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**Wilson, Hannah K, Bernert, Lina, Spillane, Padraig, Squires, Emma, Crawford, Lorna, Piasecki, Jessica, Julian, Ross ORCID logo ORCID: <https://orcid.org/0000-0002-8558-7132>, Wilhelm, Eurico N, Hicks, Kirsty M and Ansdell, Paul (2025) The effect of sex on the cardiopulmonary and neuromuscular response to high-intensity interval exercise. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 329 (1). R123-R133. doi:10.1152/ajpregu.00306.2024**

<https://doi.org/10.1152/ajpregu.00306.2024>

Official URL: <https://doi.org/10.1152/ajpregu.00306.2024>

DOI: <http://dx.doi.org/10.1152/ajpregu.00306.2024>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/15059>

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1     **The effect of sex on the cardiopulmonary and neuromuscular response**  
2                                   **to high-intensity interval exercise**

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5

6     Hannah K Wilson<sup>1,2\*</sup>, Lina Bernert<sup>1,3\*</sup>, Pdraig Spillane<sup>1</sup>, Emma Squires<sup>1</sup>, Lorna Crawford<sup>1,4</sup>,  
7     Jessica Piasecki<sup>5</sup>, Ross Julian<sup>3,6</sup>, Eurico N Wilhelm<sup>1</sup>, Kirsty M Hicks<sup>1</sup>, Paul Ansdell<sup>1</sup>

8  
9  
10

11    <sup>1</sup> Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences,  
12    Northumbria University, UK

13    <sup>2</sup> School of Psychology and Sport Science, College of Medicine and Health, Bangor  
14    University, Wales, UK

15    <sup>3</sup> Institute of Sport and Exercise Sciences, University of Münster, Germany

16    <sup>4</sup> Orreco Ltd, Business Innovation Centre, University of Galway, Ireland

17    <sup>5</sup> Musculoskeletal Physiology Research Group, Sport, Health and Performance  
18    Enhancement Research Centre, Nottingham Trent University, UK

19    <sup>6</sup> School of Sport and Exercise, Exercise and Sport Research Centre, University of  
20    Gloucestershire, UK

21

22    \* Equal contribution to the manuscript

23

24    **Running title:** Sex differences in the response to interval exercise

25

26    **Corresponding author:**

27    Paul Ansdell Ph.D, FHEA  
28    Department of Sport, Exercise and Rehabilitation  
29    Faculty of Health and Life Sciences  
30    Northumbria University  
31    Newcastle upon Tyne  
32    Tyne and Wear  
33    NE1 8ST  
34    United Kingdom

35    [p.ansdell@northumbria.ac.uk](mailto:p.ansdell@northumbria.ac.uk)

36    +44 191 227 4398

37 **Abstract:**

38 Sex differences exist in the integrative response to exercise, however, these are typically  
39 researched during incremental and constant-load exercise. Interval exercise involves high-  
40 intensity efforts interspersed with recovery periods to repeatedly stress physiological  
41 systems, and it is currently unknown whether the response to this form of exercise differs  
42 between sexes.

43

44 Ten males and ten females (age:  $25 \pm 3$  years) completed two experimental visits. First, an  
45 incremental treadmill exercise test was performed to obtain submaximal (lactate threshold)  
46 and maximal ( $\dot{V}O_{2\text{peak}}$ ) data. Thereafter, visit two involved  $4 \times 3$ -min running intervals at 90%  
47 of the final incremental test velocity ( $v\dot{V}O_{2\text{peak}}$ ), with 90 secs rest between intervals. Before  
48 exercise and after each interval, maximal voluntary contraction (MVC), quadriceps  
49 potentiated twitch ( $Q_{\text{tw.pot}}$ ), and voluntary activation (VA) were recorded. The rates of oxygen  
50 uptake ( $\dot{V}O_2$ ), carbon dioxide production ( $\dot{V}CO_2$ ) and ventilation ( $\dot{V}_E$ ) were continuously  
51 recorded throughout.

52

53 There was no sex difference in relative  $\dot{V}O_{2\text{peak}}$  (males:  $47.2 \pm 6.0$  vs. females:  $44.4 \pm 5.8$  ml·kg<sup>-1</sup>·min<sup>-1</sup>,  $p=0.292$ ). When expressed relative to peak values, there were no sex differences in  
54 the  $\dot{V}O_2$  or  $\dot{V}CO_2$  response to the interval task ( $p \geq 0.781$ ). Females had greater  $\dot{V}_E/\dot{V}O_2$ , and  
55  $\dot{V}_E/\dot{V}CO_2$  values during the first ( $p \leq 0.034$ ) and second ( $\dot{V}_E/\dot{V}CO_2$ ,  $p=0.006$ ) intervals, with a  
56 sex  $\times$  time interaction effect ( $p \leq 0.046$ ). There were no sex differences in the reductions in  
57 MVC,  $Q_{\text{tw.pot}}$ , and VA during the interval task ( $p \geq 0.150$ ), however females had lesser  
58 reductions in  $Q_{\text{tw.pot}}$  values post-exercise ( $-24 \pm 9$  vs.  $-15 \pm 8\%$ ,  $p=0.044$ ).

59

60  
61 Sex differences exist in the physiological response to interval exercise. Compared to males,  
62 females experienced greater hyperpnoea during the initial stages, and had lesser decreases  
63 in contractile function post-exercise.

64

65 **New and Noteworthy:**

66 This study determined that males and females differ in the physiological response to high-  
67 intensity interval exercise. Specifically, females had poorer ventilatory efficiency during the  
68 first half of the task, but greater knee-extensor fatigue resistance following the task. These  
69 data build upon previous observations from constant-load exercise, demonstrating that  
70 physiological sex differences are observed during an ecologically valid exercise task  
71 commonly prescribed by practitioners in clinical and athletic populations.

72

73 **Key words:** Fatigue, female, gender, HIIT, male, ventilation.

74 **Introduction**

75 Females have historically been under-represented in sport science studies for a multitude of  
76 reasons [1, 2]. As a result, assumptions about exercise training have been generalised from  
77 male-dominated research and applied to females. It is becoming increasingly evident that  
78 the acute physiological responses to various modalities of exercise differ between the sexes  
79 [3], meaning this approach might not be optimal for the prescription of exercise to females.  
80 Research is needed to identify whether males and females respond to commonly prescribed  
81 forms of exercise similarly, in order to optimise training and performance for both sexes.

82

83 Morphological and anatomical sex differences in key physiological systems are thought to  
84 lead to the differences in the integrative response to exercise between sexes. For instance,  
85 males typically have a greater quantity of muscle mass and can generate larger maximal  
86 force, but experience greater proportional occlusion of limb blood flow during muscle  
87 contraction [4]. Whilst not consistent across all muscle groups [5, 6], females have  
88 consistently demonstrated a higher proportional area of type I muscle fibres of the knee-  
89 extensors [7], as well as greater capillary density [8], and vasodilatory response of the  
90 femoral artery [9]. These physiological sex differences have previously been suggested to  
91 aid in oxygen delivery and help delay the onset of fatigue in females [3, 10]. Despite the  
92 potentially superior aerobic muscular phenotype, it has been established that females have  
93 smaller lung volumes, airway size, and alveolar surface for gas exchange, even when  
94 matched for stature [11-14]. These morphological differences lead to a greater work and  
95 oxygen cost of breathing at elevated ventilatory rates [14, 15], resulting in an increased  
96 fraction of whole-body oxygen uptake ( $\dot{V}O_2$ ) originating from the respiratory musculature in  
97 females compared to males [16]. This, combined with lower cardiac output and haemoglobin  
98 concentrations [17], means that females have a poorer  $O_2$  carrying capacity during exercise  
99 [18, 19]. The balance between the importance of  $O_2$  delivery and utilisation depends on the  
100 physiological determinants of the task being performed, which is one reason why sex  
101 differences in the integrative response to exercise are not uniform and require further  
102 investigation [3, 20].

103

104 Previous studies considering single-limb contractions found that males experienced greater  
105 rates of fatigue than females [21-23] as well as males demonstrating a slower recovery time  
106 than females [23, 24]. Indeed, more recent evidence suggests that these sex differences in  
107 the response to single-limb exercise appear to be related to the proportion of myosin heavy  
108 chain I isoform and mitochondrial protein abundance [25]. Despite this evidence in single-  
109 limb models, these findings do not necessarily translate into whole-body locomotion [26]. In  
110 particular, the literature comparing the physiological response to locomotor exercise

111 between sexes is less comprehensive. Several studies have demonstrated that female  
112 knee-extensors experience less fatigue following cycling to task failure at equivalent relative  
113 exercise intensities [27-29], or following fixed duration running [30] and cycling [31] tasks.  
114 Although these studies point towards a consistent mechanism for the sex difference in knee  
115 extensor fatigability, the tasks employed are limited in generalisability to athletic training  
116 outside of the controlled lab environment. Constant-load tasks, particularly to task failure,  
117 are rarely employed by those prescribing exercise for athletic enhancement. Recently, we  
118 demonstrated that the sex difference in knee extensor fatigability was evident following a  
119 self-paced 5 km running time trial [32], whilst others have demonstrated similar sex  
120 differences following longer distance running tasks longer than 40 km [33, 34]. Although  
121 Boccia, Dardanello [35] did not observe a sex difference in fatigability following a half  
122 marathon, suggesting that the difference is intensity and/or duration dependent.

123

124 High intensity interval training is a method often prescribed to enhance aerobic and  
125 anaerobic capacity [36]. Research that has previously investigated sex differences in the  
126 response to interval exercise typically utilises repeated sprints (5-6 secs bouts) interspersed  
127 with prolonged (25-30 secs) rest [37, 38]. This modality of training has implications for  
128 intermittent and team sport athletes, and researchers have previously suggested that the  
129 lesser fatigue experienced by females is related to lower absolute mechanical work [39].  
130 Longer duration intervals are typically prescribed at submaximal, yet supra-threshold  
131 intensities (e.g., 2-6 min at 3 km-10 km race pace, Parmar *et al.*, 2021). In terms of exercise  
132 intensity domains, the intention of such intervals is to intersperse severe intensity bouts with  
133 periods of moderate intensity recovery or rest, and repeatedly elicit a state of metabolic  
134 stress [41]. Given that evidence from constant-load exercise indicates that female skeletal  
135 muscle is more resistant to fatigue during metabolically challenging tasks [27-29], it is  
136 important to understand how interspersing high-intensity bouts with recovery periods  
137 mediates the sex difference in fatigability and the integrative physiological response to  
138 exercise. The little evidence that does exist from cycling exercise suggests that there might  
139 also be perceptual, but not physiological sex differences in the response to high-intensity  
140 interval exercise [42]. As highlighted, both exercise modality and intensity mediate the  
141 influence of sex on the responses to exercise, therefore this study aimed to compare the  
142 physiological responses to, and recovery from, a bout of high-intensity interval running  
143 between sexes. We hypothesised that both sexes would experience a similar metabolic  
144 response to exercise, but female knee-extensors would be less fatigable.

145

146 **Methods**

147 *Ethical Approval*

148 This study received institutional ethical approval from the Northumbria University Health and  
149 Life Sciences Research Ethics Committee (submission reference: 2022-0094-368) and was  
150 conducted according to all aspects of the Declaration of Helsinki, apart from pre-registration  
151 in a public database. Participants volunteered for the study and provided written informed  
152 consent.

