2016) suggested that in single-limb  
602 exercise,  $W'$  likely constitutes of substrate depletion and metabolite accumulation, whereas during  
603 whole-body exercise,  $W'$  is likely influenced by cardiopulmonary limiting factors to exercise. Indeed,  
604 despite a sex difference present for the cardiovascular response to isometric exercise (HR and  $\dot{Q}$ , Table  
605 2), the present study showed submaximal end-exercise values for these parameters, implying that  
606 oxygen delivery was not the limiting factor to exercise, but rather oxygen extraction determined  
607 exercise tolerance. Therefore, whether the conclusions of the present study apply to whole-body  
608 exercise remains to be determined.

609

610 To further support the notion that females possess more fatigue-resistant knee extensors, the rise in  
611 rmsEMG/ $M_{\max}$  was smaller compared to males during the +10% task (Table 2). Despite the known  
612 limitations (Farina *et al.*, 2014; Enoka & Duchateau, 2015) associated with surface EMG, increases are  
613 suggested to reflect additional neural drive and recruitment of further motor units, as the contractile  
614 apparatus become fatigued (Gandevia, 2001). Therefore the smaller increase in rmsEMG/ $M_{\max}$  could  
615 suggest that female musculature was able to sustain the required intensity with a reduced need for  
616 additional neural drive and motor unit activation. This could also explain the smaller decrease in  
617  $Q_{\text{tw,pot}}$  experienced during the tasks, further supporting the notion that the sex differences in skeletal  
618 muscle properties influence fatigability during intermittent isometric exercise. Further research could  
619 employ the use of high density EMG, which is capable of discerning motor unit properties (Merletti *et al.*  
620 *et al.*, 2008), without the limitations associated with bipolar surface EMG (Farina *et al.*, 2014; Enoka &  
621 Duchateau, 2015).

622

### 623 *Conclusions*

624 The present study is the first to demonstrate that females can sustain a greater relative work intensity  
625 compared with males during single limb exercise, as shown by the greater critical intensity.  
626 Importantly, when exercise intensity was normalised to this threshold, females out-performed males  
627 during the open-ended task (+10%), and showed reduced fatigability during a fixed workload task  
628 (-10%). These sex differences in the intensity-duration relationship and fatigue resistance are likely  
629 related to a greater ability to preserve oxygen availability within the knee-extensors during exercise,  
630 as demonstrated by the NIRS data. Following exercise, a faster rate of recovery was observed for  
631 contractile function in females, suggesting that, in addition to possessing more fatigue-resistant  
632 skeletal muscle, females are able to resolve exercise-induced dysfunction at a faster rate. These data  
633 explain previous findings related to sex differences in fatigability tasks, whilst providing the first sex-  
634 comparison of fatigability during work normalised to a metabolic threshold. Furthermore, the

635 difference between sexes highlights the importance of individualising exercise and recovery  
636 prescription to males and females, rather than generalising from previously generated male-only data  
637 within the literature.

638

639

640

641 **Conflicts of Interest**

642 No conflicts of interest, financial or otherwise, are declared by the authors.

643

644 **Author Contributions**

645 PA, GH, KT, and SG conceived and designed the experiments; PA, CB, and JS performed the  
646 experiments; PA analysed the data; PA, KMH, KT, GH, SKH, and SG interpreted the results of the  
647 experiments; PA drafted the manuscript; PA, CB, JS, KMH, GH, KT, SKH, and SG edited the manuscript;  
648 all authors approved the final manuscript and agree to be accountable for all aspects of the work.

649

650 **Funding**

651 No funding was received for the completion of this work.

652

653 **Acknowledgements**

654 We thank Mr Tom Pearson of Cambridge Electronic Design Ltd for his assistance creating scripts that  
655 facilitated data analysis.

656

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819

820

821 **Table 1: Intensity, times to task failure and impulse for the critical intensity estimation**  
 822 **trials. Values are mean  $\pm$  SD**

Trial	% MVC	Males		Females		
		TTF (s)	Impulse (N·s <sup>-1</sup> )	% MVC	TTF (s)	Impulse (N·s <sup>-1</sup> )
1	61 $\pm$ 2	217 $\pm$ 38	50,188 $\pm$ 10,600	71 $\pm$ 3*	216 $\pm$ 139	52,353 $\pm$ 22,164
2	56 $\pm$ 2	335 $\pm$ 102	74,185 $\pm$ 27,746	66 $\pm$ 3*	355 $\pm$ 158	70,105 $\pm$ 23,355
3	51 $\pm$ 2	427 $\pm$ 117	82,614 $\pm$ 22,044	61 $\pm$ 3*	486 $\pm$ 163	94,263 $\pm$ 33,207
4	46 $\pm$ 2	647 $\pm$ 186	120,318 $\pm$ 33,089	57 $\pm$ 4*	760 $\pm$ 148	141,504 $\pm$ 4,7839
r <sup>2</sup>		0.98 $\pm$ 0.03		0.99 $\pm$ 0.01		

MVC: maximal voluntary contraction, TTF: time to task failure; \* = greater than males ( $P < 0.001$ )

823

824 Table 2: Neuromuscular and cardiovascular function throughout the fatigue and recovery periods in both +10% and -10% trials.

