

1 **Title:** Effects of prior upper body exercise on the 3-minute all-out cycling test in men

2

3 **Authors:** Michael A. Johnson, Neil C. Williams, Ashley W. Graham, Luke A.L. Ingram,
4 Simon B. Cooper, Graham R. Sharpe

5 **Institutional affiliation:** Exercise and Health Research Group, Sport, Health and Performance
6 Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham
7 Trent University, Nottingham, UK

8

9 **Corresponding author**

10 Dr Michael A. Johnson

11 School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS,
12 UK

13 Telephone: +44 (0)115 8483362

14 E-mail: Michael.johnson@ntu.ac.uk

15

16

17

18

19

20

21

22 **ABSTRACT**

23 **Introduction** Prior upper body exercise reduces the curvature constant (W') of the hyperbolic
24 power-duration relationship without affecting critical power. This study tested the hypothesis
25 that prior upper body exercise reduces the work done over the end-test power (WEP; analogue
26 of W') during a 3-min all-out cycling test (3MT) without affecting the end-test power (EP;
27 analogue of critical power). **Methods** Ten endurance-trained men ($\dot{V}O_{2\max} = 62 \pm 5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)
28 performed a 3MT without (CYC) and with (ARM-CYC) prior severe-intensity,
29 intermittent upper body exercise. EP was calculated as the mean power output over the last 30-
30 s of the 3MT, whereas WEP was calculated as the power-time integral above EP. **Results** At
31 the start of the 3MT, plasma $[\text{La}^-]$ (1.8 ± 0.4 vs. $14.1 \pm 3.4 \text{ mmol}\cdot\text{L}^{-1}$) and $[\text{H}^+]$ (42.8 ± 3.1 vs.
32 $58.6 \pm 5.5 \text{ nmol}\cdot\text{L}^{-1}$) were higher, whereas the strong ion difference ([SID]) (41.4 ± 2.2 vs. 30.9
33 $\pm 4.6 \text{ mmol}\cdot\text{L}^{-1}$) and $[\text{HCO}_3^-]$ (27.0 ± 1.9 vs. $16.9 \pm 3.2 \text{ mmol}\cdot\text{L}^{-1}$) were lower, during ARM-
34 CYC than CYC ($P < 0.010$). EP was 12% lower during the 3MT of ARM-CYC ($298 \pm 52 \text{ W}$)
35 than CYC ($338 \pm 60 \text{ W}$) ($P < 0.001$), whereas WEP was not different (CYC: $12.8 \pm 3.3 \text{ kJ}$ vs.
36 ARM-CYC: $13.5 \pm 4.1 \text{ kJ}$, $P = 0.312$). EP in CYC was positively correlated with the peak $[\text{H}^+]$
37 ($r = 0.78$, $P = 0.008$), and negatively correlated with the lowest $[\text{HCO}_3^-]$ ($r = -0.74$, $P = 0.015$).
38 **Conclusion** These results suggest that EP during a 3MT in endurance-trained men is sensitive
39 to fatigue-related ionic perturbation.

40 **Key words:** Power-duration relationship, prior exercise, critical power, metabolic perturbation

41

42

43

44

45 INTRODUCTION

46 The hyperbolic power-duration relationship is conventionally determined using three to five
47 constant power exercise tests lasting 2-15-min and performed until task failure (1,2). The
48 asymptote of this relationship represents critical power (CP), whereas the curvature constant
49 (termed W') represents a finite work capacity that is utilized when the power output exceeds
50 CP (3). CP represents an important threshold of intramuscular metabolic control that defines
51 the highest oxidative metabolic rate that can be sustained without utilization of W' (3). Thus,
52 CP demarcates the heavy and severe exercise intensity domains, with exercise $<CP$
53 characterized by stability in intramuscular metabolism and pulmonary oxygen uptake ($\dot{V}O_2$),
54 and exercise $>CP$ characterized by inexorable metabolic perturbation and continued utilization
55 of W' (3,4). The determinants of W' appear multifactorial and task specific (3). During whole-
56 body exercise the magnitude of W' may be related to intramuscular energy stores ([PCr] and
57 [glycogen]) (5,6), fatigue-related metabolic and ionic perturbation (P_i , Na^+ , K^+ , Cl^- , La^- , H^+ ,
58 $H_2PO_4^-$, and HCO_3^-) (1,7,8) and the extent of associated muscle fatigue (4) and inefficiency
59 (9,10).

60 The power-duration relationship has great utility for those performing endurance and
61 intermittent exercise (2,11), but the required number of exercise tests may deter some
62 individuals. Therefore, a single 3-min all-out cycling test (3MT) was developed to expedite the
63 estimation of CP and W' (12). Hypothetically, the all-out nature of the 3MT results in complete
64 depletion of W' within ~2-min. The mean power output over the final 30-s of the 3MT, termed
65 end-test power (EP), estimates CP, whereas the work done above EP (WEP) estimates W' (12).
66 It is reported that EP and WEP are valid estimates of CP and W' in recreationally active
67 individuals (12,13) but not in trained cyclists (14,15). Furthermore, W' and WEP do not always

68 correlate (13,16) and may respond differently to training (16), thus whether they are
69 mechanistically equivalent remains uncertain.