153

154 *Sample Size Calculation*

155 An *a priori* sample size calculation was performed using GPower (v3.0.0) using the effect  
156 size from Ansdell, Škarabot [27] for the sex difference in contractile dysfunction following  
157 high-intensity cycling ( $\eta^2 = 0.344$ ), and reliability data for the same variable from Ansdell,  
158 Brownstein [43]. With the parameters of  $\alpha = 0.001$  and  $1-\beta = 0.99$ , the minimum sample size  
159 required was 12 participants (6 males, 6 females). Therefore, to maximise statistical power,  
160 10 participants of each sex were recruited.

161

162 *Participant Characteristics*

163 Ten healthy males (mean  $\pm$  SD age:  $27 \pm 4$  years, stature:  $180 \pm 6$  cm, body mass:  $83.5 \pm$   
164  $12.1$  kg) and ten healthy females (age:  $23 \pm 2$  years, stature:  $163 \pm 6$  cm, body mass:  $62.9 \pm$   
165  $9.1$  kg) whose gender matched their sex assigned at birth, volunteered for the study. All  
166 participants were free from musculoskeletal conditions, as well as neurological, respiratory,  
167 and cardiovascular disease. Hormonal status was not an exclusion criterion or controlled for  
168 in this study, based on evidence that the menstrual cycle or hormonal contraceptive usage  
169 do not influence the cardiopulmonary or neuromuscular responses to whole-body exercise  
170 [44, 45]. However, it must be acknowledged that perceptual responses to exercise might be  
171 influenced by menstrual cycle phase [46]. Five females were naturally cycling, with their  
172 second visits occurring on self-reported days 5, 8, 16, 19, and 23 of their menstrual cycle.  
173 Three females were using combined oral contraceptive pills; one female had a non-  
174 hormonal (or copper) intrauterine device, and one had a contraceptive implant. A screening  
175 questionnaire was used to ensure the participants met the inclusion criteria and  
176 training/activity status. Male and female participants reported  $6.5 \pm 4.1$  hours and  $5.8 \pm 2.1$   
177 hours of structured physical activity per week (respectively) and  $4.3 \pm 1.8$  hours and  $2.8 \pm$   
178  $1.9$  hours of unstructured physical activity per week (respectively). All participants were  
179 considered to be recreationally active as per the U.K. Chief Medical Officer's guidelines of at  
180 least 150 min of moderate intensity exercise or 75 min of vigorous intensity exercise.

181

182

183 *Experimental Design*

184 Participants visited the laboratory on two occasions. The first visit involved familiarisation  
185 with neuromuscular measures, followed by a treadmill incremental exercise test to the limit  
186 of tolerance. The second visit was the performance of a high-intensity interval exercise  
187 protocol, involving 4 × 3-min intervals at 90% of final incremental test velocity ( $\dot{V}O_{2peak}$ ),  
188 interspersed with 90 sec rest periods. This exercise paradigm was chosen as it has high  
189 ecological validity [40] as well as being an effective method of improving aerobic capacity  
190 over 4-8 weeks [47, 48]. Before exercise, and in each rest period, participants performed a  
191 neuromuscular assessment while blood lactate was sampled, and heart rate and rating of  
192 perceived exertion (RPE) were recorded. Following exercise, the neuromuscular measures  
193 were repeated at 10, 20, and 30 min.

194

195 *Visit 1: Familiarisation & Incremental Exercise Testing*

196 Participants were firstly familiarised with the neuromuscular stimulation techniques. This  
197 began with the determination of the femoral nerve stimulation threshold at rest (see details  
198 below), then the performance of warm up contractions increasing from 50% perceived effort  
199 to 90%. Participants then performed a neuromuscular function assessment (see details  
200 below).

201

202 Prior to the incremental exercise test, participants provided a capillary blood lactate sample  
203 from the earlobe, then performed a five-min warm up at an intensity corresponding to the  
204 first stage of the incremental test. Depending on each participant's sex and training history,  
205 the speed for the first stage was set between 6 – 10 km·h<sup>-1</sup>. This permitted a similar number  
206 of subsequent stages to be completed prior to the limit of tolerance for males and females (6  
207 ± 2 vs. 6 ± 1, respectively,  $p = 0.756$ ). After the warmup, participants were given one minute  
208 rest, where another blood lactate sample was taken. The incremental test then involved  
209 three-min stages, with the treadmill speed increased by 1 km·h<sup>-1</sup> each stage [49]. Between  
210 stages, participants had 1 min of rest, where they provided further blood lactate samples.  
211 The treadmill was kept at a constant elevation of 1% throughout all testing and subsequent  
212 visits. Instructions were provided to “complete as many stages as possible”, and participants  
213 were informed that they should jump to the sides of the treadmill if they could not maintain  
214 the running speed any longer. The speed of the final complete stage was recorded as  
215  $\dot{V}O_{2peak}$ , however, if participants were able to run for longer than 90 secs during the final  
216 incomplete stage, 0.5 km·h<sup>-1</sup> was added to the speed at the final complete stage when  
217 recording  $\dot{V}O_{2peak}$ . Throughout the incremental exercise test, breath-by-breath gas  
218 exchange was recorded continuously, with heart rate (Polar T31-coded chest strap & FT1  
219 watch, Polar O.Y., Finland) and RPE (6-20 scale) recorded at the end of each stage.

220 Blood lactate samples were analysed immediately to determine lactate threshold and  
221 turnpoint (Biosen C-Line, EKF Diagnostics, Cardiff, UK). Lactate threshold was determined  
222 as the first work rate at which a non-linear increase in blood lactate concentration was  
223 observed, while lactate turnpoint was identified as the work rate that elicited a sudden and  
224 sustained increase in blood lactate concentration [50]. The two lactate thresholds were  
225 analysed independently by two experimenters, and if any disagreement occurred, a third  
226 experimenter was consulted in order to mediate.

227

### 228 *Visit 2: High Intensity Interval Exercise*

229 This visit began with the determination of the femoral nerve stimulation threshold, then warm  
230 up contractions increasing from 50% perceived effort to 90% were performed. Hereafter, a  
231 neuromuscular assessment was performed (see details below), before participants moved to  
232 the treadmill and provided a resting blood lactate sample.

233

234 Participants began the high-intensity interval exercise with a warmup consisting of a 5-min  
235 stage at 50%  $\dot{V}O_{2\text{peak}}$ , and the final minute at 90%  $\dot{V}O_{2\text{peak}}$ . After the warmup, participants  
236 rested for one min, while providing a blood lactate sample. The interval exercise involved 4 ×  
237 3-min intervals at 90%  $\dot{V}O_{2\text{peak}}$ , interspersed with 90 secs of rest. At the end of each  
238 interval, participants moved from the treadmill, with the gas exchange mask still attached, to  
239 an isometric dynamometer, and performed three MVCs with femoral nerve stimulation, as  
240 well as providing a blood lactate sample, before returning to the treadmill. Heart rate and  
241 RPE were recorded at the end of each interval. Upon completion of the intervals,  
242 participants repeated the neuromuscular assessments immediately, as well as at 10-, 20-,  
243 and 30-min post-exercise. All participants completed the four intervals.

244

### 245 *Experimental Techniques*

#### 246 *Breath by Breath Gas Exchange*

247 During all visits, expired gas was analysed breath-by-breath using an online system (Vyntus  
248 CPX, Jaeger, CareFusion, Germany). Oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) concentrations  
249 were analysed via a paramagnetic chemical fuel cell and non-dispersive infrared cell  
250 respectively. Before each test, the analysers were calibrated using ambient air and a gas of  
251 known  $O_2$  (15.00%) and  $CO_2$  (4.97%) concentrations. Ventilatory volumes were inferred from  
252 measurement of gas flow using a digital turbine transducer (volume 0 to 10 L, resolution 3  
253 mL, flow 0 to 15  $L \cdot s^{-1}$ ), which was calibrated prior to each visit (Hans Rudolph Inc. Kansas  
254 City, USA).

255

256

257 *Neuromuscular Assessments*

258 Pre-exercise neuromuscular assessments consisted of five MVCs separated by 30 secs.  
259 During the final three MVCs, femoral nerve stimulation was delivered at peak force of the  
260 MVC and 2 secs after. This was used to quantify MVC force, voluntary activation (VA), and  
261 potentiated twitch force ( $Q_{tw,pot}$ ). Following each interval, the same neuromuscular  
262 assessment was repeated but with three MVCs instead of five, as prior potentiation of  
263 twitches was not required [51]. For the assessments 10-, 20-, and 30-min post exercise, the  
264 baseline assessment was repeated including the two additional MVCs for potentiation of  
265 resting twitches.

266

267 *Femoral Nerve Stimulation*

268 Electrical stimuli (200  $\mu$ s duration) were delivered to the femoral nerve via 32 mm-diameter  
269 surface electrodes (CF3200; Nidd Valley Medical, North Yorkshire, UK) using a constant-  
270 current stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode  
271 was placed high in the femoral triangle over the nerve, and the anode was positioned  
272 midway between the greater trochanter and the iliac crest. The cathode was repositioned if  
273 necessary, to the location that elicited the largest quadriceps twitch amplitude ( $Q_{tw}$ ). Stimuli  
274 began at 20 mA, and increased by 20 mA until a plateau in  $Q_{tw}$  occurred; this stimulus  
275 intensity was then increased by 30% to ensure supramaximal stimulations during the  
276 neuromuscular assessments.

277

278 *Force and Electromyography*

279 For neuromuscular assessments, participants were seated on a custom-built MVC chair,  
280 with force (N) measured using a calibrated load cell (MuscleLab force sensor 300, Ergotest  
281 technology, Norway). The load cell was attached to the participant's dominant leg, 2 cm  
282 superior to the ankle malleoli, using a non-compliant cuff. The load cell height was adjusted  
283 to ensure a direct line with the applied force for each participant. Participants were sat  
284 upright with knee and hip angles kept at 90° flexion. Force was sampled continuously (1000  
285 Hz), and acquired for off-line analysis (Spike 2, Cambridge Electronic Design, Cambridge,  
286 UK).

287

288 *Blood Lactate Sampling*

289 Blood lactate was sampled via capillary puncture technique with a 10  $\mu$ l sample taken from  
290 the earlobe of each participant. Samples were immediately analysed for the concentration of  
291 lactate ( $\text{mmol}\cdot\text{L}^{-1}$ ) and used for the calculation of lactate threshold and lactate turnpoint.

292

293

294 *Data Analysis*

295 All MVCs were recorded, with the average of the peak forces during the three contractions  
296 used for further analyses. Voluntary activation assessed with nerve stimulation was  
297 calculated using the twitch interpolation method:  $VA (\%) = (1 - [SIT/Q_{tw,pot}] \times 100)$ , where SIT  
298 is the amplitude of the superimposed twitch force measured during MVC, and  $Q_{tw,pot}$  is the  
299 amplitude of the resting potentiated twitch force assessed 2 secs post-MVC.

300

301 Pulmonary gas exchange during both visits was exported in 5 secs bins, and the greatest 30  
302 secs average recorded in visit 1 was used as peak values for each variable ( $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and  
303  $\dot{V}_E$ ). Data from visit two was exported in the same manner, with the final 30 secs of data  
304 during each interval expressed in absolute ( $L \cdot min^{-1}$ ) and relative (%peak) units. Ventilatory  
305 equivalents of  $\dot{V}O_2$  and  $\dot{V}CO_2$  ( $\dot{V}_E/\dot{V}O_2$  and  $\dot{V}_E/\dot{V}CO_2$ ) and respiratory exchange ratio  
306 ( $\dot{V}CO_2/\dot{V}O_2$ ) were also calculated.