		110% Critical Intensity					90% Critical Intensity				
		<i>Neuromuscular Function</i>									
		<i>Pre</i>	<i>Post</i>	<i>Post 15</i>	<i>Post 30</i>	<i>Post 45</i>	<i>Pre</i>	<i>Post</i>	<i>Post15</i>	<i>Post30</i>	<i>Post 45</i>
<b>ERT (N)</b>	Males	167 ± 66	130 ± 67*	135 ± 60#	133 ± 66	132 ± 63	167 ± 63	124 ± 52*	122 ± 47	120 ± 44	110 ± 39
	Females	151 ± 43	101 ± 23*	134 ± 27#	127 ± 23	129 ± 45	149 ± 33	131 ± 37*	123 ± 37	135 ± 39	132 ± 30
<b>M<sub>MAX</sub> (mV)</b>	Males	6.55 ± 2.57	5.89 ± 2.54	5.86 ± 2.28	5.95 ± 2.51	6.41 ± 2.56	6.33 ± 2.44	5.52 ± 2.58*	5.61 ± 2.39	5.75 ± 2.48	5.19 ± 2.32
	Females	5.18 ± 2.95	4.75 ± 2.28	4.36 ± 2.32	4.47 ± 2.07	4.59 ± 2.80	4.98 ± 2.40	4.39 ± 1.66	4.43 ± 1.57	4.27 ± 1.63	4.2 ± 1.52
<b>Pre-Stimulus rmsEMG (% M<sub>MAX</sub>)</b>	Males	0.62 ± 0.27	0.65 ± 0.28	0.67 ± 0.28	0.70 ± 0.29	0.66 ± 0.30	0.64 ± 0.23	0.84 ± 0.34	0.80 ± 0.33	0.81 ± 0.32	0.90 ± 0.36
	Females	0.75 ± 0.34	0.8 ± 0.77	0.82 ± 0.56	0.87 ± 0.49	0.87 ± 0.64	0.73 ± 0.39	0.85 ± 0.53	0.79 ± 0.54	0.79 ± 0.46	0.77 ± 0.46
<b>rmsEMG during task (% M<sub>MAX</sub>)</b>	Males	<i>1<sup>st</sup> Set</i> 16 ± 8	<i>25% TTF</i> 22 ± 13*	<i>50% TTF</i> 24 ± 12*	<i>75% TTF</i> 25 ± 10* <sup>§</sup>	<i>100% TTF</i> 27 ± 11* <sup>§</sup>	<i>1<sup>st</sup> Set</i> 8 ± 3	<i>25% TTF</i> 8 ± 3	<i>50% TTF</i> 8 ± 3	<i>75% TTF</i> 8 ± 4	<i>100% TTF</i> 8 ± 3
	Females	16 ± 5	19 ± 8	17 ± 5	17 ± 4	17 ± 4	8 ± 4	9 ± 3	9 ± 4	8 ± 4	9 ± 4
		<i>Cardiovascular Function</i>									
		<i>Pre</i>	<i>25% TTF</i>	<i>50% TTF</i>	<i>75% TTF</i>	<i>100% TTF</i>	<i>Pre</i>	<i>25% TTF</i>	<i>50% TTF</i>	<i>75% TTF</i>	<i>100% TTF</i>
<b>Heart Rate (bpm)</b>	Males	78 ± 5	95 ± 12*	99 ± 10*	108 ± 16*	116 ± 16* <sup>§</sup>	71 ± 11*	88 ± 20*	92 ± 18*	91 ± 19*	91 ± 17*
	Females	80 ± 13	91 ± 18*	94 ± 21*	94 ± 21*	96 ± 19*	81 ± 14	86 ± 13	87 ± 13	87 ± 12	87 ± 12
<b>Cardiac Output (L·min<sup>-1</sup>)</b>	Males	8.1 ± 2.2* <sup>§</sup>	10.0 ± 2.3* <sup>§</sup>	10.3 ± 2.0* <sup>§</sup>	10.6 ± 2.2* <sup>§</sup>	10.4 ± 2.2* <sup>§</sup>	6.8 ± 1.6	7.4 ± 1.8	7.5 ± 1.7*	7.6 ± 1.8*	7.6 ± 1.7*
	Females	6.0 ± 1.8	7.2 ± 1.9*	7.0 ± 1.6*	6.9 ± 1.6	6.8 ± 1.5	6.0 ± 1.3	6.2 ± 1.3	6.3 ± 1.4	6.3 ± 1.4	6.3 ± 1.2
<b>Mean Arterial Pressure (mmHg)</b>	Males	90 ± 13	98 ± 13	100 ± 15	104 ± 18*	107 ± 15*	94 ± 8	94 ± 10	95 ± 13	97 ± 10	101 ± 13
	Females	93 ± 11	104 ± 12*	104 ± 12	101 ± 12	105 ± 11*	93 ± 14	98 ± 15	99 ± 13	100 ± 13	100 ± 15

\* = significantly different from Pre (P < 0.05), # = significantly different from Post (P < 0.05), <sup>§</sup> = significantly greater than Females. ERT: estimated resting twitch; M<sub>max</sub>: maximal compound action potential; rmsEMG: root mean squared EMG; TTF: time to task failure.

826 **Figure Captions:**

827

828 **Figure 1: Characteristics of the intensity duration relationship for males and females.** Panel  
829 A: The linear relationships between impulse and time to task failure across the four estimation  
830 trials. Panel B: Critical intensities expressed as a percentage of MVC. Panel C:  $W'$  in both sexes.

831

832 **Figure 2: Pre-post changes in neuromuscular function across the four estimation trials.** A:  
833 maximum voluntary contraction (MVC); B: voluntary activation (assessed with motor nerve  
834 stimulation,  $VA_{MNS}$ ); C: potentiated quadriceps twitch force ( $Q_{tw.pot}$ ). TTF: time to task failure.

835

836 **Figure 3: Time to task failure during intermittent, isometric knee extensor exercise at 110%**  
837 **of critical intensity.** Individual participants are represented as the dots, and group mean and  
838 standard deviations are illustrated by the horizontal bars.

839

840 **Figure 4: Changes in neuromuscular parameters assessed during the +10% exercise task and**  
841 **recovery period.** Panel A: maximum voluntary contraction (MVC); Panel B: potentiated  
842 quadriceps twitch force ( $Q_{tw.pot}$ ); Panel C: voluntary activation assessed with motor nerve  
843 stimulation ( $VA_{MNS}$ ). Filled lines and circles represent the group mean values, and the dashed  
844 lines represent individual participants. \* = different from Pre ( $P < 0.05$ ), \$ = significantly  
845 different from Post ( $P < 0.05$ ), # = different between males and females ( $P < 0.05$ ).

846

847 **Figure 5: Neuromuscular changes across the fatiguing task (+10%) and the recovery period.**  
848 A: voluntary activation (transcranial magnetic stimulation, VATMS); B: motor evoked  
849 potentials (normalised to  $M_{max}$ ,  $MEP/M_{max}$ ), C: short interval intracortical inhibition (SICI). \* =  
850 different from Pre ( $P < 0.05$ ), \$ = significantly different from Post ( $P < 0.05$ ), # = different  
851 between males and females ( $P < 0.05$ ).

852

853 **Figure 6: Near Infrared Spectroscopy variables throughout the fatiguing task (+10%).** A:  
854 Oxyhaemoglobin ( $O_2Hb$ ), B: Deoxyhaemoglobin (HHb), C: Tissue oxygenation index (TOI). # =  
855 significantly different between males and females ( $P < 0.05$ ), \* = significantly different from  
856 Pre ( $P < 0.05$ ).