70 Insight into the mechanistic determinants of the power-duration relationship has been
71 provided by studies using prior exercise to induce a pre-existing metabolic and ionic
72 perturbation. Prior severe intensity cycling exercise followed by minimal recovery (≤ 2 -min)
73 reduced both W' (-34–64%) and WEP (-21–45%) without affecting CP or EP (1,17,18). When
74 prior exercise was followed by a 15-min recovery interval, WEP was fully restored (18), which
75 suggests that the effects of prior exercise on WEP are sensitive to the intervening recovery
76 duration. However, since prior exercise was performed using homologous muscles, reductions
77 in W' and WEP may have resulted from pre-existing intramuscular energy store depletion
78 and/or metabolite and ionic perturbation. Interestingly, 2-h of heavy intensity cycling exercise
79 reduced both EP (-11%) and WEP (-20%), which was not explained by pre-existing muscle
80 glycogen depletion (19). Previously in the Journal, we reported on the effects of ionic
81 perturbation, *per se*, on CP and W' by preceding the constant power prediction trials with severe
82 intensity upper body (arm-cranking) exercise (i.e. using non-homologous muscles) (8) which
83 has the advantage of not concomitantly causing quadriceps muscle fatigue (20) or reducing leg
84 intramuscular energy stores (21). Upper body exercise increased plasma $[La^-]$ and $[H^+]$ to ~ 12
85 $mmol \cdot L^{-1}$ and $\sim 53 \text{ nmol} \cdot L^{-1}$, respectively, accelerated plasma K^+ accumulation during
86 subsequent cycling exercise, and consistent with the effects of prior exercise using homologous
87 muscles, reduced W' by 32% without affecting CP (8).

88 If EP and WEP are mechanistically equivalent to CP and W' , it can be hypothesized
89 that prior upper body exercise would reduce WEP without affecting EP. However, since the
90 determinants of performance fatiguability are task specific (22), EP/WEP and CP/ W' may
91 respond differently to interventions that modulate fatiguability and exercise tolerance. Indeed,

92 it is noteworthy that W' was reduced (-22%) by mental fatigue (23) and increased (+26%) after
93 creatine monohydrate supplementation (5), whereas WEP was unaffected (24,25).

94 Therefore, the aim of this study was to examine the effects of prior severe intensity
95 upper body exercise on the cycling 3MT in endurance-trained men. Based on the assumption
96 that EP and WEP are mechanistically equivalent to CP and W' , we tested the hypothesis that
97 prior upper body exercise would reduce WEP without affecting EP.

98 **METHODS**

99 **Participants and ethical approval**

100 Ten healthy, non-smoking men (age: 27 ± 9 years; height: 178 ± 8 cm; body mass: 73 ± 8 kg)
101 provided written informed consent to participate in the study. Participants included competitive
102 ($N = 6$) and recreational ($N = 2$) cyclists, one competitive triathlete and one competitive middle-
103 distance runner. The study was approved by the institutional Human Ethics Committee and all
104 procedures conformed to the standard set by the Declaration of Helsinki.

105 **Experimental design**

106 Participants attended the laboratory on seven separate occasions, at a similar time of day,
107 separated by at least 48-h. During visit 1, participants performed a cycling ramp incremental
108 test for determination of gas exchange threshold (GET) and $\dot{V}O_{2\max}$. During visit 2, participants
109 performed a 3MT, which served as a familiarization trial. During visits 3 and 4, participants
110 performed a 3MT to evaluate the repeatability of EP and WEP. During visit 5, participants
111 performed a 3MT preceded by severe-intensity upper body exercise, which served as a
112 familiarization trial. During visits 6 and 7, participants performed in a randomized order a 3MT
113 without and with prior severe-intensity upper body exercise (hereafter, these trials are termed
114 CYC and ARM-CYC, respectively). Participants refrained from caffeine on test days, and

115 alcohol and strenuous exercise the day preceding and day of a test. Participants reported to the
116 laboratory at least 2-h post-prandial.

117 **Equipment and measurements**

118 Exercise was performed using electromagnetically braked cycle (Excalibur Sport; Lode,
119 Groningen, The Netherlands) and arm-cranking (Angio; Lode) ergometers. Participants fitted
120 their own pedals to the cycle ergometer and the position of the seat and handlebars was
121 replicated for all tests. Ventilatory and pulmonary gas exchange variables were measured
122 breath-by-breath (ZAN 600USB CPX incorporating GPI V3.0 software; Nspire Health,
123 Oberthulba, Germany). Participants wore a facemask (model 7940; Hans Rudolph, Missouri,
124 USA) connected to a low resistance ($0.51 \text{ cmH}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$ at $<14 \text{ L}\cdot\text{s}^{-1}$) flow sensor (ZAN
125 variable orifice pneumotach; Nspire Health) with a combined dead space of 67 mL. The flow
126 sensor was calibrated using a 3-L syringe. Gas concentrations were sampled ($50 \text{ mL}\cdot\text{min}^{-1}$) at
127 the mouth via a 2-m capillary line and analyzed using fast responding laser diode absorption
128 spectroscopy sensors that were calibrated using ambient air and gases of known concentration
129 (5% CO_2 , 15% O_2 , balance N_2 ; BOC, Guilford, UK). Volume and concentration signals were
130 time aligned by accounting for the transit delay in the gas capillary line and the analyzer rise
131 time ($T_{10-90} < 90\text{-ms}$) relative to the volume signal.