307

308 *Statistical Analysis*

309 Data are presented as mean  $\pm$  standard deviation within the text and figures. Normal  
310 distribution of data was confirmed with the Shapiro-Wilk test. As all variables had normally  
311 distributed data, males and females were compared with independent samples t tests for  
312 variables with a single value or time point. For repeated measures variables assessed  
313 during and after exercise, a two-way (sex  $\times$  time) repeated measures ANOVA was  
314 performed. To assess fatigue during exercise, neuromuscular variables (normalised to %  
315 baseline) were assessed using a 2  $\times$  5 (sex  $\times$  time) ANOVA (time points: pre, interval 1, 2, 3,  
316 and 4). Whereas to assess recovery, neuromuscular variables (normalised to % baseline)  
317 were assessed with a 2  $\times$  4 (sex  $\times$  time) ANOVA (time points: post, +10, +20, and +30 min).  
318 Main and interaction effects were adjusted according to the Greenhouse-Geisser correction  
319 if the assumption of sphericity was violated, and significant effects were followed with  
320 Bonferroni-corrected *post-hoc* tests. The significance level for all statistical tests was set at  $\alpha$   
321  $< 0.05$ .

322

323 **Results**

324 *Incremental Exercise Testing*

325 Absolute and relative data recorded during the incremental exercise test are presented in  
 326 Table 1. As expected, males had greater values for absolute  $\dot{V}O_{2peak}$  ( $p < 0.001$ ), however,  
 327 when values were normalized to body mass, there was no sex difference in  $\dot{V}O_{2peak}$  ( $p =$   
 328  $0.292$ ). Both sexes completed a similar number of incremental test stages before reaching  
 329 exhaustion (males:  $6.3 \pm 1.6$  stages vs females:  $6.5 \pm 1.3$  stages,  $p = 0.756$ ). The velocity at  
 330 which lactate threshold occurred, expressed as a percentage of  $v\dot{V}O_{2peak}$ , was not different  
 331 between sexes (males:  $67 \pm 7\%$  vs females:  $68 \pm 9\%$ ,  $p = 0.773$ ).

332

333 *Table 1: Submaximal and peak data recorded during the incremental exercise test.*

	<b>Males (n = 10)</b>	<b>Females (n = 10)</b>	<b>P value</b>
$\dot{V}O_{2peak}$ ( $l \cdot min^{-1}$ )	$3.901 \pm 0.433$	$2.763 \pm 0.323$	$< 0.001$
$\dot{V}O_{2peak}$ ( $ml \cdot kg^{-1} \cdot min^{-1}$ )	$47.2 \pm 6.0$	$44.4 \pm 5.8$	$0.292$
$\dot{V}_{Epeak}$ ( $l \cdot min^{-1}$ )	$152.9 \pm 20$	$105.8 \pm 11.3$	$< 0.001$
$RER_{peak}$	$1.06 \pm 0.05$	$1.05 \pm 0.07$	$0.798$
$HR_{peak}$ (bpm)	$200 \pm 4$	$200 \pm 6$	$0.874$
$v\dot{V}O_{2peak}$ ( $km \cdot h^{-1}$ )	$14.9 \pm 1.7$	$13.6 \pm 1.6$	$0.090$
<b>Lactate threshold (<math>km \cdot h^{-1}</math>)</b>	$10.0 \pm 1.8$	$9.2 \pm 1.5$	$0.317$

HR: heart rate; RER: respiratory exchange ratio;  $\dot{V}_E$ : minute ventilation;  $\dot{V}O_2$ : rate of oxygen uptake;  $v\dot{V}O_{2peak}$ : velocity at the maximal rate of oxygen uptake.

334

335

336 *Metabolic & Cardiopulmonary Responses to Interval Exercise*

337 During the interval task, heart rate, RPE, and blood lactate progressively increased (main  
 338 effects of time: all  $p < 0.001$ ). No main effects of sex were observed for heart rate ( $p =$   
 339  $0.601$ ), RPE ( $p = 0.497$ ) or blood lactate ( $p = 0.203$ ). A sex  $\times$  time interaction effect was  
 340 observed for heart rate (Figure 1A,  $F_{2,7, 45.5} = 3.470$ ,  $p = 0.028$ ,  $\eta^2 = 0.170$ ), with *post-hoc*  
 341 comparisons revealing females had greater heart rate at the end of the warmup ( $p = 0.041$ ),  
 342 but no other time points ( $p \geq 0.696$ ). No sex  $\times$  time interaction effects were observed for  
 343 either RPE (Figure 1B,  $p = 0.137$ ) or blood lactate (Figure 1C,  $p = 0.183$ ).

344

345

\*\*\* *Figure 1 here* \*\*\*

346

347 While males demonstrated greater absolute values ( $p < 0.001$ ), when  $\dot{V}O_2$  and  $\dot{V}CO_2$  were  
 348 expressed as a percentage of peak values (Figure 2A and 2B) from the incremental exercise

349 test, no sex ( $p \geq 0.631$ ) or sex  $\times$  time interaction effects ( $p \geq 0.781$ ) were observed. Similarly,  
350 no sex ( $p = 0.330$ ) or sex  $\times$  time interaction effects ( $p = 0.710$ ) were observed for RER.

351

352

\*\*\* Figure 2 here \*\*\*

353

354 In absolute values, males had greater  $\dot{V}_E$  throughout the interval task ( $p < 0.001$ ). When  
355 expressed as a percentage of peak values (Figure 2C), no main effect of sex was observed  
356 ( $p = 0.187$ ), however, there was a sex  $\times$  time interaction ( $F_{3,54} = 3.269$ ,  $p = 0.042$ ,  $\eta^2 =$   
357  $0.154$ ) for  $\dot{V}_E$ . *Post-hoc* comparisons revealed no significant sex differences at either the first  
358 ( $p = 0.067$ ), second ( $p = 0.050$ ), third ( $p = 0.212$ ), or fourth interval ( $p = 1.000$ ). Similarly,  
359  $\dot{V}_E/\dot{V}O_2$  demonstrated no main effect of sex (Figure 3A,  $p = 0.317$ ), but a sex  $\times$  time  
360 interaction effect ( $F_{3,54} = 4.831$ ,  $p = 0.005$ ,  $\eta^2 = 0.212$ ); with *post-hoc* comparisons revealing  
361 greater female values compared to males during the first interval ( $p = 0.034$ ).  $\dot{V}_E/\dot{V}CO_2$  also  
362 demonstrated no main effect of sex (Figure 3B,  $p = 0.096$ ), but a sex  $\times$  time interaction effect  
363 ( $F_{3,54} = 2.853$ ,  $p = 0.046$ ,  $\eta^2 = 0.137$ ). *Post-hoc* comparisons revealed that females had  
364 greater values than males during the first interval for both  $\dot{V}_E/\dot{V}O_2$  and  $\dot{V}_E/\dot{V}CO_2$  ( $p \leq 0.034$ )  
365 and second ( $p = 0.006$ ) intervals for  $\dot{V}_E/\dot{V}CO_2$ .

366

367

\*\*\* Figure 3 here \*\*\*

368

### 369 *Fatigability and Recovery*

370 At baseline, males produced greater maximal force than females ( $667 \pm 35$  vs.  $471 \pm 98$  N,  $p$   
371  $< 0.001$ ). Throughout the interval task, a main effect of time was observed for MVC ( $F_{2,8,51.6} =$   
372  $9.897$ ,  $p < 0.001$ ,  $\eta^2 = 0.355$ ),  $Q_{tw,pot}$  ( $F_{2,0,36.1} = 30.481$ ,  $p < 0.001$ ,  $\eta^2 = 0.629$ ), and VA ( $F_{2,4,$   
373  $43.0} = 10.884$ ,  $p < 0.001$ ,  $\eta^2 = 0.377$ ). *Post-hoc* comparisons revealed significant reductions  
374 in each variable after interval one compared to baseline ( $p \leq 0.016$ ), however, after the first  
375 interval, no further reductions were observed ( $p \geq 0.506$ ). No sex or sex  $\times$  time interaction  
376 effects were observed for MVC ( $p \geq 0.150$ ),  $Q_{tw,pot}$  ( $p \geq 0.184$ ), or VA ( $p = 0.461$ ).

377

378

\*\*\* Figure 4 here \*\*\*

379

380 In the 30-min recovery period after the interval task, no main effect of time was observed for  
381 MVC ( $p = 0.226$ ), whereas as main effect of time was observed for  $Q_{tw,pot}$  ( $F_{2,3, 42.0} = 12.824$ ,  
382  $p < 0.001$ ,  $\eta^2 = 0.416$ ) and VA ( $F_{2,1,37.9} = 6.531$ ,  $p = 0.003$ ,  $\eta^2 = 0.266$ ). No sex or sex  $\times$   
383 time interaction effects were observed for MVC ( $p \geq 0.256$ ) or VA ( $p \geq 0.598$ ), and while  
384 there was no sex  $\times$  time interaction for  $Q_{tw,pot}$  ( $p = 0.567$ ), there was a main effect of sex  
385 ( $F_{1,18} = 4.679$ ,  $p = 0.044$ ,  $\eta^2 = 0.206$ ). *Post-hoc* comparisons indicated that females had

386 greater  $Q_{tw,pot}$  amplitudes than males at 20 ( $p = 0.027$ ) and 30 minutes ( $p = 0.030$ ) after the  
387 interval task.

388

389

## 390 **Discussion**

391 This study aimed to compare the neuromuscular and cardiopulmonary responses to high-  
392 intensity interval running exercise between sexes. It was hypothesised that there would be  
393 no sex differences in the metabolic response to the task, but females would experience  
394 lesser declines in voluntary and evoked contractions. The lack of sex differences in the  
395 relative  $\dot{V}O_2$ , RER, and blood lactate response to the task confirm the similarity of the  
396 metabolic response, whilst the sex difference in  $Q_{tw,pot}$  following the interval task indicated  
397 more fatigue-resistant female knee extensors. In addition, females demonstrated poorer  
398 ventilatory efficiency in the first half of the interval task compared to males, which was not  
399 evident in the second half of the task. Combined, these data demonstrate that the  
400 cardiopulmonary and neuromuscular responses to high-intensity interval running differ  
401 between sexes, adding to the growing evidence base that suggests practitioners should  
402 consider sex when prescribing exercise.