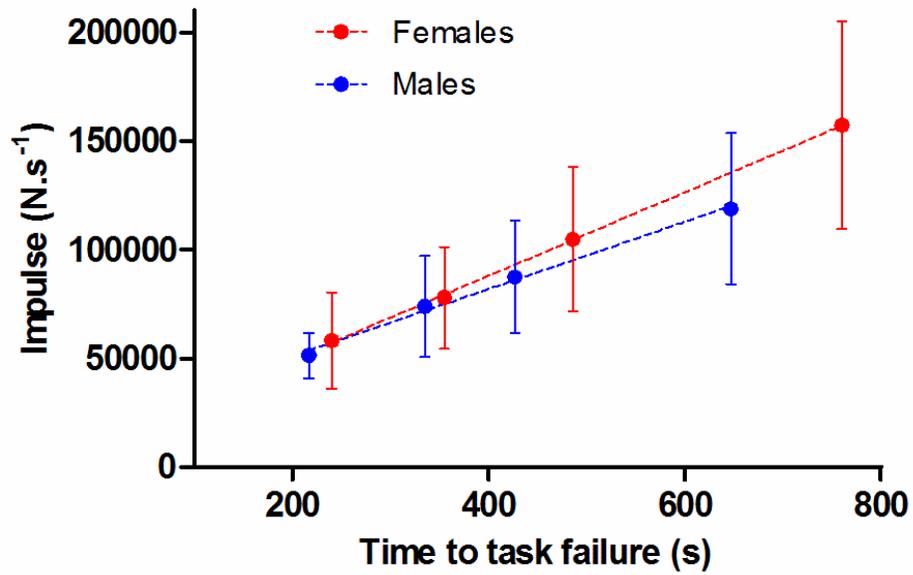
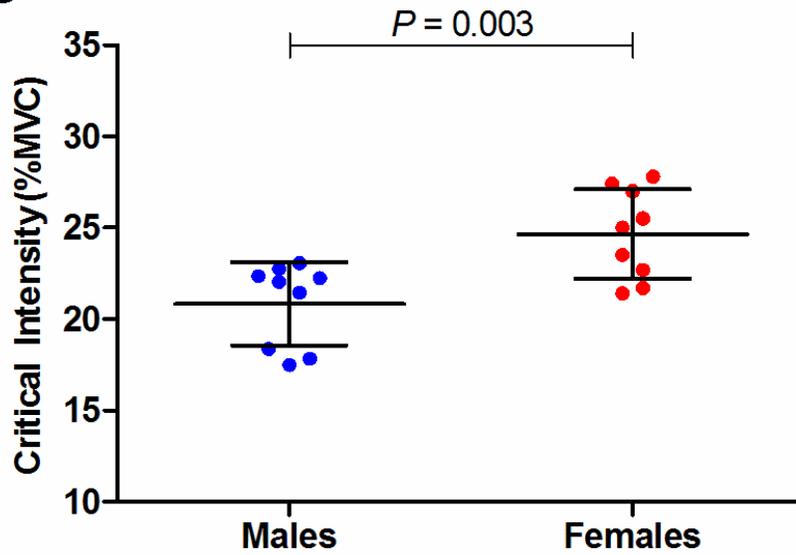
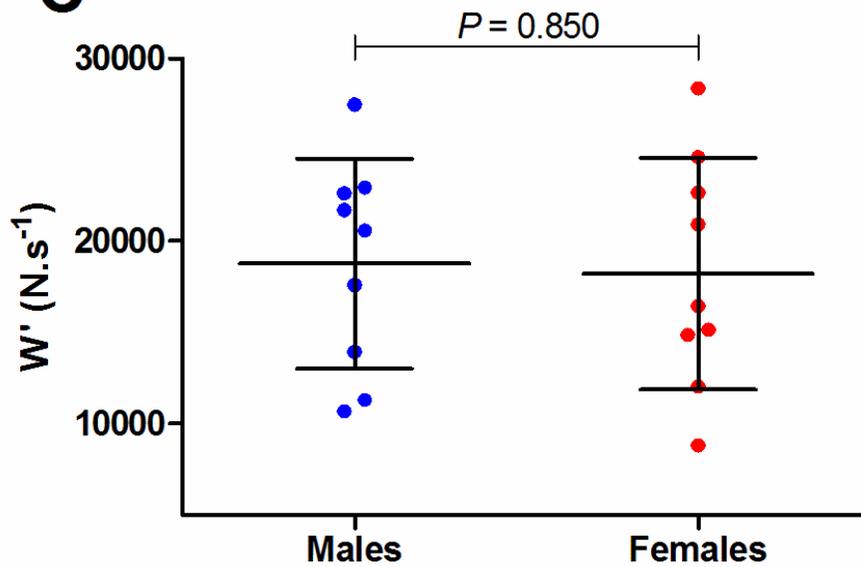
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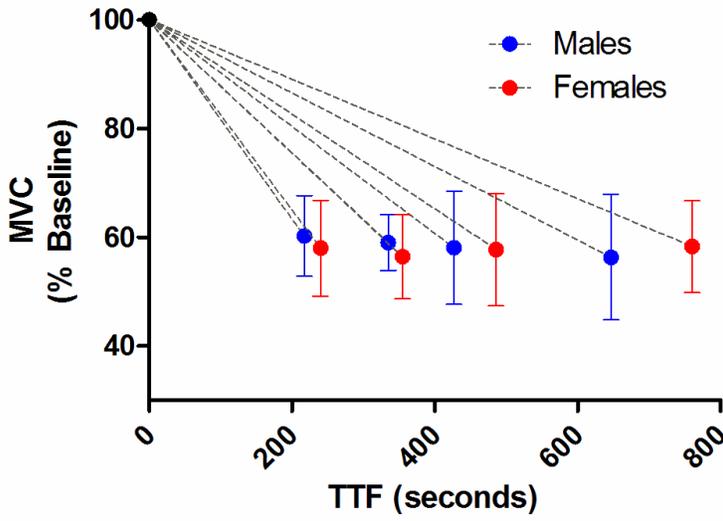
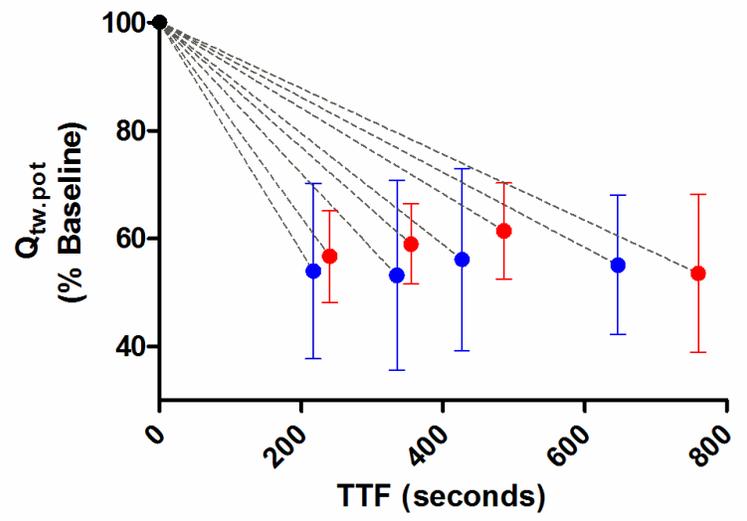
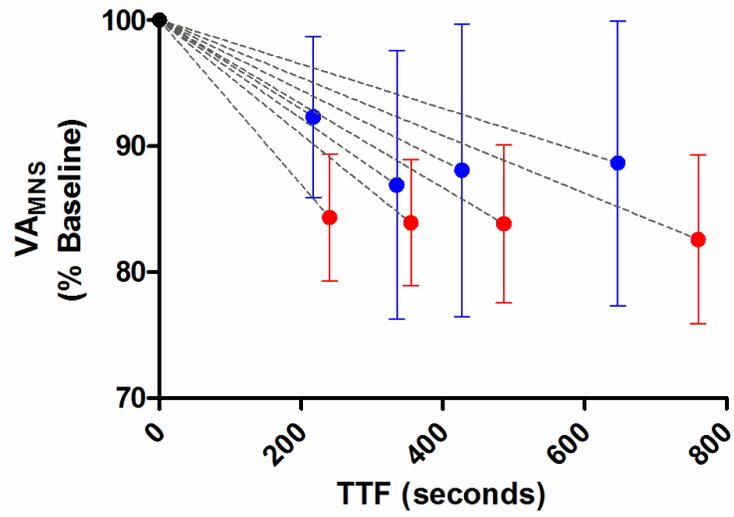
858 **Figure 7: Neuromuscular changes across the fatiguing task (-10%) and the recovery period.**  
859 A: maximal voluntary contraction, B: voluntary activation (transcranial magnetic stimulation),  
860 C: potentiated twitch force, D: motor evoked potential amplitude normalised to  $M_{MAX}$ , E:  
861 voluntary activation (motor nerve stimulation), F: short interval intracortical inhibition. \* =  
862 significantly different from Pre ( $P < 0.05$ ), \$ = significantly different from Post ( $P < 0.05$ ).