132 Heart rate was measured using short-range telemetry (Polar RS400; Polar Electro, Kempele,
133 Finland). Arterial oxygen saturation (SpO_2) was estimated using a pulse oximeter (Model 8500;
134 Nonin Medical, Plymouth, MN) and an adhesive forehead reflectance sensor (Model 8000R;
135 Nonin Medical). Arterialized venous blood (1.5-mL) was drawn from a heated dorsal hand vein
136 using an indwelling 21-G cannula and a syringe containing dry electrolyte-balanced heparin
137 (*safePICO*, Radiometer, Copenhagen, Denmark). Blood was analyzed immediately (ABL90
138 FLEX; Radiometer) for [Hb] and pH, PCO_2 , $[\text{HCO}_3^-]$, $[\text{K}^+]$, $[\text{Na}^+]$, $[\text{Ca}^{2+}]$, and $[\text{Cl}^-]$. 0.5-mL of
139 each blood sample was then immediately centrifuged for 10-min at 3000g. The plasma

140 supernatant was removed and analyzed for $[La^-]$ using enzymatic amperometry (Biosen C_Line
141 Sport; EKF Diagnostics, Barleben, Germany), and total plasma protein concentration ($[PPr^-]$)
142 using colorimetry (Biuret method) (ABX Pentra 400; Horiba, Northampton, UK). The total
143 concentration of weak acids ($[A_{tot}]$) was calculated as $2.45 \times [PPr^-]$ (8). $[H^+]$ was derived from
144 the measured pH as the antilog. The strong ion difference ($[SID]$) was calculated as the sum of
145 the strong cations minus the sum of the strong anions: $[SID] = ([Na^+] + [K^+] + [Ca^{2+}]) - ([Cl^-]$
146 $+ [La^-])$ (8,26). This physicochemical approach to acid-base balance describes $[H^+]$ and
147 $[HCO_3^-]$ as dependent variables that are determined by the independent variables $[SID]$, $[A_{tot}]$,
148 and PCO_2 (26). Changes in blood volume from baseline were calculated from changes in $[Hb]$
149 (27).

150 **Cycling ramp incremental test**

151 Participants performed 3-min of unloaded cycling followed by an incremental ramp protocol
152 ($30 \text{ W} \cdot \text{min}^{-1}$), at their preferred cadence, until the limit of tolerance or task failure (cadence
153 below 60 rpm). Pulmonary gas exchange data were reduced to 10-s rolling averages. The GET
154 was determined using the V-slope method and the $\dot{V}O_{2max}$ was taken as the highest 10-s rolling
155 average (28).

156 **The 3MT**

157 The 3MT was preceded by 3-min of unloaded cycling. During the last 3-s of unloaded cycling
158 participants gradually increased their cadence so that maximum cadence was achieved at the
159 start of the 3MT. Participants were instructed to maintain their cadence as high as possible for
160 the duration of the 3MT. The resistance to pedaling was set using the linear mode of the cycle
161 ergometer so that each participant reached a power output halfway between their GET and
162 $\dot{V}O_{2max}$ on reaching their preferred cadence (recorded during the ramp incremental test). Strong
163 verbal encouragement was provided, and participants were blinded from the elapsed time to

164 prevent pacing. The EP was calculated as the mean power output over the last 30-s of the 3MT,
165 whereas WEP was calculated as the power-time integral above EP (12). The $\dot{V}O_{2max}$ during
166 the 3MT taken as the highest 10-s rolling average (28).

167 **Reliability of the 3MT**

168 Participants performed two 3MT (as described above) to evaluate the repeatability of EP and
169 WEP. These tests included the same battery of measurements taken during CYC, although
170 these data were not used for further analysis.

171 **CYC and ARM-CYC**

172 After 3-min of seated rest (baseline), participants remained seated and either rested for 11.5-
173 min (CYC) or performed severe-intensity, intermittent upper body exercise (ARM-CYC)
174 comprising eight 1-min arm-cranking exercise intervals, interspersed by 30-s rest intervals, at
175 an intensity of 1.5-2.0 W·kg⁻¹ body mass (129 ± 29 W) and cadence of 100-130 rpm (8,20).
176 Seated rest / arm-cranking was followed by a 1-min rest interval during which participants
177 transferred to the adjacent cycle ergometer. Thereafter, 3-min of unloaded cycling commenced
178 followed by the 3MT. Blood samples were taken at baseline, at the start and end of the 3MT,
179 and 3- and 6-min into recovery. Heart rate and SpO₂ were measured at baseline and at the end
180 of each arm-cranking exercise interval in ARM-CYC, or at an equivalent time point during
181 CYC (latter data not reported). Thereafter, heart rate was measured every second during
182 unloaded cycling and the 3MT, and SpO₂ was measured every 30-s during the 3MT. The O₂
183 cost of exercise was determined using the $\dot{V}O_2$ gain ($\dot{V}O_2$ /power) at 10-s intervals.