403

### 404 *Fatigability & Recovery Following Interval Exercise*

405 The lack of sex difference in the decline in neuromuscular function during exercise  
406 contradicts previous literature utilising high-intensity constant load [27-29] and self-paced  
407 [32] locomotor exercise. Additionally, the data contradict those reported following repeated  
408 sprint exercise [37, 38], reinforcing the notion that sex differences in fatigability are task-  
409 specific [52]. A recent study that employed a similar task to the present study (4 minute  
410 intervals with 3 minutes rest) also demonstrated similar fatigability between sexes [42],  
411 suggesting that the specific demands of high-intensity interval exercise do not permit sex  
412 differences in fatigability from manifesting. The duration of the intervals used in the present  
413 study (3 min) likely resulted in a substantial metabolic disturbance within the working  
414 muscles. Jones, Wilkerson [53] demonstrated that after 3.6 min of exercise at 110% of  
415 critical power, depletion of phosphocreatine (PCr) stores, inorganic phosphate accumulation,  
416 and pH decreases were all exaggerated compared to exercise below critical power. The rest  
417 periods between intervals (90 secs) likely permitted a degree of metabolic recovery of the  
418 working muscles, with near complete recovery of PCr stores and intramuscular pH being  
419 observed 90-120 secs after single-limb exercise [54, 55] and 6 minutes following all-out  
420 sprinting [56]. However, it should be acknowledged that the rest periods in the present study  
421 included the performance of MVCs, which may have delayed the metabolic recovery. The  
422 magnitude of knee-extensor fatigability that this repeated metabolic stress and recovery

423 elicited was moderate, with MVC reductions (between 5-10%) less than was reported in  
424 males following a 5 km running time trial [32], and substantially less than typically reported  
425 reductions following severe intensity cycling (~20% reduction in MVC, [57, 58]). One  
426 potential explanation why the fatigability induced by high-intensity interval running was not  
427 different between sexes is that the magnitude was too small to detect sex differences.  
428 Indeed, compared to previous data in single-limb and cycling exercise [27-29], where sex  
429 differences were observed, the present study observed approximately half the degree of  
430 MVC reduction in both sexes.

431

432 Although sex differences in fatigability were not evident during the interval task, a sex  
433 difference was observed in the 30 min recovery period, whereby females demonstrated  
434 greater  $Q_{tw,pot}$  amplitudes relative to pre-exercise. Although this post-exercise period was  
435 termed the 'recovery period', no recovery was observed for either sex for MVC or  $Q_{tw,pot}$ .  
436 Although central and peripheral contributions to fatigability are thought to mostly recover  
437 within 30 minutes following short duration exercise, recovery from running is further  
438 complicated by the presence of muscle damage caused by repeated stretch-shortening  
439 cycles [58, 59]. Studies employing high- and low-frequency electrical stimulation to profile  
440 fatigue and recovery following damaging exercise reveal that low-frequency evoked  
441 contractions remain depressed, whereas high-frequency contractions recover [60].  
442 Depression of low-frequency evoked contractions is thought to be underpinned by impaired  
443 intracellular calcium ion ( $Ca^{2+}$ ) release and/or reduced  $Ca^{2+}$  sensitivity of myofibrils [61, 62],  
444 with the former being mechanistically linked to the reduced contractility of muscle fibres  
445 following damaging exercise [63]. It is likely that the prolonged depression of MVC and  $Q_{tw,pot}$   
446 in the present study was underpinned by altered  $Ca^{2+}$  handling within the knee-extensors,  
447 which also provides insight into the lesser relative reductions experienced by females  
448 compared to males. Evidence from Harmer, Ruell [64] demonstrated sex differences in  $Ca^{2+}$   
449 regulation before and after high-intensity exercise, with  $Ca^{2+}$ ATPase activity reduced in  
450 males, but increased in females following exercise. Therefore, it is possible that the lesser  
451 relative reductions in  $Q_{tw,pot}$  experienced by females following high-intensity interval exercise  
452 in the present study were related to a lesser disruption to  $Ca^{2+}$  regulation within the knee-  
453 extensors. One additional consideration is that the patellar tendon stiffness has been  
454 demonstrated to be lower in females, which results in a greater mechanical buffer, and  
455 lesser knee-extensor fascicle lengthening for females during eccentric contractions [65].  
456 Conceivably, this could also contribute to the lesser reductions in contractile function  
457 observed post-exercise in the present study.

458

459 *Metabolic and Cardiopulmonary Responses to Interval Exercise*

460 The  $\dot{V}O_2$  recorded during all four intervals exceeded 90% of  $\dot{V}O_{2peak}$ , with values of ~95% in  
461 both sexes by the final interval. The lack of sex difference in relative  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , RER, and  
462 blood lactate provides evidence that the metabolic response to high-intensity interval  
463 exercise was similar between males and females. This agrees with similar data recorded  
464 during constant-load exercise in the severe intensity domain, where no sex differences in the  
465 aforementioned variables were observed [27]. While sex differences in substrate utilisation  
466 have been observed previously, these appear to be limited to steady-state exercise (i.e.,  
467 moderate and heavy intensity domains) rather than the intensities typically utilized during  
468 high-intensity interval exercise [66, 67]. In response to the similar metabolic demands, males  
469 and females employed different respiratory strategies across the interval task, with females  
470 demonstrating poorer ventilatory efficiency during the first two intervals. While it must be  
471 acknowledged that females did not reach criteria for clinical diagnosis of 'ventilatory  
472 inefficiency' [68], poorer ventilatory efficiency is thought to reflect poorer matching of lung  
473 ventilation to perfusion, and therefore impaired gas exchange [69]. Sex differences are well-  
474 established in the structure and function of the respiratory system [70], with larger airway  
475 diameters and lung volume, as well as greater alveolar surface area observed in males,  
476 even when height matched [71]. The lesser alveolar surface area in females results in  
477 poorer oxygen diffusing capacity during exercise compared to height-matched males,  
478 although matching for lung volume negates this sex difference [72]. The lower lung volume  
479 necessitates greater relative  $\dot{V}_E$  in females to achieve the same relative  $\dot{V}O_2$  and  $\dot{V}CO_2$ ,  
480 which is driven primarily through increases in breathing frequency in females [73]. In addition  
481 to the present study that observed this sex difference during high-intensity interval exercise,  
482 greater relative  $\dot{V}_E$  were observed in females during constant-load exercise in the heavy and  
483 severe intensity domains [27]. Interestingly, in the present study this ventilatory sex  
484 difference had dissipated by the third and fourth intervals, implying that both sexes ultimately  
485 experienced a loss of ventilatory efficiency as exercise progressed. Furthermore, the sex  
486 differences in ventilatory efficiency did not appear to affect perceptual variables in the  
487 present study, although future research should investigate dyspnoea more directly.

488

489

490

#### 491 *Further Considerations*

492 High-intensity interval exercise is often prescribed to improve an individual's  $\dot{V}O_{2max}$  via  
493 positive adaptations to skeletal muscle capillary density, maximum stroke volume and  
494 cardiac output, and blood volume [74]. How sex influences physiological adaptation to  
495 exercise is unclear, with some evidence suggesting that  $\dot{V}O_{2max}$  adaptation is blunted in  
496 females compared to males [75]. Physiological adaptation to exercise is multi-factorial, with

497 a variety of signalling pathways activated by disruptions to homeostasis in various  
498 physiological systems [76]. In the present study, the relative cardiovascular and metabolic  
499 perturbations were similar between sexes, but females experienced lesser contractile  
500 impairment following the task. Disruptions to intramuscular  $\text{Ca}^{2+}$  homeostasis that cause  
501 contractile impairment also activate  $\text{Ca}^{2+}$ -calmodulin-dependent kinases (CaMK, [77]).  
502 CaMKs play an important role in regulating oxidative enzyme expression, as well as  
503 peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1a) and therefore  
504 mitochondrial biogenesis [78]. While speculative, it could be suggested that the lesser  
505 reductions in contractile function experienced by females in the present study might result in  
506 a lesser stimulus for adaptation, however this hypothesis should be directly tested with  
507 appropriate methodologies.

508

### 509 *Limitations*

510 The present study employed a high-intensity interval task that was performed at a  
511 percentage of each individual's  $\dot{V}\text{O}_{2\text{peak}}$ , which has previously been suggested to lead to  
512 greater heterogeneity of physiological responses compared to threshold-based approaches  
513 [79]. The approach of threshold-anchored exercise prescription has led to sex differences in  
514 fatigability and cardiopulmonary function being observed previously [27-29]. However, the  
515 present study observed similar sex differences to previous research when work rate was  
516 normalized to maximum capacity, rather than submaximal thresholds. Given that both sexes  
517 performed the task at ~140% of their lactate threshold (Table 1), and reached  $\dot{V}\text{O}_2$  values of  
518 ~95%  $\dot{V}\text{O}_{2\text{peak}}$ , peak blood lactate concentrations of ~ 8  $\text{mmol}\cdot\text{l}^{-1}$ , and peak RERs >1.05  
519 during the interval task, it is likely that the task took place in the severe domain, and  
520 observed sex differences were not related to differences in the relative work rate of the task.

521

522 Participants completed an incremental exercise test to exhaustion in visit 1, however they  
523 did not complete a verification phase to determine whether the peak  $\dot{V}\text{O}_2$  data were a valid  
524 estimate of  $\dot{V}\text{O}_{2\text{max}}$  [80]. While the concern here is that  $\dot{V}\text{O}_{2\text{peak}}$  might not be an accurate  
525 reflection of  $\dot{V}\text{O}_{2\text{max}}$  and its associated variables, Chidnok, DiMenna [81] demonstrated that  
526 in healthy, active participants,  $\dot{V}\text{O}_{2\text{peak}}$  does consistently provide equivalent outcomes when  
527 compared to verification tests.

528

529 Finally, our *a priori* sample size calculation estimated that n=12 participants would be  
530 necessary to detect a sex difference in fatigability, yet that calculation used an effect size  
531 from constant-load cycling [27]. As discussed above, the magnitude of fatigability  
532 experienced by participants was smaller than previous research, for a variety of reasons,  
533 therefore one potential reason as to why a sex difference in fatigability *during* interval

534 exercise was not observed could be that the study was underpowered, and a sample size  
535 greater than the  $n=20$  we tested would be required to detect differences. Similarly, the  
536 anticipated sex difference in relative  $\dot{V}O_{2peak}$  ( $\sim 10\%$ , [82]) was not statistically detected in this  
537 study (6% difference,  $p = 0.292$ ). Given the study was powered to detect changes in  
538 fatigability, rather than between group differences in  $\dot{V}O_{2peak}$ , as well as the similar self-  
539 reported training volume in males and females, as well as similar relative velocities at  
540 submaximal thresholds, it is unlikely that training status was different between groups in the  
541 present study.

542

### 543 **Conclusions**

544 This study demonstrated that males and females experienced similar cardiovascular and  
545 metabolic responses to a high-intensity interval exercise task, however females  
546 demonstrated poorer ventilatory efficiency in the first half of the task, and lesser reductions  
547 in knee-extensor contractile function following the task. Much like previous research that  
548 observed integrative sex differences during constant-load exercise, this study demonstrated  
549 that females and males do not experience the same responses to interval exercise. The  
550 exercise task employed in the present study is akin to those typically used for the  
551 enhancement of athletic performance, highlighting that those prescribing exercise should be  
552 cognisant that the sex of participants will influence the acute physiological responses.