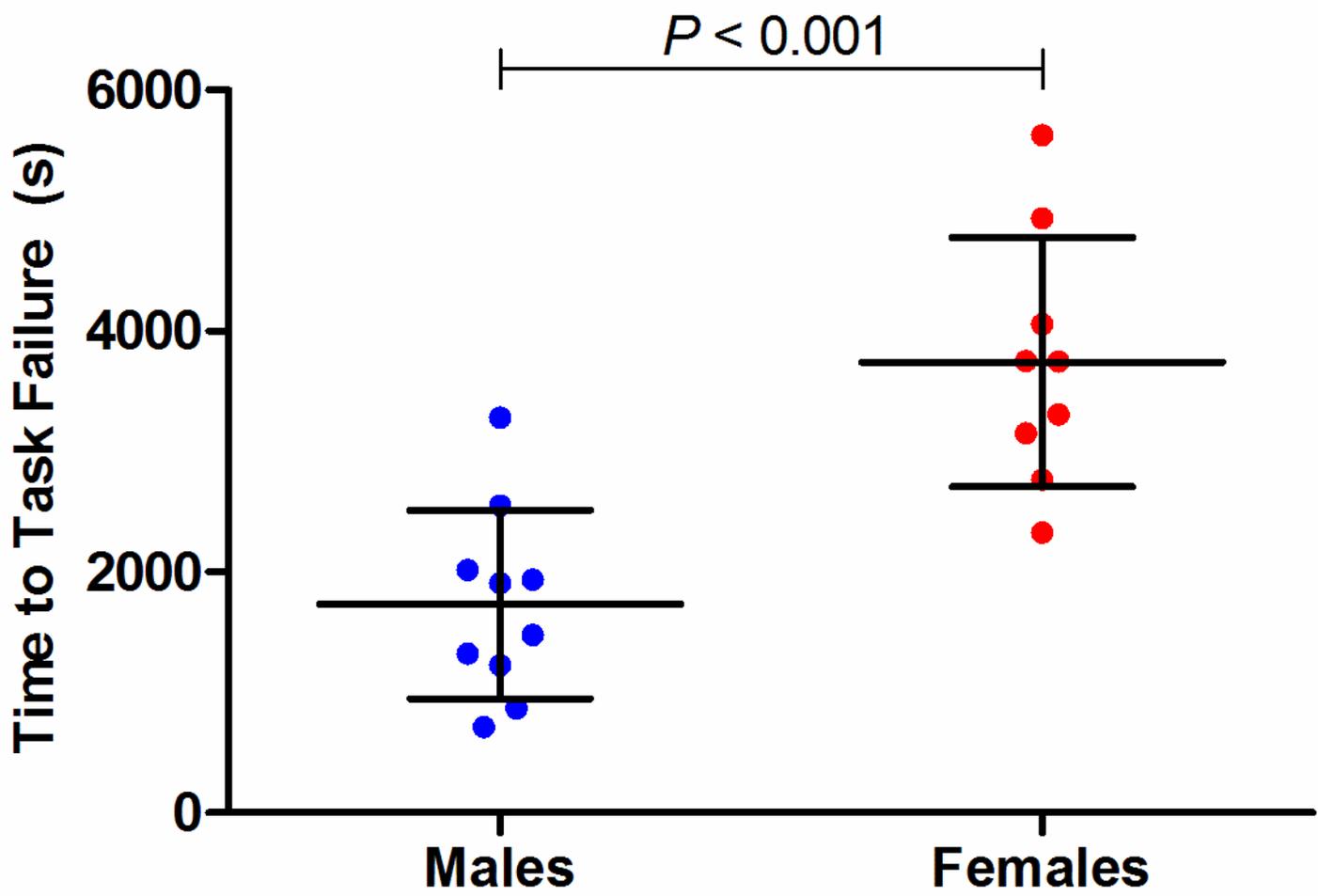
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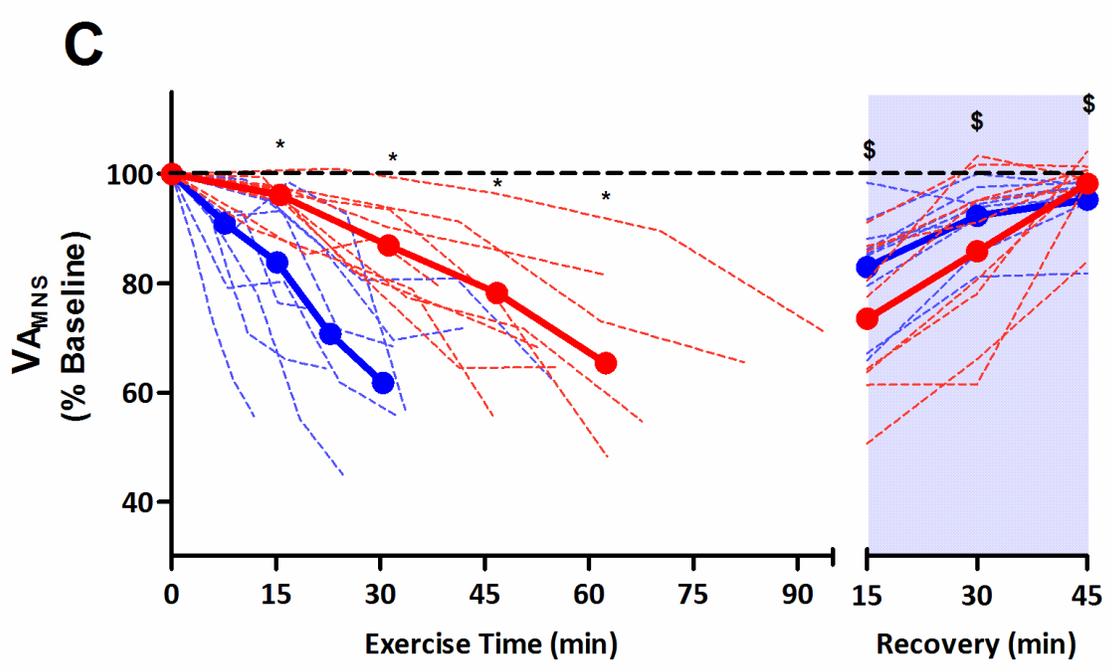
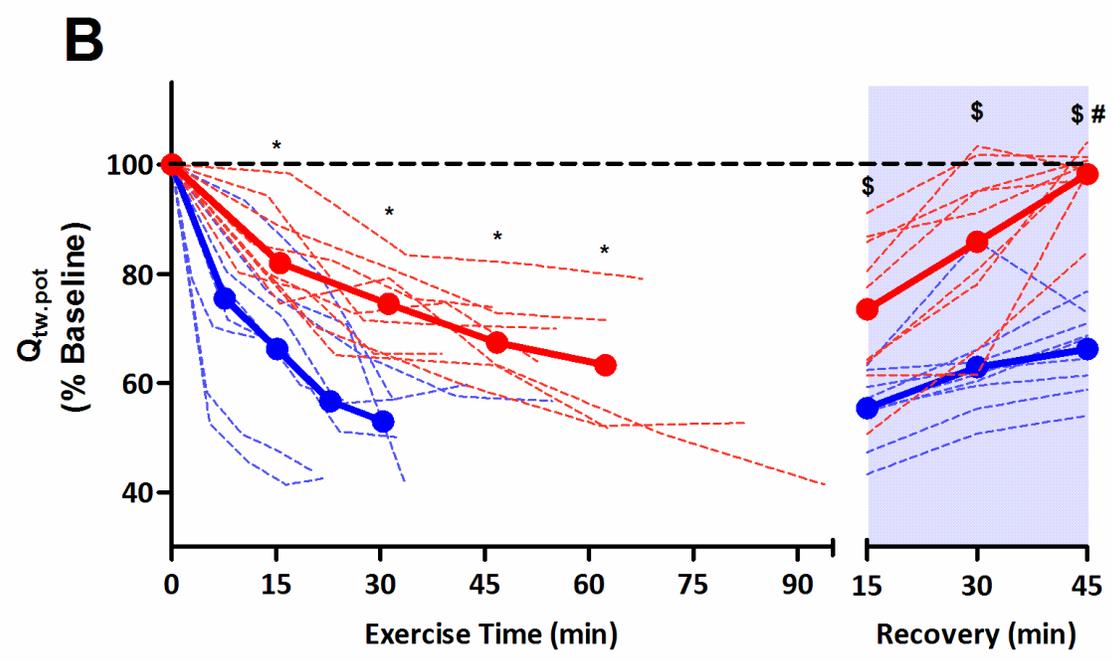
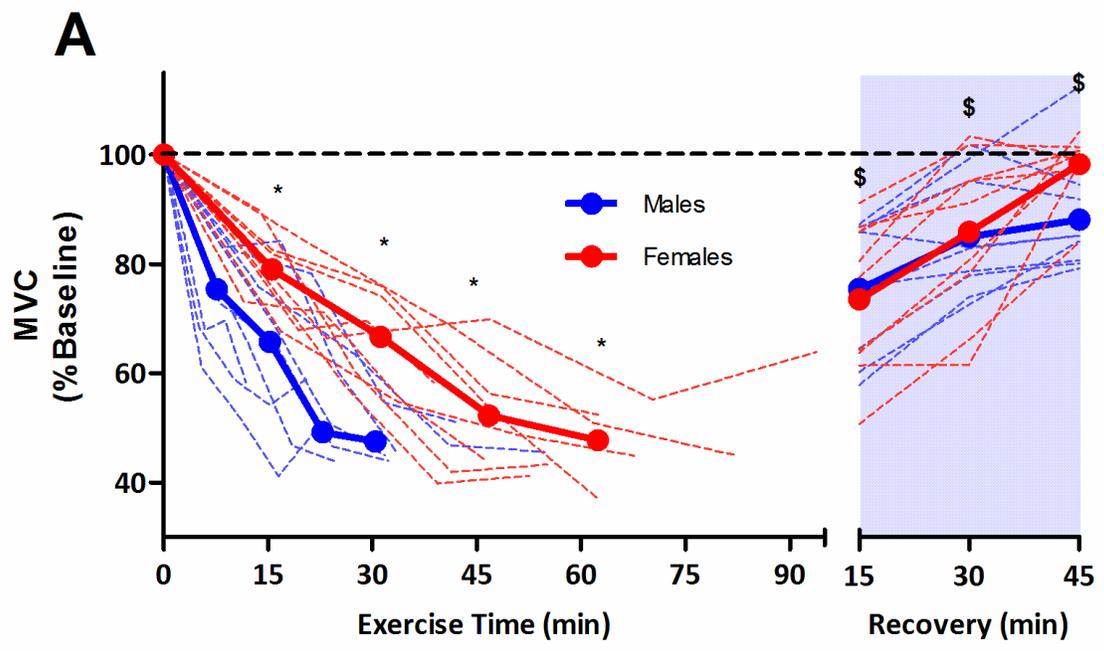
864 **Figure 8: Near Infrared Spectroscopy variables throughout the fatiguing task (-10%).** A:  
865 Oxyhaemoglobin, B: Deoxyhaemoglobin, C: Tissue oxygenation index. # = significantly  
866 different between males and females ( $P < 0.05$ ).