184 **Statistical analyses**

185 Normality of the data was confirmed by the Shapiro-Wilk test. For the reliability trials, EP and
186 WEP were compared using Student's paired t-tests. Trial-to-trial variation in EP and WEP was

187 calculated as the within-participant coefficient of variation (CV). Measurement error and
188 repeatability of EP and WEP were calculated, along with the smallest meaningful change.
189 Student's paired t-tests were used to evaluate differences between CYC and ARM-CYC for
190 peak power output and the corresponding cadence, time to peak power output, EP, WEP, and
191 total work done during the 3MT. One-way repeated measures ANOVA followed by Tukey's
192 post-hoc test was used to evaluate differences in $\dot{V}O_{2\max}$ between the cycling ramp incremental
193 test and the 3MT in CYC and ARM-CYC. For heart rate, ventilatory, and pulmonary gas
194 exchange responses during the 3MT, data were averaged into 10-s bins. These data, along with
195 SpO_2 , [Hb], and acid-base variables, were then analyzed using a two-way (trial \times time) repeated
196 measures ANOVA. Significant interactions and main effects were explored by determining
197 between-trial differences at individual time-points using Student's paired t-tests. Effect sizes
198 are reported as partial eta-squared (η_p^2) for ANOVA and Cohen's d_z for Student's paired t-tests.
199 The Pearson product moment correlation coefficient (r) was calculated to determine the
200 relationship between selected variables. Statistical significance was set at $P < 0.05$. Data were
201 analyzed using IBM SPSS Statistics V24.0, except for Cohen's d_z which was calculated using
202 G*Power 3 software. Results are presented as mean \pm SD unless otherwise indicated.

203 **RESULTS**

204 **Incremental cycling ramp test**

205 Peak power output was 419 ± 73 W (5.7 ± 0.5 W \cdot kg $^{-1}$), and $\dot{V}O_{2\max}$ was 4.53 ± 0.65 L \cdot min $^{-1}$
206 (62 ± 5 mL \cdot kg $^{-1}\cdot$ min $^{-1}$). The GET occurred at 2.82 ± 0.52 L \cdot min $^{-1}$ (240 ± 44 W). The participants
207 were therefore classified as 'trained' ($\dot{V}O_{2\max}$ between 55.0 – 64.9 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) ($n = 6$) or
208 'well-trained' ($\dot{V}O_{2\max}$ between 65.0 – 71.0 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) ($n = 4$) (29).

209 **Reliability of the 3MT**

210 There was no difference between the reliability trials for EP (328 ± 53 vs. 337 ± 57 W; $t_9 =$
211 1.42 , $P = 0.189$, $d_z = 0.44$) or WEP (13.1 ± 4.4 vs. 13.3 ± 2.9 kJ; $t_9 = 0.24$, $P = 0.820$, $d_z = 0.07$).
212 For EP and WEP respectively, there was a day-to-day CV of 4% and 12%, measurement error
213 of 14 W and 2.1 kJ, reproducibility of 40 W and 5.9 kJ, and a smallest meaningful change of
214 20 W and 2.9 kJ.

215 **Peak power output, EP, WEP and total work done during 3MT of CYC and ARM-CYC**

216 Peak power output ($t_9 = 2.02$, $P = 0.074$, $d_z = 0.62$), the corresponding cadence ($t_9 = 2.03$, $P =$
217 0.073 , $d_z = 0.53$), and the time to peak power output ($t_9 = 0.52$, $P = 0.614$, $d_z = 0.19$) during the
218 3MT were not different between CYC and ARM-CYC (Fig. 1), although a medium effect size
219 was observed for peak power output. EP was 12% lower in ARM-CYC than CYC (mean
220 difference = 40 ± 13 W, 95% CI [31, 49 W]; $t_9 = 2.94$, $P < 0.001$, $d_z = 3.07$), whereas WEP
221 was not different ($t_9 = 1.07$, $P = 0.312$, $d_z = 0.34$) (Fig. 1). The total work done was 9% lower
222 in ARM-CYC (67.2 ± 12.7 kJ) than CYC (73.7 ± 13.7 kJ) ($t_9 = 7.34$, $P < 0.001$, $d_z = 2.31$).

223 **Cardiorespiratory responses during the 3MT of CYC and ARM-CYC**

224 Pulmonary gas exchange, heart rate and SpO₂ responses are shown in Figure 2. For $\dot{V}O_2$, there
225 was a trial \times time interaction effect due to $\dot{V}O_2$ being ~ 0.26 L \cdot min⁻¹ (5-7%) lower during ARM-
226 CYC than CYC over the final 50-s of the 3MT ($t_9 = 2.46 - 3.74$, $P = 0.005 - 0.036$, $d_z = 0.77 -$
227 1.16). There was a main effect of test on $\dot{V}O_{2\max}$ ($F_{2,18} = 9.20$, $P < 0.001$, $\eta_p^2 = 0.51$). The
228 $\dot{V}O_{2\max}$ during the cycling ramp incremental test and the 3MT of CYC (4.41 ± 0.62 L \cdot min⁻¹)
229 was not different ($P = 0.549$), whereas both were 5-8% higher than $\dot{V}O_{2\max}$ during the 3MT of
230 ARM-CYC (4.17 ± 0.64 L \cdot min⁻¹) ($P = 0.002$ and 0.017 , respectively). The relative reduction
231 in $\dot{V}O_{2\max}$ during ARM-CYC vs. CYC was negatively correlated with the between-trial
232 difference in peak [H⁺] ($r = -0.86$, $P = 0.001$). For the $\dot{V}O_2$ gain (Fig. 3), there was a trial \times