553 **Acknowledgements**

554 The researchers would like to thank the participants of the present study for their time and  
555 efforts.

556

557 **Conflicts of Interest**

558 The authors report no conflicts, financial or otherwise.

559

560 **Funding**

561 No funding was received for this study.

562

563 **Data Availability**

564 Data are available upon request to the corresponding author.

565

566 **Reference List**

- 567 1. Cowley, E.S., et al., *"Invisible Sportswomen": The Sex Data Gap in Sport and Exercise*  
568 *Science Research*. Women in Sport and Physical Activity Journal, 2021. **29**(2): p. 146-  
569 151.
- 570 2. James, J.J., et al., *Underrepresentation of women in exercise science and physiology*  
571 *research is associated with authorship gender*. Journal of Applied Physiology, 2023.  
572 **135**(4): p. 932-942.
- 573 3. Ansdell, P., et al., *Physiological sex differences affect the integrative response to*  
574 *exercise: acute and chronic implications*. Experimental Physiology, 2020. **105**(12): p.  
575 2007-2021.
- 576 4. Hammer, S.M., et al., *Sex differences in muscle contraction-induced limb blood flow*  
577 *limitations*. European Journal of Applied Physiology, 2023.
- 578 5. Coggan, A.R., et al., *Histochemical and enzymatic comparison of the gastrocnemius*  
579 *muscle of young and elderly men and women*. J Gerontol, 1992. **47**(3): p. B71-6.
- 580 6. Porter, M.M., et al., *Capillary supply of the tibialis anterior muscle in young, healthy,*  
581 *and moderately active men and women*. J Appl Physiol (1985), 2002. **92**(4): p. 1451-  
582 7.
- 583 7. Staron, R.S., et al., *Fiber Type Composition of the Vastus Lateralis Muscle of Young*  
584 *Men and Women*. Journal of Histochemistry & Cytochemistry, 2000. **48**(5): p. 623-  
585 629.
- 586 8. Roepstorff, C., et al., *Sex differences in hormone-sensitive lipase expression, activity,*  
587 *and phosphorylation in skeletal muscle at rest and during exercise*. American Journal  
588 of Physiology-Endocrinology and Metabolism, 2006. **291**(5): p. E1106-E1114.
- 589 9. Parker, B.A., et al., *Sex differences in leg vasodilation during graded knee extensor*  
590 *exercise in young adults*. Journal of Applied Physiology, 2007. **103**(5): p. 1583-1591.
- 591 10. Hunter, S.K., *Sex differences in human fatigability: mechanisms and insight to*  
592 *physiological responses*. Acta Physiologica, 2014. **210**(4): p. 768-789.
- 593 11. Mead, J., *Dysanapsis in Normal Lungs Assessed by the Relationship between Maximal*  
594 *Flow, Static Recoil, and Vital Capacity*. American Review of Respiratory Disease,  
595 1980. **121**(2): p. 339-342.

- 596 12. Crapo, R.O., A.H. Morris, and R.M. Gardner, *Reference values for pulmonary tissue*  
597 *volume, membrane diffusing capacity, and pulmonary capillary blood volume.*  
598 *Bulletin europeen de physiopathologie respiratoire*, 1982. **18**(6): p. 893-899.
- 599 13. Martin, T.R., et al., *Airway size is related to sex but not lung size in normal adults.*  
600 *Journal of Applied Physiology*, 1987. **63**(5): p. 2042-2047.
- 601 14. Guenette, J.A., et al., *Sex differences in the resistive and elastic work of breathing*  
602 *during exercise in endurance-trained athletes.* *American Journal of Physiology-*  
603 *Regulatory, Integrative and Comparative Physiology*, 2009. **297**(1): p. R166-R175.
- 604 15. Sheel, A.W., P.B. Dominelli, and Y. Molgat-Seon, *Revisiting dysanapsis: sex-based*  
605 *differences in airways and the mechanics of breathing during exercise.* *Experimental*  
606 *Physiology*, 2016. **101**(2): p. 213-218.
- 607 16. Dominelli, P.B., et al., *Oxygen cost of exercise hyperpnoea is greater in women*  
608 *compared with men.* *The Journal of Physiology*, 2015. **593**(8): p. 1965-1979.
- 609 17. Murphy, W.G., *The sex difference in haemoglobin levels in adults — Mechanisms,*  
610 *causes, and consequences.* *Blood Reviews*, 2014. **28**(2): p. 41-47.
- 611 18. Harms, C.A., et al., *Exercise-induced arterial hypoxaemia in healthy young women.*  
612 *The Journal of Physiology*, 1998. **507**(2): p. 619-628.
- 613 19. Diaz-Canestro, C., et al., *Sex differences in cardiorespiratory fitness are explained by*  
614 *blood volume and oxygen carrying capacity.* *Cardiovascular Research*, 2022. **118**(1):  
615 p. 334-343.
- 616 20. Hunter, S.K., *Sex differences in fatigability of dynamic contractions.* *Experimental*  
617 *Physiology*, 2016. **101**(2): p. 250-255.
- 618 21. Hunter, S.K., et al., *Men are more fatigable than strength-matched women when*  
619 *performing intermittent submaximal contractions.* *Journal of Applied Physiology*,  
620 2004. **96**(6): p. 2125-2132.
- 621 22. Ansdell, P., et al., *Contraction intensity and sex differences in knee-extensor*  
622 *fatigability.* *Journal of Electromyography and Kinesiology*, 2017. **37**: p. 68-74.
- 623 23. Senefeld, J., et al., *Sex Differences in Mechanisms of Recovery after Isometric and*  
624 *Dynamic Fatiguing Tasks.* *Med Sci Sports Exerc*, 2018. **50**(5): p. 1070-1083.
- 625 24. Ansdell, P., et al., *Sex differences in fatigability and recovery relative to the intensity–*  
626 *duration relationship.* *The Journal of Physiology*, 2019. **597**(23): p. 5577-5595.
- 627 25. McDougall, R.M., et al., *The influence of skeletal muscle mitochondria and sex on*  
628 *critical torque and performance fatigability in humans.* *The Journal of Physiology*,  
629 2023. **601**(23): p. 5295-5316.
- 630 26. Sidhu, S.K., A.G. Cresswell, and T.J. Carroll, *Corticospinal Responses to Sustained*  
631 *Locomotor Exercises: Moving Beyond Single-Joint Studies of Central Fatigue.* *Sports*  
632 *Medicine*, 2013. **43**(6): p. 437-449.
- 633 27. Ansdell, P., et al., *Sex differences in fatigability following exercise normalised to the*  
634 *power–duration relationship.* *The Journal of Physiology*, 2020. **598**(24): p. 5717-5737.
- 635 28. Azevedo, R.d.A., et al., *Slight power output manipulations around the maximal*  
636 *lactate steady state have a similar impact on fatigue in females and males.* *Journal of*  
637 *Applied Physiology*, 2021. **130**(6): p. 1879-1892.
- 638 29. Azevedo, R.D.E.A., et al., *Time Course of Performance Fatigability during Exercise*  
639 *below, at, and above the Critical Intensity in Females and Males.* *Medicine and*  
640 *science in sports and exercise*, 2022. **54**(10): p. 1665-1677.

- 641 30. Glace, B.W., M.P. McHugh, and G.W. Gleim, *Effects of a 2-Hour Run on Metabolic*  
642 *Economy and Lower Extremity Strength in Men and Women*. Journal of Orthopaedic  
643 & Sports Physical Therapy, 1998. **27**(3): p. 189-196.
- 644 31. Glace, B.W., I.J. Kremenec, and M.P. McHugh, *Sex differences in central and*  
645 *peripheral mechanisms of fatigue in cyclists*. European Journal of Applied Physiology,  
646 2013. **113**(4): p. 1091-1098.
- 647 32. Solleiro Pons, M., S.K. Hunter, and P. Ansdell, *Sex differences in fatigability and*  
648 *recovery following a 5 km running time trial in recreationally active adults*. European  
649 Journal of Sport Science, 2023. **23**(12): p. 2349-2356.
- 650 33. Besson, T., et al., *Sex Differences in Neuromuscular Fatigue and Changes in Cost of*  
651 *Running after Mountain Trail Races of Various Distances*. Med Sci Sports Exerc, 2021.  
652 **53**(11): p. 2374-2387.
- 653 34. Temesi, J., et al., *Are Females More Resistant to Extreme Neuromuscular Fatigue?*  
654 *Medicine and science in sports and exercise*, 2015. **47**(7): p. 1372-1382.
- 655 35. Boccia, G., et al., *Women show similar central and peripheral fatigue to men after*  
656 *half-marathon\**. European Journal of Sport Science, 2018. **18**(5): p. 695-704.
- 657 36. Laursen, P.B. and D.G. Jenkins, *The Scientific Basis for High-Intensity Interval*  
658 *Training*. Sports Medicine, 2002. **32**(1): p. 53-73.
- 659 37. Brooks, S., et al., *The hormonal responses to repetitive brief maximal exercise in*  
660 *humans*. European Journal of Applied Physiology and Occupational Physiology, 1990.  
661 **60**(2): p. 144-148.
- 662 38. Billaut, F. and K. Smith, *Sex alters impact of repeated bouts of sprint exercise on*  
663 *neuromuscular activity in trained athletes*. Applied Physiology, Nutrition, and  
664 Metabolism, 2009. **34**(4): p. 689-699.
- 665 39. Billaut, F. and D.J. Bishop, *Mechanical work accounts for sex differences in fatigue*  
666 *during repeated sprints*. European Journal of Applied Physiology, 2012. **112**(4): p.  
667 1429-1436.
- 668 40. Parmar, A., T. Jones, and P. Hayes, *The use of interval-training methods by coaches of*  
669 *well-trained middle- to long-distance runners*. International Journal of Strength and  
670 Conditioning, 2021. **1**(1).
- 671 41. Jones, A.M. and A. Vanhatalo, *The 'Critical Power' Concept: Applications to Sports*  
672 *Performance with a Focus on Intermittent High-Intensity Exercise*. Sports Medicine,  
673 2017. **47**(1): p. 65-78.
- 674 42. Tripp, Thomas R., et al., *The Effect of Duration on Performance and Perceived*  
675 *Fatigability During Acute High-Intensity Interval Exercise in Young, Healthy Males*  
676 *and Females*. Scandinavian Journal of Medicine & Science in Sports, 2024. **34**(7): p.  
677 e14692.
- 678 43. Ansdell, P., et al., *Menstrual cycle-associated modulations in neuromuscular function*  
679 *and fatigability of the knee extensors in eumenorrhic women*. Journal of Applied  
680 Physiology, 2019. **126**(6): p. 1701-1712.
- 681 44. Mattu, A.T., et al., *Menstrual and oral contraceptive cycle phases do not affect*  
682 *submaximal and maximal exercise responses*. Scandinavian Journal of Medicine &  
683 Science in Sports, 2020. **30**(3): p. 472-484.
- 684 45. Georgescu, V.P., et al., *The female menstrual cycle: impact on cardiovascular,*  
685 *ventilatory and neuromuscular responses to whole body exercise*. The FASEB Journal,  
686 2020. **34**(S1): p. 1-1.