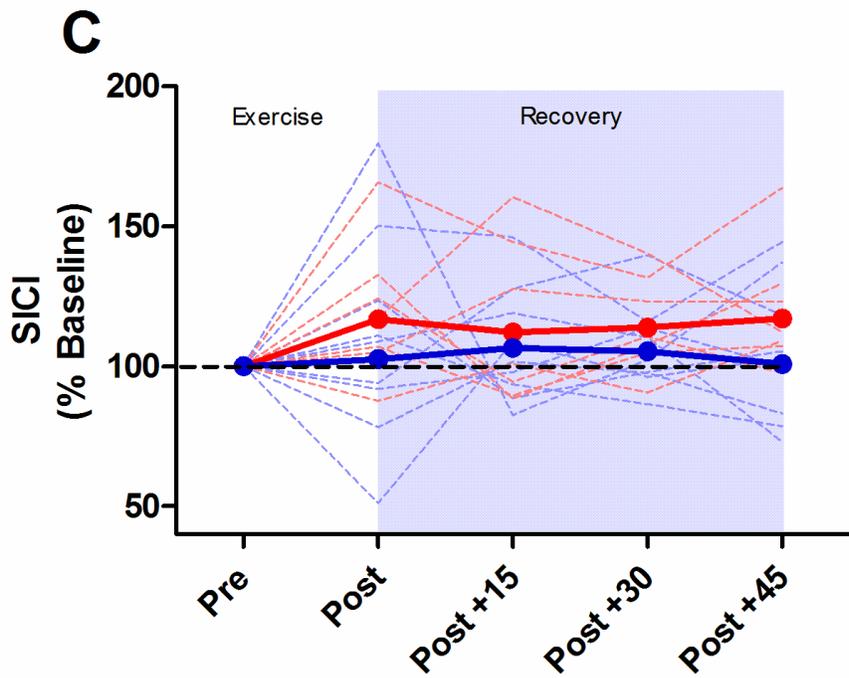
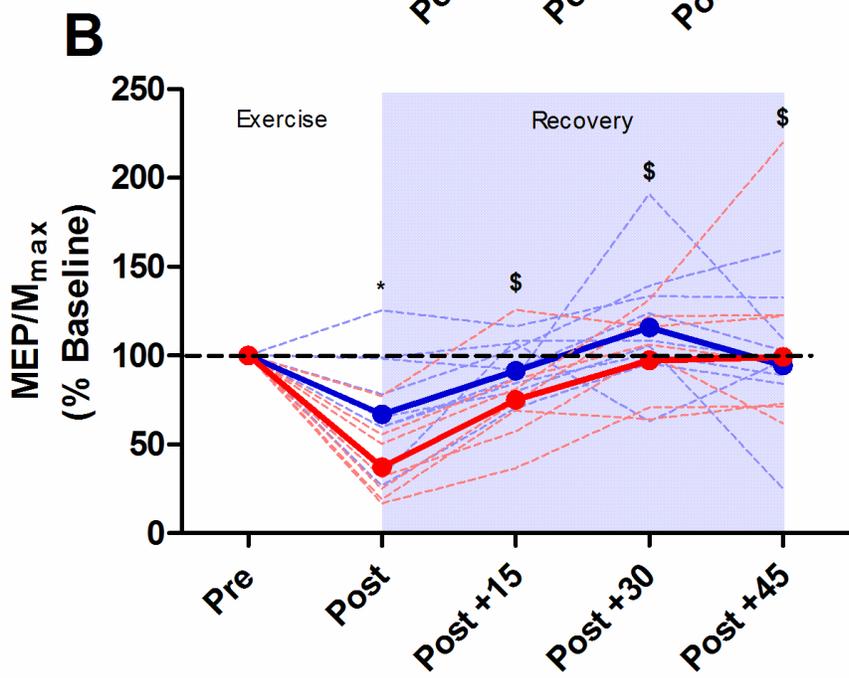
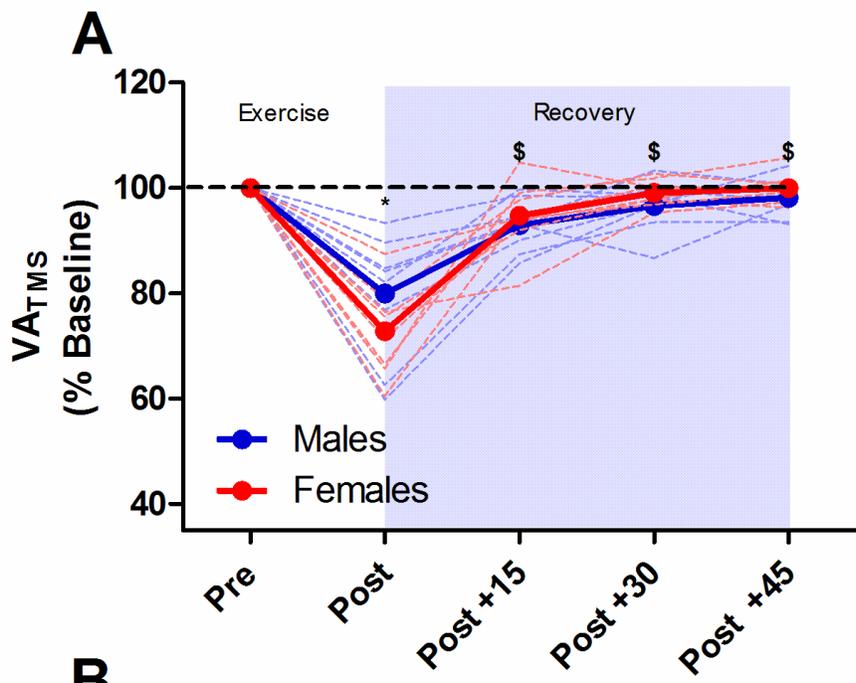
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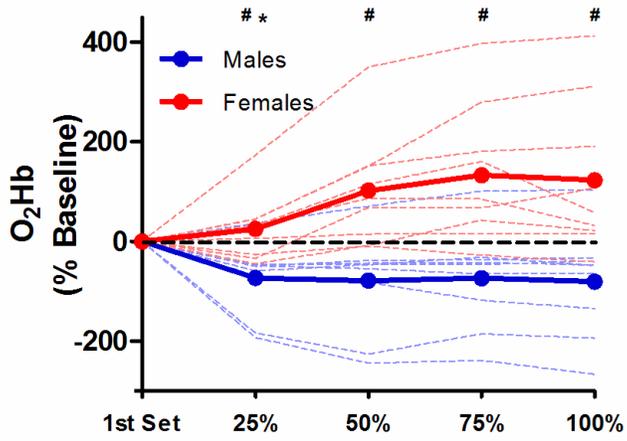
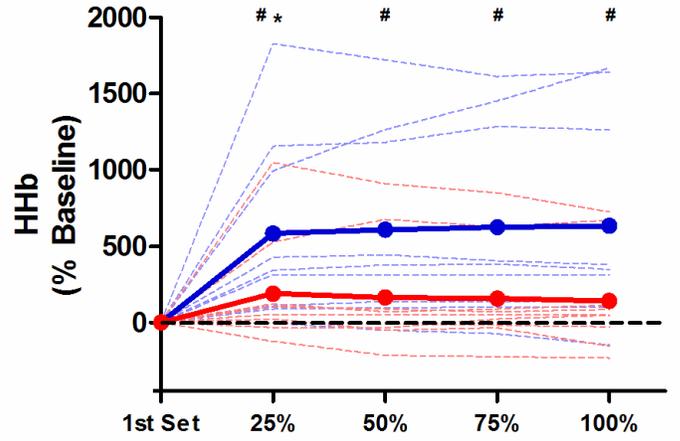
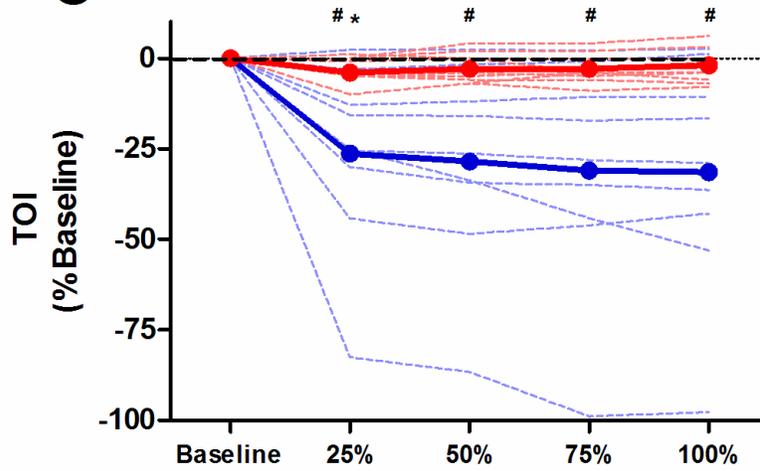
**A****B****C**