233 time interaction effect. The $\dot{V}O_2$ gain was $0.9 \text{ mL}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ (7%) higher during ARM-CYC
234 than CYC at 130-s of the 3MT ($t_9 = 2.62, P = 0.028, d_z = 0.83$). $\dot{V}CO_2$ was $0.25 \text{ L}\cdot\text{min}^{-1}$ higher
235 at the start of the 3MT in ARM-CYC than CYC ($t_9 = 2.77, P = 0.022, d_z = 0.88$). Thereafter,
236 except for the first 30-s of the 3MT, $\dot{V}CO_2$ was $\sim 0.78 \text{ L}\cdot\text{min}^{-1}$ ($\sim 15\%$) lower during ARM-
237 CYC than CYC ($t_9 = 3.55 - 6.11, P = <0.001 - 0.006, d_z = 0.98 - 1.92$). Heart rate was ~ 9
238 $\text{beats}\cdot\text{min}^{-1}$ ($\sim 6\%$) higher throughout the 3MT of ARM-CYC than CYC ($t_9 = 2.34 - 11.01, P =$
239 $<0.001 - 0.044, d_z = 0.73 - 3.54$). SpO_2 fell during the 3MT and was $\sim 2\%$ lower during ARM-
240 CYC than CYC after 150-s ($t_9 = 2.51, P = 0.033, d_z = 0.79$) and 180-s ($t_9 = 2.41, P = 0.039, d_z$
241 $= 0.76$).

242 **Ventilatory responses during the 3MT of CYC and ARM-CYC**

243 Ventilatory response are shown in Figure 4. For \dot{V}_E , the trial \times time interaction effect was due
244 to an $\sim 19 \text{ L}\cdot\text{min}^{-1}$ higher \dot{V}_E during ARM-CYC than CYC at the start ($t_9 = 3.89, P = 0.003, d_z$
245 $= 1.24$) and after 10-s ($t_9 = 2.99, P = 0.015, d_z = 0.94$) of the 3MT. Thereafter, \dot{V}_E was similar
246 between CYC and ARM-CYC, although a relative tachypnea (i.e. lower V_T and higher f_R) was
247 observed in ARM-CYC. Due to \dot{V}_E being higher at the start of the 3MT of ARM-CYC than
248 CYC, $\dot{V}_E/\dot{V}O_2$ was also higher by ~ 8 ($t_9 = 5.30, P < 0.001, d_z = 1.67$). Conversely, the higher
249 $\dot{V}_E/\dot{V}O_2$ during ARM-CYC than CYC from 110-180-s of the 3MT ($t_9 = 2.35 - 6.19, P = <0.001$
250 $- 0.043, d_z = 0.74 - 1.98$) was due to the lower $\dot{V}O_2$ in ARM-CYC. The lower $\dot{V}CO_2$ during
251 the 3MT of ARM-CYC resulted in $\dot{V}_E/\dot{V}CO_2$ being higher during ARM-CYC than CYC ($t_9 \geq$
252 $3.27 - 8.60, P = <0.001 - 0.009, d_z = 1.02 - 2.44$). $P_{ET}CO_2$ was $\sim 6 \text{ mmHg}$ ($\sim 17\%$) lower
253 throughout the 3MT of ARM-CYC than CYC ($t_9 = 3.40 - 9.26, P = <0.001 - 0.008, d_z = 0.96$
254 $- 2.74$).

255 **Blood volume and plasma acid-base balance during CYC and ARM-CYC**

256 During the 3MT of CYC blood volume fell by ~8%, as reflected by the $1.4 \pm 0.3 \text{ g}\cdot\text{dL}^{-1}$ increase
257 in [Hb] from baseline (Table 1). The 3MT of ARM-CYC commenced with a pre-existing ~8%
258 reduction in blood volume from baseline, as reflected by the $1.3 \pm 0.2 \text{ g}\cdot\text{dL}^{-1}$ increase in [Hb].
259 A further reduction in blood volume (~12% from baseline) was observed at the end of the 3MT
260 in ARM-CYC, as reflected by a further $0.8 \pm 0.2 \text{ g}\cdot\text{dL}^{-1}$ increase in [Hb]. The greater [Hb] at
261 the start ($t_9 = 6.74$, $P < 0.001$, $d_z = 2.12$) and end ($t_9 = 3.51$, $P = 0.007$, $d_z = 1.14$) of the 3MT
262 of ARM-CYC than CYC reflects a lower blood volume during ARM-CYC.