- 687 46. Freemans, J.A., et al., *Exercise Performance Is Impaired during the Midluteal Phase of*  
688 *the Menstrual Cycle*. Med Sci Sports Exerc, 2021. **53**(2): p. 442-452.
- 689 47. Granata, C., et al., *Training intensity modulates changes in PGC-1 $\alpha$  and p53 protein*  
690 *content and mitochondrial respiration, but not markers of mitochondrial content in*  
691 *human skeletal muscle*. The FASEB Journal, 2016. **30**(2): p. 959-970.
- 692 48. Henriksson, J. and J.S. Reitman, *Time Course of Changes in Human Skeletal Muscle*  
693 *Succinate Dehydrogenase and Cytochrome Oxidase Activities and Maximal Oxygen*  
694 *Uptake with Physical Activity and Inactivity*. Acta Physiologica Scandinavica, 1977.  
695 **99**(1): p. 91-97.
- 696 49. Peserico, C.S., A.M. Zagatto, and F.A. Machado, *Evaluation of the Best-designed*  
697 *Graded Exercise Test to Assess Peak Treadmill Speed*. Int J Sports Med, 2015. **36**(09):  
698 p. 729-734.
- 699 50. Faude, O., W. Kindermann, and T. Meyer, *Lactate Threshold Concepts*. Sports  
700 Medicine, 2009. **39**(6): p. 469-490.
- 701 51. Kufel, T.J., L.A. Pineda, and M.J. Mador, *Comparison of potentiated and*  
702 *unpotentiated twitches as an index of muscle fatigue*. Muscle & Nerve, 2002. **25**(3):  
703 p. 438-444.
- 704 52. Hunter, S.K., *Sex Differences and Mechanisms of Task-Specific Muscle Fatigue*.  
705 Exercise and Sport Sciences Reviews, 2009. **37**(3): p. 113-122.
- 706 53. Jones, A.M., et al., *Muscle metabolic responses to exercise above and below the*  
707 *"critical power" assessed using 31P-MRS*. American Journal of Physiology-Regulatory,  
708 Integrative and Comparative Physiology, 2008. **294**(2): p. R585-R593.
- 709 54. Layec, G., et al., *Effects of exercise-induced intracellular acidosis on the*  
710 *phosphocreatine recovery kinetics: a 31P MRS study in three muscle groups in*  
711 *humans*. NMR in Biomedicine, 2013. **26**(11): p. 1403-1411.
- 712 55. Iotti, S., et al., *Kinetics of post-exercise phosphate transport in human skeletal*  
713 *muscle: An in vivo 31P-MR spectroscopy study*. Biochemical and Biophysical Research  
714 Communications, 1991. **176**(3): p. 1204-1209.
- 715 56. Bogdanis, G.C., et al., *Recovery of power output and muscle metabolites following 30*  
716 *s of maximal sprint cycling in man*. The Journal of Physiology, 1995. **482**(2): p. 467-  
717 480.
- 718 57. Thomas, K., et al., *Intensity-Dependent Contribution of Neuromuscular Fatigue after*  
719 *Constant-Load Cycling*. Med Sci Sports Exerc, 2016. **48**(9): p. 1751-60.
- 720 58. Brownstein, C.G., G.Y. Millet, and K. Thomas, *Neuromuscular responses to fatiguing*  
721 *locomotor exercise*. Acta Physiologica, 2021. **231**(2): p. e13533.
- 722 59. Carroll, T.J., J.L. Taylor, and S.C. Gandevia, *Recovery of central and peripheral*  
723 *neuromuscular fatigue after exercise*. Journal of Applied Physiology, 2017. **122**(5): p.  
724 1068-1076.
- 725 60. Skurvydas, A., et al., *Mechanisms of force depression caused by different types of*  
726 *physical exercise studied by direct electrical stimulation of human quadriceps muscle*.  
727 European Journal of Applied Physiology, 2016. **116**(11): p. 2215-2224.
- 728 61. Bruton, J.D., J. Lannergren, and H. Westerblad, *Mechanisms underlying the slow*  
729 *recovery of force after fatigue: importance of intracellular calcium*. Acta Physiologica  
730 Scandinavica, 1998. **162**(3): p. 285-293.
- 731 62. Kamandulis, S., et al., *Prolonged force depression after mechanically demanding*  
732 *contractions is largely independent of Ca<sup>2+</sup> and reactive oxygen species*. The FASEB  
733 Journal, 2017. **31**(11): p. 4809-4820.

- 734 63. Gehlert, S., et al., *Intense Resistance Exercise Induces Early and Transient Increases in*  
735 *Ryanodine Receptor 1 Phosphorylation in Human Skeletal Muscle*. PLOS ONE, 2012.  
736 **7**(11): p. e49326.
- 737 64. Harmer, A.R., et al., *Effects of type 1 diabetes, sprint training and sex on skeletal*  
738 *muscle sarcoplasmic reticulum Ca<sup>2+</sup> uptake and Ca<sup>2+</sup>-ATPase activity*. The Journal of  
739 *Physiology*, 2014. **592**(3): p. 523-535.
- 740 65. Hicks, K.M., et al., *Gender differences in fascicular lengthening during eccentric*  
741 *contractions: the role of the patella tendon stiffness*. *Acta Physiologica*, 2013. **209**(3):  
742 p. 235-244.
- 743 66. Devries, M.C., et al., *Menstrual cycle phase and sex influence muscle glycogen*  
744 *utilization and glucose turnover during moderate-intensity endurance exercise*.  
745 *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*,  
746 2006. **291**(4): p. R1120-R1128.
- 747 67. Tarnopolsky, L.J., et al., *Gender differences in substrate for endurance exercise*.  
748 *Journal of Applied Physiology*, 1990. **68**(1): p. 302-308.
- 749 68. Phillips, D.B., S.É. Collins, and M.K. Stickland, *Measurement and Interpretation of*  
750 *Exercise Ventilatory Efficiency*. *Frontiers in Physiology*, 2020. **11**.
- 751 69. Sun, X.-G., et al., *Ventilatory Efficiency during Exercise in Healthy Subjects*. *American*  
752 *Journal of Respiratory and Critical Care Medicine*, 2002. **166**(11): p. 1443-1448.
- 753 70. Molgat-Seon, Y., C.M. Peters, and A.W. Sheel, *Sex-differences in the human*  
754 *respiratory system and their impact on resting pulmonary function and the*  
755 *integrative response to exercise*. *Current Opinion in Physiology*, 2018. **6**: p. 21-27.
- 756 71. Dominelli, P.B. and Y. Molgat-Seon, *Sex, gender and the pulmonary physiology of*  
757 *exercise*. *European Respiratory Review*, 2022. **31**(163): p. 210074.
- 758 72. Bouwsema, M.M., V. Tedjasaputra, and M.K. Stickland, *Are there sex differences in*  
759 *the capillary blood volume and diffusing capacity response to exercise?* *Journal of*  
760 *Applied Physiology*, 2017. **122**(3): p. 460-469.
- 761 73. White, D.P., et al., *Sexual influence on the control of breathing*. *J Appl Physiol Respir*  
762 *Environ Exerc Physiol*, 1983. **54**(4): p. 874-9.
- 763 74. MacInnis, M.J. and M.J. Gibala, *Physiological adaptations to interval training and the*  
764 *role of exercise intensity*. *The Journal of Physiology*, 2017. **595**(9): p. 2915-2930.
- 765 75. Diaz-Canestro, C. and D. Montero, *Sex Dimorphism of VO<sub>2</sub>max Trainability: A*  
766 *Systematic Review and Meta-analysis*. *Sports Medicine*, 2019. **49**(12): p. 1949-1956.
- 767 76. Furrer, R., J.A. Hawley, and C. Handschin, *The molecular athlete: exercise physiology*  
768 *from mechanisms to medals*. *Physiological Reviews*, 2023. **103**(3): p. 1693-1787.
- 769 77. Coffey, V.G. and J.A. Hawley, *The Molecular Bases of Training Adaptation*. *Sports*  
770 *Medicine*, 2007. **37**(9): p. 737-763.
- 771 78. Chin, E.R., *Role of Ca<sup>2+</sup>/calmodulin-dependent kinases in skeletal muscle plasticity*.  
772 *Journal of Applied Physiology*, 2005. **99**(2): p. 414-423.
- 773 79. Meyler, S., et al., *Variability in exercise tolerance and physiological responses to*  
774 *exercise prescribed relative to physiological thresholds and to maximum oxygen*  
775 *uptake*. *Experimental Physiology*, 2023. **108**(4): p. 581-594.
- 776 80. Poole, D.C. and A.M. Jones, *Measurement of the maximum oxygen uptake  $\dot{V}O_{2max}$ :*  
777  *$\dot{V}O_{2peak}$  is no longer acceptable*. *Journal of Applied Physiology*, 2017. **122**(4): p. 997-  
778 1002.

- 779 81. Chidnok, W., et al.,  $\dot{V}_{\text{O}_2\text{max}}$  is not altered by self-  
780 pacing during incremental exercise. *European Journal of Applied Physiology*, 2013.  
781 **113**(2): p. 529-539.  
782 82. Joyner, M.J., *Physiological limits to endurance exercise performance: influence of sex*.  
783 *The Journal of Physiology*, 2017. **595**(9): p. 2949-2954.  
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786 **List of Figures:**