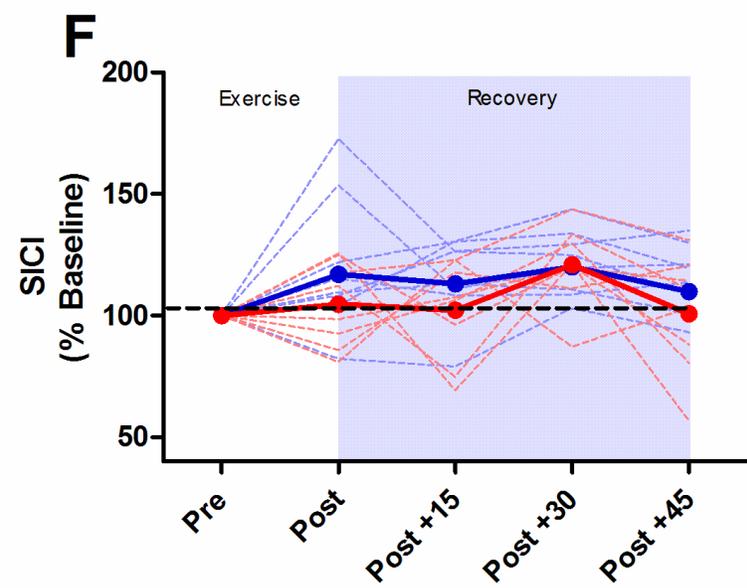
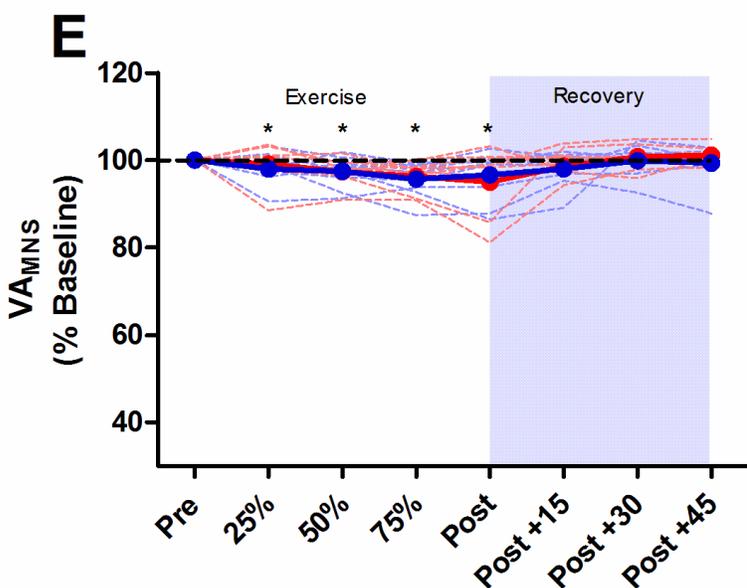
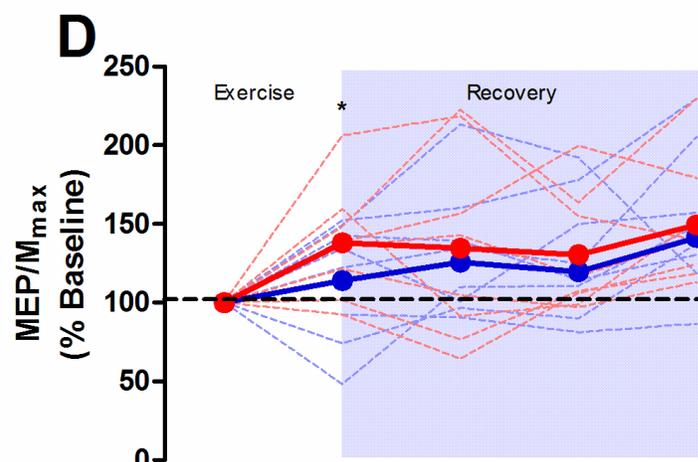
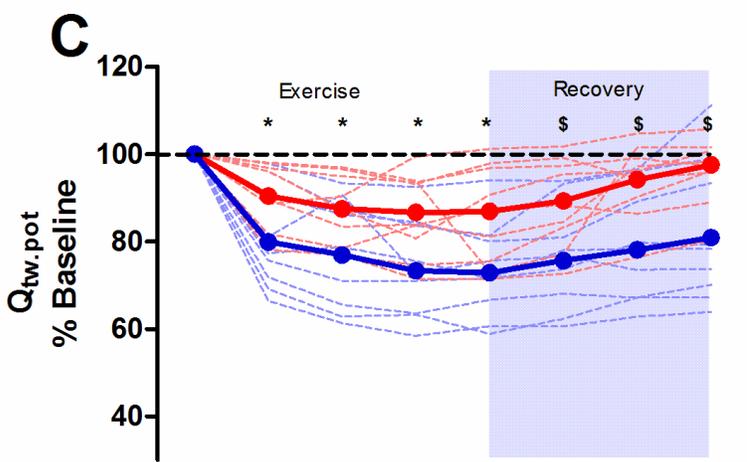
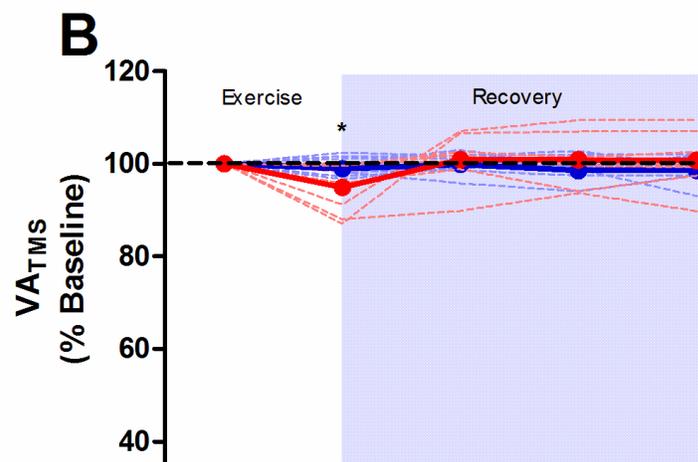
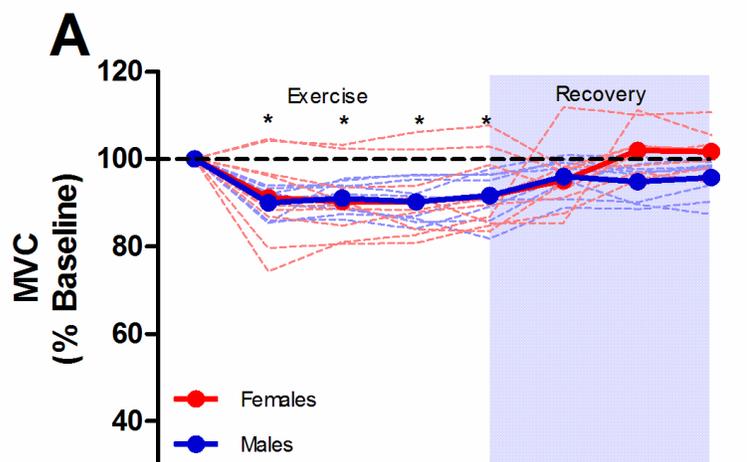
**A****B****C**