263 Plasma acid-base variables at baseline were not different between CYC and ARM-CYC
264 and therefore these data were pooled (Table 1). The $[\text{La}^-]$, $[\text{Na}^+]$, $[\text{Cl}^-]$, and $[\text{PPr}^-]$ were higher
265 at the start and end of the 3MT of ARM-CYC than CYC. The net increase in $[\text{La}^-]$ during the
266 3MT was smaller during ARM-CYC ($7.8 \pm 4.0 \text{ mmol}\cdot\text{L}^{-1}$) than CYC ($13.2 \pm 3.5 \text{ mmol}\cdot\text{L}^{-1}$) (t_9
267 $= 4.51$, $P = 0.001$, $d_z = 1.42$). The between-trial differences in ion concentrations and $[\text{PPr}^-]$
268 resulted in between-trial differences for the independent acid-base variables $[\text{SID}]$ and $[\text{A}_{\text{tot}}]$.
269 Specifically, $[\text{SID}]$ was lower during ARM-CYC than CYC at the start ($\sim -10.5 \text{ mmol}\cdot\text{L}^{-1}$) and
270 end ($\sim -5.2 \text{ mmol}\cdot\text{L}^{-1}$) of the 3MT, whereas $[\text{A}_{\text{tot}}]$ was higher during ARM-CYC than CYC at
271 the start ($\sim 2.3 \text{ mmol}\cdot\text{L}^{-1}$) and end ($\sim 1.4 \text{ mmol}\cdot\text{L}^{-1}$) of the 3MT. PCO_2 was ~ 12.0 - 13.0 mmHg
272 lower during ARM-CYC than CYC at the start and end of the 3MT. The between-trial
273 differences in the independent acid-base variables resulted in between-trial differences in the
274 dependent acid-base variables $[\text{H}^+]$ and $[\text{HCO}_3^-]$. Specifically, $[\text{H}^+]$ was higher and $[\text{HCO}_3^-]$ was
275 lower during ARM-CYC than CYC at the start ($\sim 15.8 \text{ nmol}\cdot\text{L}^{-1}$ and $\sim 10.1 \text{ mmol}\cdot\text{L}^{-1}$) and end
276 ($\sim 10.4 \text{ nmol}\cdot\text{L}^{-1}$ and $\sim 4.0 \text{ mmol}\cdot\text{L}^{-1}$) of the 3MT. The net increase in $[\text{H}^+]$ during the 3MT was
277 smaller during ARM-CYC ($22.6 \pm 10.2 \text{ nmol}\cdot\text{L}^{-1}$) than CYC ($28.0 \pm 10.6 \text{ nmol}\cdot\text{L}^{-1}$) ($t_9 = 3.43$,
278 $P = 0.008$, $d_z = 1.06$).

279 The EP and WEP (normalized to body mass) in CYC were positively correlated with
280 the peak $[H^+]$, and negatively correlated with the lowest $[HCO_3^-]$ (Fig. 5).

281 **DISCUSSION**

282 **Main findings**

283 In contrast to our hypothesis, the main finding of the present study was that prior upper body
284 exercise, which caused marked pre-existing ionic perturbation, reduced EP by 12% (40 W)
285 without affecting WEP. Importantly, the 40 W reduction in EP and the associated true score
286 change 95% CI exceeded the smallest meaningful change (20 W). Moreover, the EP in CYC
287 was correlated with the dependent acid-base variables, namely peak $[H^+]$ and minimum
288 $[HCO_3^-]$. These novel observations are the first to suggest that EP during the 3MT is sensitive
289 to fatigue-related ionic perturbation.

290 **Prior upper body exercise and the power-duration relationship: 3MT vs. conventional** 291 **protocol**

292 Previously in the Journal we showed that prior severe intensity upper body exercise reduced
293 W' by 32% without affecting CP (8). We therefore examined the effects of an identical prior
294 upper body exercise protocol, causing comparable pre-existing ionic perturbation, on EP (the
295 analogue of CP) and WEP (the analogue of W') to test the hypothesis that these parameters are
296 mechanistically equivalent to CP and W' . In stark contrast to this hypothesis, prior upper body
297 exercise reduced EP without affecting WEP. This suggests that EP and WEP are not
298 mechanistically equivalent to CP and W' . Moreover, these discrepancies suggest that the
299 determinants of EP and WEP are specific to the 3MT and, in part, different from the
300 determinants of CP and W' established using severe intensity constant power exercise.
301 Important methodological differences between the 3MT and the conventional protocol may

302 explain why the parameter estimates are task specific, including: (1) type of exercise test (all-
303 out closed-end vs. constant power open-end); (2) test duration (3-min vs. 3-15-min); (3)
304 ergometer mode (linear vs. hyperbolic), which dictates power and cadence profiles and
305 therefore power-velocity relationships; and (4) different exercise intensity domains (extreme
306 and severe domains vs. severe domain only), which mediates intramuscular metabolic
307 perturbation (4,28). By applying the EP and WEP measured in CYC to the hyperbolic power-
308 duration model, the lower boundary of the extreme domain (i.e. the power output eliciting task
309 failure in 120-s) can be estimated at 445 ± 85 W. Participants therefore spent the first 36 ± 4 s
310 (20%) of the 3MT in the extreme domain, which may explain why plasma $[H^+]$ at the end of
311 the 3MT was $10 \text{ nmol}\cdot\text{L}^{-1}$ (16%) greater than that previously observed at task failure during
312 severe intensity constant power cycling exercise (8). The significance of performing extreme
313 intensity exercise initially during the 3MT remains uncertain and warrants further investigation.
314 Importantly, participants spent the latter ~80% of the 3MT in the severe intensity domain,
315 which suggests the mechanisms by which prior upper body exercise reduces EP are related to
316 changes in the physiology of severe intensity exercise. Moreover, from a practical perspective,
317 the findings of the present study suggest that if sport and exercise science practitioners use the
318 3MT for athlete evaluation, especially if it is part of a testing battery, it should be performed in
319 a ‘fresh’ state to ensure that test validity is not compromised and to avoid underestimating CP.