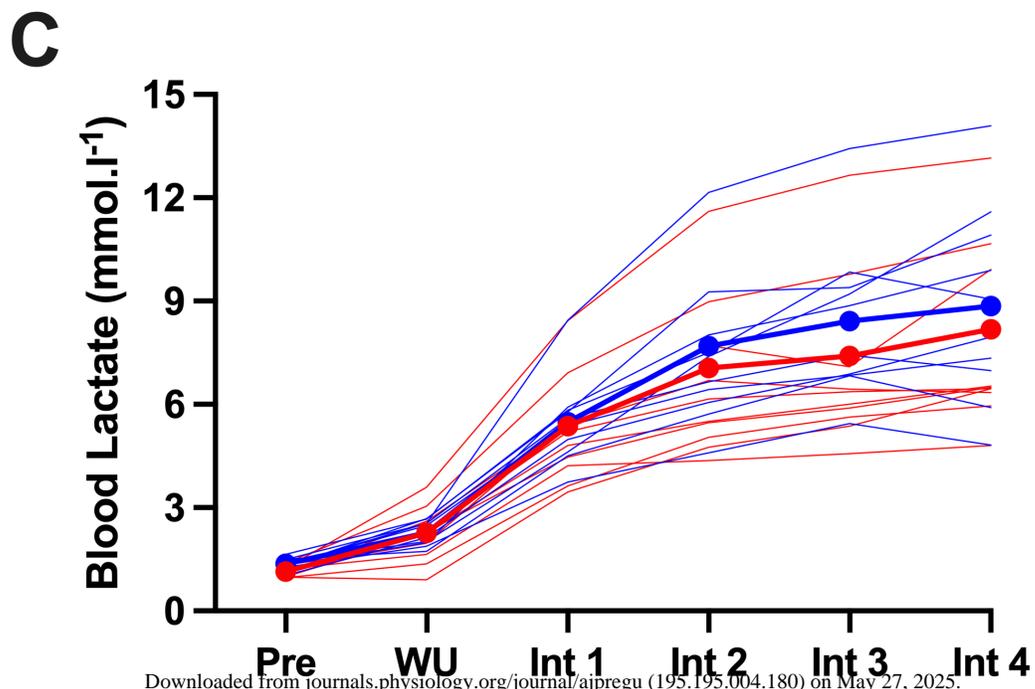
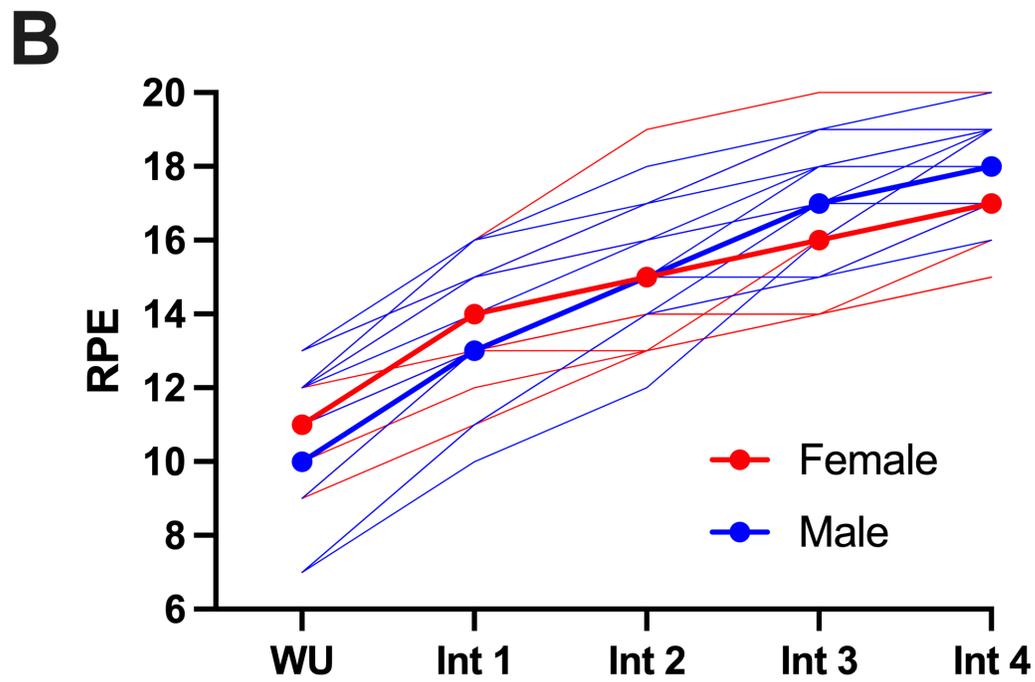
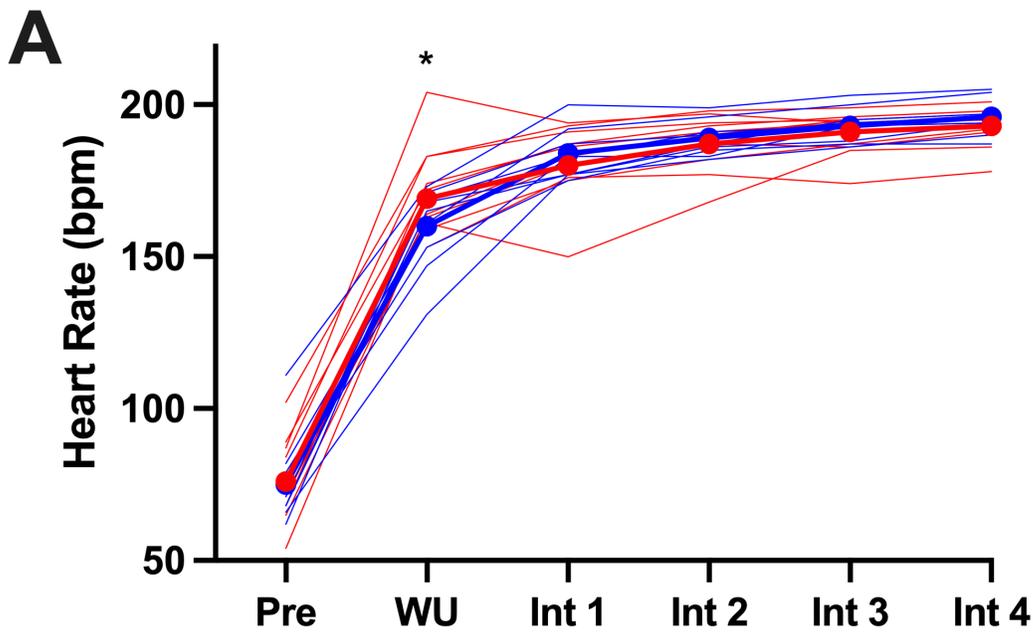
787 Figure 1: Heart rate (Panel A), rating of perceived exertion (RPE, Panel B), and blood lactate  
788 concentration (Panel C) at rest, following the warmup (WU), and at the end of each interval (Int).

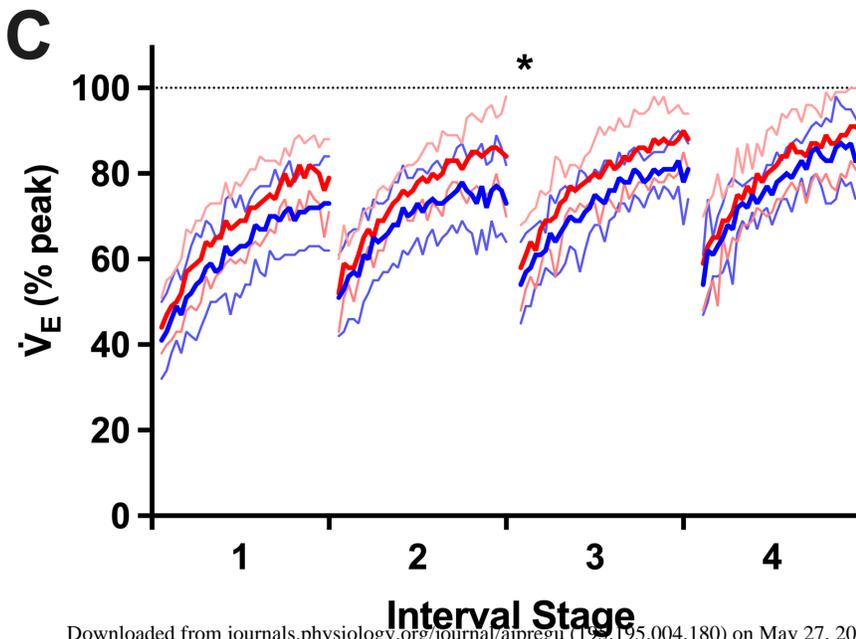
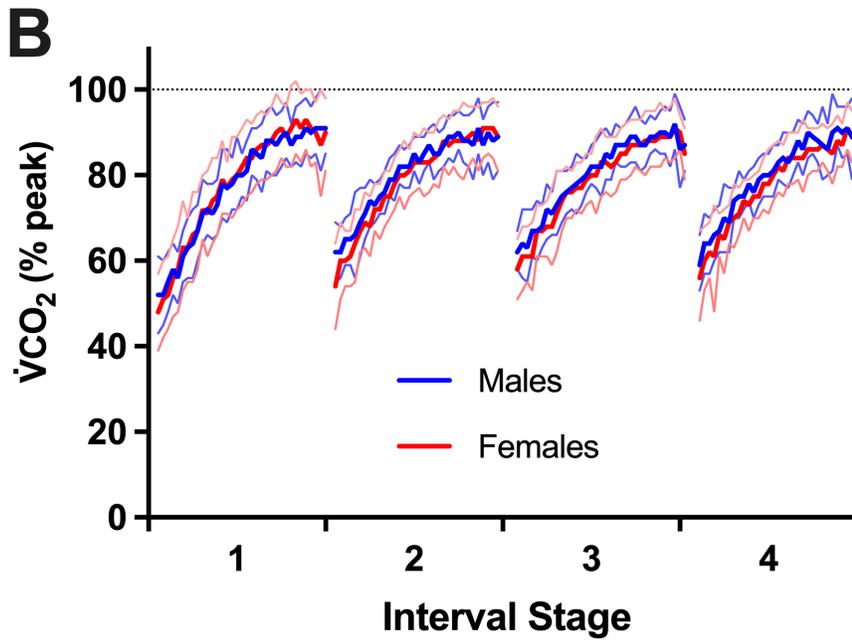
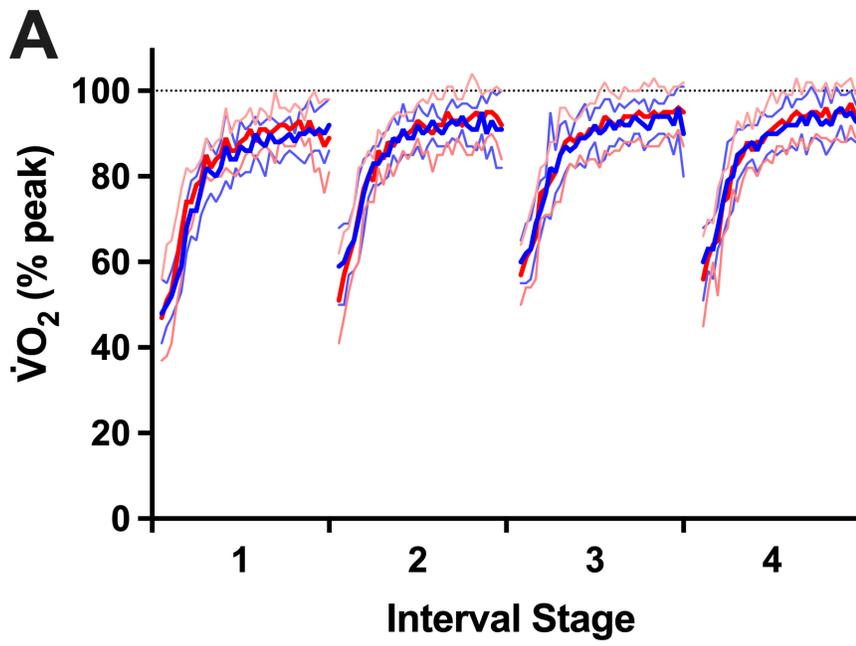
789 \* = females greater than males ( $p < 0.05$ ).  
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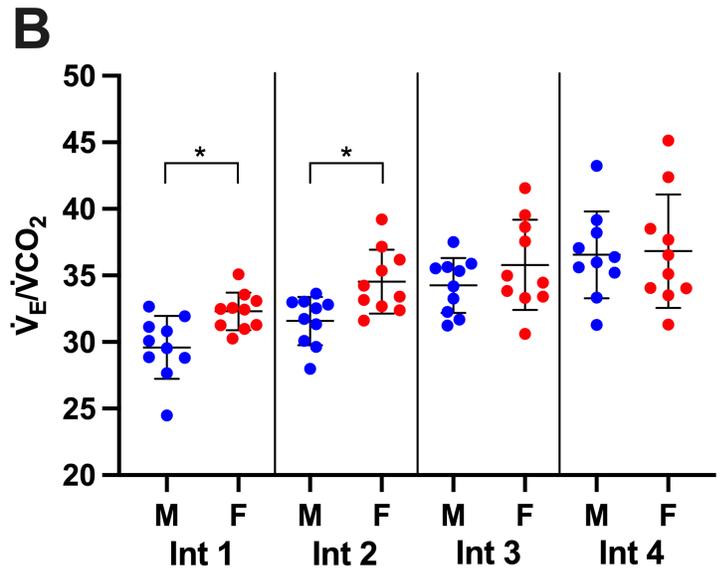
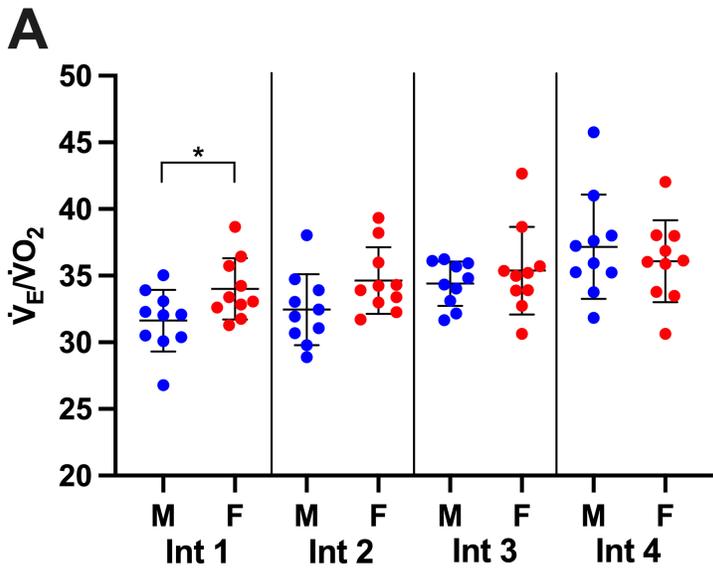
791 Figure 2: Rate of oxygen uptake ( $\dot{V}\text{O}_2$ , Panel A), carbon dioxide production ( $\dot{V}\text{CO}_2$ , Panel B), and  
792 ventilation ( $\dot{V}_E$ , Panel C) during the four interval stages. Thick lines represent the mean data for  
793 each sex, while thin lines represent the standard deviation. Horizontal dashed lines indicate peak  
794 values attained during the incremental exercise test. \* = sex x time interaction effect ( $p < 0.05$ ).  
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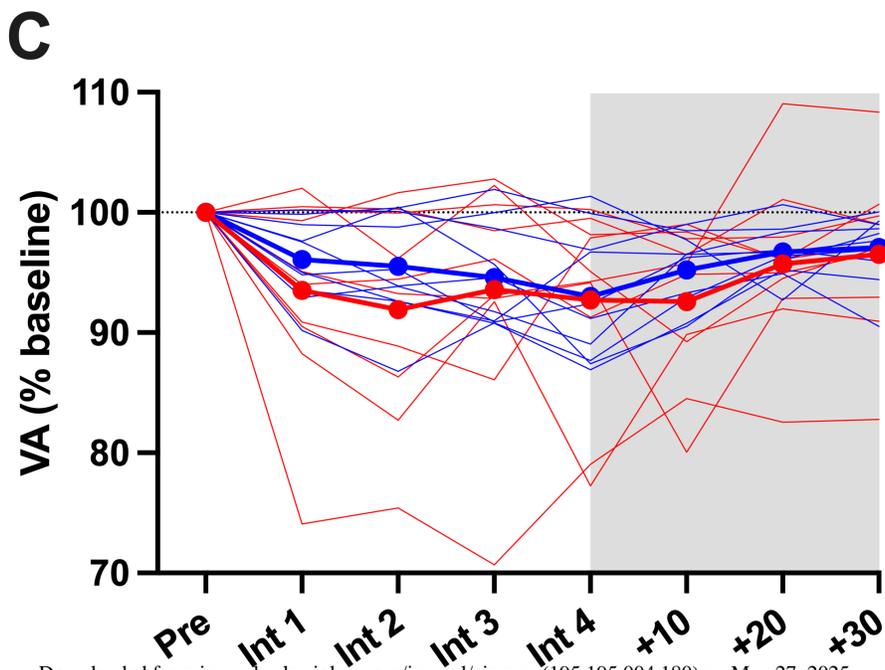
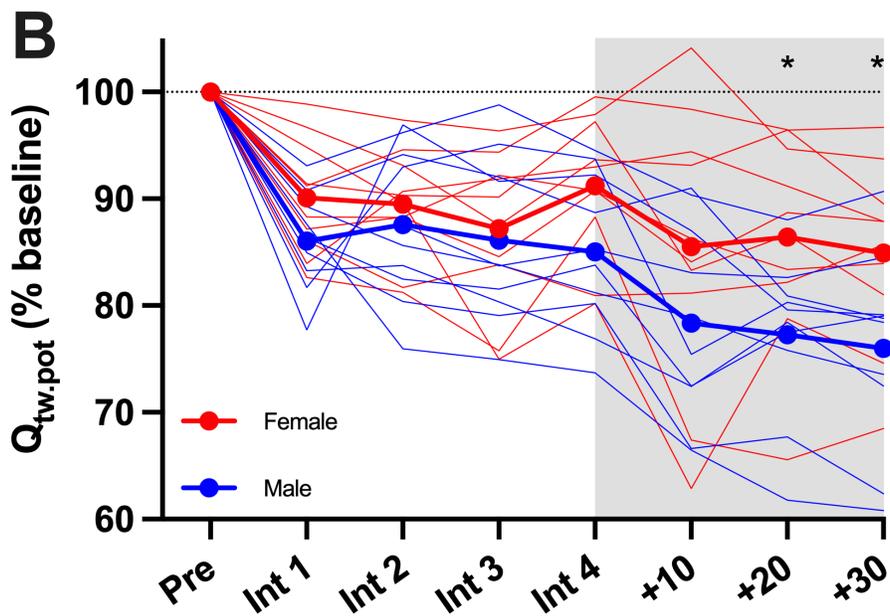
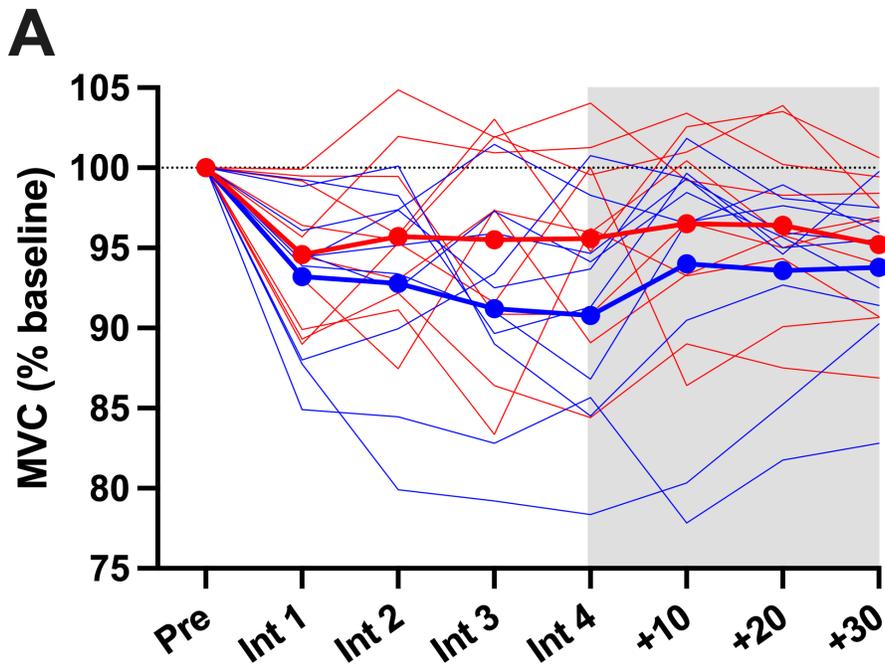
796 Figure 3: Ventilatory equivalents of oxygen uptake ( $\dot{V}_E/\dot{V}\text{O}_2$ , Panel A) and carbon dioxide  
797 production ( $\dot{V}_E/\dot{V}\text{CO}_2$ , Panel B) during the final 30 secs of each interval (Int). Blue dots represent  
798 individual males, while red dots represent individual females. \* = females greater than males ( $p <$   
799  $0.05$ ).  
800