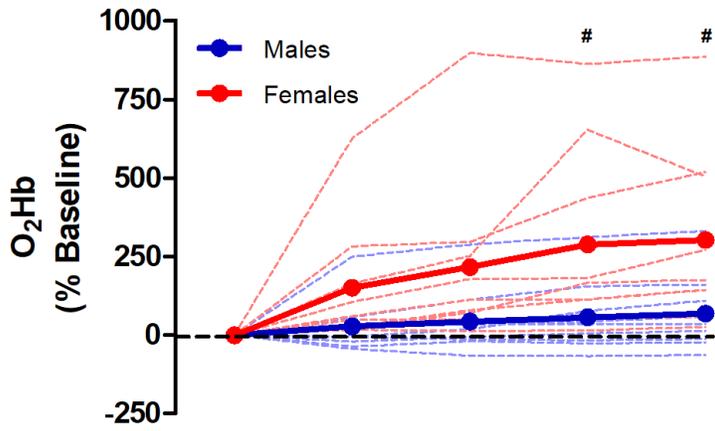
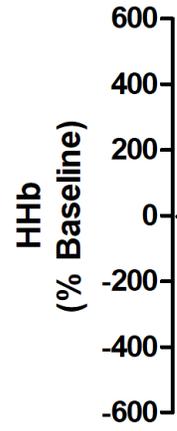






**A****B****C**



**A****B****C**