320 **Prior exercise and the power-duration relationship: homologous vs. non-homologous** 321 **muscles**

322 The findings of the present study, along with those of Johnson et al. (8), show that prior exercise
323 using non-homologous muscles reduces EP and W' without affecting CP or WEP. Interestingly,
324 prior exercise using homologous muscles reduces both W' and WEP without affecting CP or
325 EP (1,17,18). Previous studies preceded the 3MT with 4-min of severe intensity cycling
326 exercise (17) or a 30-s all-out cycling sprint followed by 2-min recovery (18). At the start of

327 the 3MT blood $[La^-]$ was 3.7 (17) and 5.6 $mmol \cdot L^{-1}$ (18) and WEP was reduced by 45% (17)
328 and 21% (18). However, when prior exercise was followed by 15-min recovery and thus
329 probable replenishment of intramuscular [PCr], WEP was fully restored despite the 3MT
330 commencing with blood $[La^-]$ at 5.5 $mmol \cdot L^{-1}$ (18). This suggests that WEP is mediated more
331 by intramuscular [PCr] than ionic / metabolite perturbation. This may explain why WEP was
332 not reduced in ARM-CYC since upper-body exercise does not concomitantly reduce leg
333 intramuscular [PCr] (21).

334 The mechanisms by which EP is reduced by prior exercise using non-homologous, but
335 not homologous, muscles remain unclear. Possible explanations include between-study
336 differences in: (1) the magnitude of ionic / metabolite perturbation incurred; (2) peripheral and
337 / or central fatigue kinetics; and/or (3) participant training status which may affect the validity
338 of the 3MT. In the present study, WEP was similar to previous reports in trained cyclists (~11-
339 15 kJ) (14,15,30), but notably lower than W' (~20-30 kJ) (2,15,23). In trained cyclists, EP may
340 overestimate CP by 11-15%, whereas WEP may underestimate W' by 36-45% (14,15). These
341 studies raise the possibility that W' is still being expended during the final 30-s of the 3MT,
342 which results in EP being elevated at the expense of WEP. It is therefore possible that a
343 reduction in EP after prior upper body exercise reflects, in part, a reduction in W' . However,
344 evaluating the validity of the 3MT requires 3-5 additional tests to derive CP and W' , which was
345 beyond the scope of the present study.

346 **Why does prior upper body exercise reduce EP?**

347 The finding that EP is reduced after prior upper body exercise suggests that EP is partly
348 determined by ionic perturbation. This is also supported by the observation that EP correlated
349 positively with the peak $[H^+]$, and negatively with the lowest $[HCO_3^-]$. Prior upper body
350 exercise may have therefore reduced EP by affecting intramuscular ionic perturbation.

351 Specifically, during the 3MT of ARM-CYC the fall in leg intramuscular [SID] and concomitant
352 increase in $[H^+]$ may have proceeded at a faster rate (21) due to: (I) reduced La^- efflux from
353 locomotor muscles due to high plasma $[La^-]$ and $[H^+]$ (21) and low plasma $[HCO_3^-]$ (31); (II)
354 reduced La^- removal from the blood due to La^- accumulation in upper body muscle and other
355 tissues; and (III) greater K^+ release from locomotor muscles (8,21) due to an acidosis-mediated
356 increase in the opening probability of ATP-sensitive K^+ channels (32). Increased interstitial
357 $[K^+]$ and/or intramuscular $[H^+]$, which exacerbates the fatiguing effects of Pi by increasing
358 $[H_2PO_4^-]$, may have accelerated the development of peripheral locomotor muscle fatigue (33)
359 during the 3MT of ARM-CYC. Although objective fatigue measurements were not taken in
360 the present study, the higher $\dot{V}O_2$ gain during the 3MT of ARM-CYC than CYC may reflect
361 differences in muscle bioenergetics and peripheral fatigue development (9).

362 The ionic perturbation induced by prior upper body exercise may have also reduced EP
363 by exacerbating central fatigue. This is consistent with the observation that central fatigue
364 develops more quickly during severe intensity constant power cycling exercise preceded by
365 upper body exercise (20). Incidentally, central fatigue may manifest within the first 30-s of the
366 3MT (34), which differs from severe intensity constant power exercise (i.e. the conventional
367 protocol) where central fatigue manifests largely towards the end of exercise (35). At the start
368 of the 3MT in ARM-CYC, the ensemble group III/IV muscle afferent feedback, mediated by
369 intramuscular metabolic / ionic perturbation, may have been elevated due to pre-existing
370 afferent feedback originating mainly from upper body muscles (20). Increased central
371 projection of group III/IV muscle afferents provides inhibitory feedback to the central nervous
372 system, thereby reducing and/or confining central motor drive (36). This may therefore explain,
373 in part, the reduced EP after prior upper body exercise in the present study.

374 A limitation of the present study is that we did not validate our previous finding that
375 prior upper body exercise reduces the conventionally determined W' without affecting CP (8).