801 Figure 4: Neuromuscular variables recorded during and following the interval task. Thin lines  
802 represent individual participants, whereas the thick lines represent group mean data. Maximal  
803 voluntary contraction (MVC, Panel A); quadriceps potentiated twitch (Qtw.pot, Panel B); and  
804 voluntary activation (VA, Panel C). \* = females greater than males ( $p < 0.05$ ).  
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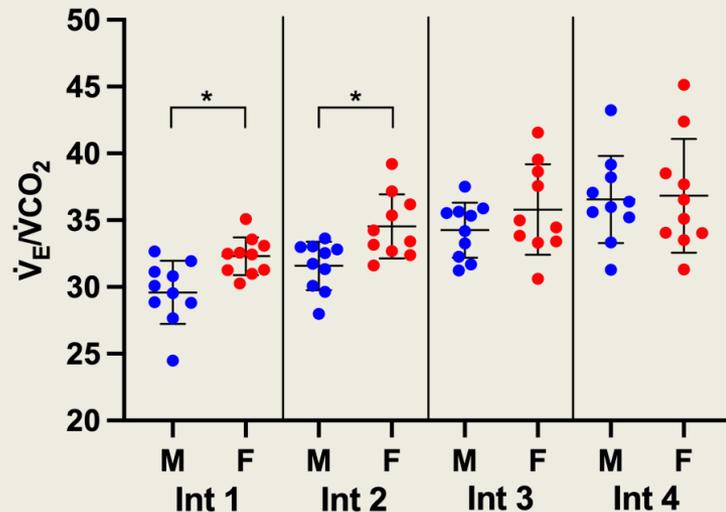
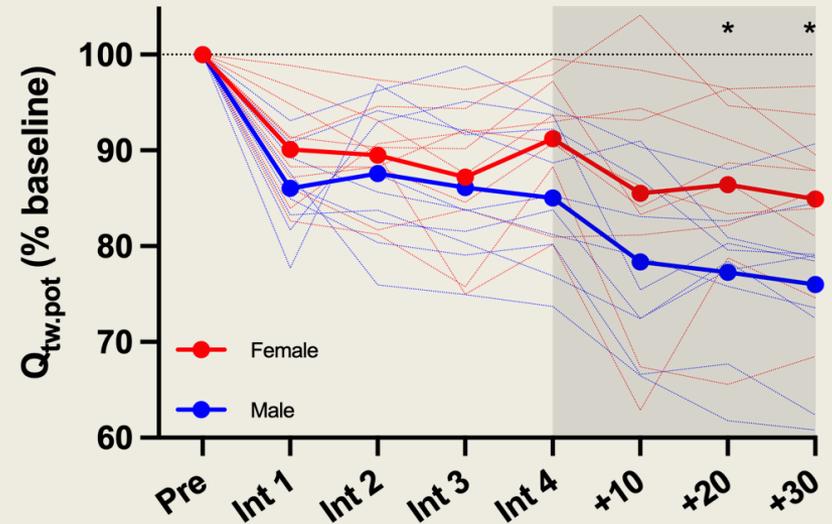
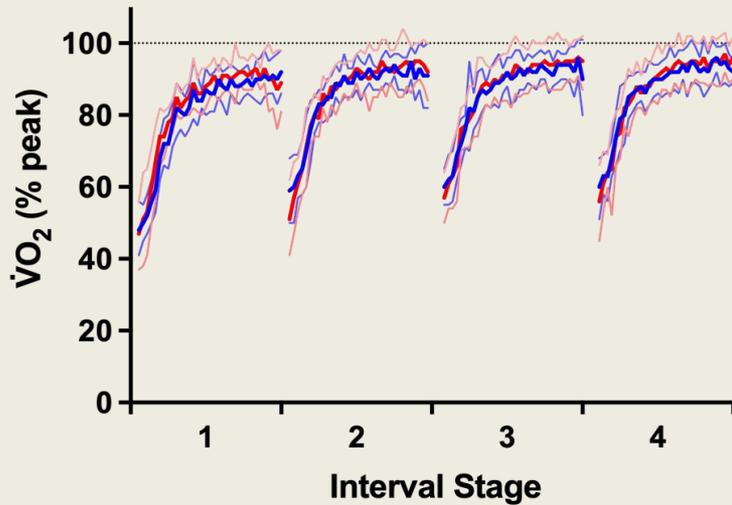








# Sex differences in the cardiopulmonary and neuromuscular response to high-intensity interval exercise



## RESULTS

In response to 4 × 3-min running intervals at 90% of the final incremental test velocity ( $v\dot{V}O_{2peak}$ ), females experienced:

- A similar metabolic response
- Greater hyperpnoea
- Lesser contractile dysfunction post-exercise