376 However, this would have required an additional 8-10 tests to establish the hyperbolic power-
377 duration relationship with and without prior upper body exercise, which was beyond the scope
378 of the present study. It is therefore possible that, in the present cohort, the reduced EP and
379 unchanged WEP after prior upper body exercise reflects a reduced CP and unchanged W'.
380 However, we consider this unlikely for three reasons: (I) one participant was common to both
381 studies, and despite their training status being strikingly similar on both occasions ($\dot{V}O_{2max} =$
382 61 vs. 62 mL·kg⁻¹·min⁻¹; GET = 3.08 vs. 3.00 L·min⁻¹) they experienced, after prior upper body
383 exercise, marked falls in W' (CYC vs. ARM-CYC: 16.1 vs. 11.1 kJ) and EP (351 vs. 304 W)
384 but not CP (279 vs. 281 W) or WEP (12.4 vs. 14.4 kJ); (II) if, in the present cohort, the reduced
385 EP after prior upper body exercise reflects a reduced CP, this would indicate that the
386 mechanistic bases of CP differ from our previous cohort, which seems unlikely given the well-
387 established mechanistic bases of CP in healthy humans (3); and (III) it is improbable that the
388 mechanistic bases of CP and W' in the present cohort would differ sufficiently from our
389 previous cohort (8) to elicit contrasting effects of prior upper body exercise on CP and W'.
390 Based on these considerations, it is likely that our previous observations (8) can be generalized
391 to the present cohort.

392 **Effects of prior upper body exercise on peak power output and $\dot{V}O_{2max}$ during the 3MT**

393 Peak power output during the 3MT was not statistically different between CYC and ARM-
394 CYC, although the effect size was medium. Interestingly, peak power output during a 30-s
395 cycling sprint was unchanged after prior arm-cranking exercise that increased blood [La⁻] to
396 11.0 mmol·L⁻¹ (37) whereas it was reduced by 5% after four sets of prior biceps curls that
397 increased blood [La⁻] by only 2.0 mmol·L⁻¹ (38). Reduced peak cycling power output after
398 prior upper body exercise may thus result from a reduced upper body contribution to peak
399 power output (38), rather than the degree of pre-existing ionic / metabolite perturbation *per se*.

400 In the present study, prior upper body exercise increased maximal heart rate but reduced
401 $\dot{V}O_{2\max}$ during the 3MT. An identical prior upper body exercise protocol reduced both $\dot{V}O_{2\max}$
402 and maximal heart rate during an incremental cycling test (8). Conversely, prior exercise using
403 homologous muscles did not affect $\dot{V}O_{2\max}$ during subsequent severe intensity constant power
404 exercise (1), or $\dot{V}O_{2\max}$ and maximal heart rate during the 3MT (17–19). This suggests that
405 autonomic function and $\dot{V}O_{2\max}$ are affected differently by prior exercise using homologous
406 and non-homologous muscles. Interestingly, the reduced $\dot{V}O_{2\max}$ during ARM-CYC correlated
407 negatively with the between-trial difference in peak $[H^+]$. Greater acidosis during ARM-CYC
408 may have reduced $\dot{V}O_{2\max}$ by inhibiting oxidative phosphorylation (39) and / or by reducing
409 SpO_2 (due to a rightward shift in the HbO_2 dissociation curve) and thereby convective oxygen
410 transport (26). Our SpO_2 data (Fig. 2D) support that oxygen delivery was reduced after prior
411 upper body exercise, although some caution is warranted given the limitations associated with
412 estimating SpO_2 using pulse oximetry (26). Nevertheless, for two reasons, it seems most likely
413 that compromised convective oxygen transport primarily explains the lower $\dot{V}O_{2\max}$ in ARM-
414 CYC: (I) $\dot{V}O_{2\max}$ in endurance-trained individuals is limited by oxygen supply rather than
415 mitochondrial respiration (40); and (II) $\dot{V}O_{2\max}$ is still reached during consecutive maximal
416 cycling exercise bouts even when intramuscular acidification is increased using bilateral leg
417 occlusion during the intervening recovery periods (41). The lower blood volume during the
418 3MT of ARM-CYC than CYC, which reflects greater plasma volume shifts from vascular to
419 intracellular compartments (26), may have also reduced stroke volume, which if not
420 compensated by the higher heart rate may have compromised cardiac output and locomotor
421 muscle perfusion. Persistent sympathetic vasoconstrictor outflow secondary to continued group
422 III/IV afferent activity originating in fatigued respiratory (evidenced by the tachypnoeic
423 breathing pattern, Fig. 4B and C) and upper body muscles may have also compromised
424 locomotor muscle perfusion. However, whether the reduced $\dot{V}O_{2\max}$ *per se* contributed to the

425 lower EP remains uncertain since between-trial differences in power output preceded
426 differences in $\dot{V}O_2$.

427 **Conclusion**

428 In conclusion, the EP derived during a 3MT was reduced by prior severe intensity upper body
429 exercise and correlated with markers of ionic perturbation. These findings therefore suggest
430 that EP is sensitive to fatigue-related ionic perturbation in endurance trained men. Since the
431 results of the present study contrast the effects of prior upper body exercise on the
432 conventionally determined CP and W' , it is possible that EP and WEP are not mechanistically
433 equivalent to CP and W' . These findings have important implications for future studies using
434 the 3MT as a framework to investigate fatigue mechanisms and the effects of experimental
435 intervention.

436 **Acknowledgements**

437 None

438 **Conflict of Interest**

439 The results of the present study do not constitute endorsement by ACSM.

440 The results of the study are presented clearly, honestly, and without fabrication, falsification,
441 or inappropriate data manipulation.

442

443

444

445

446

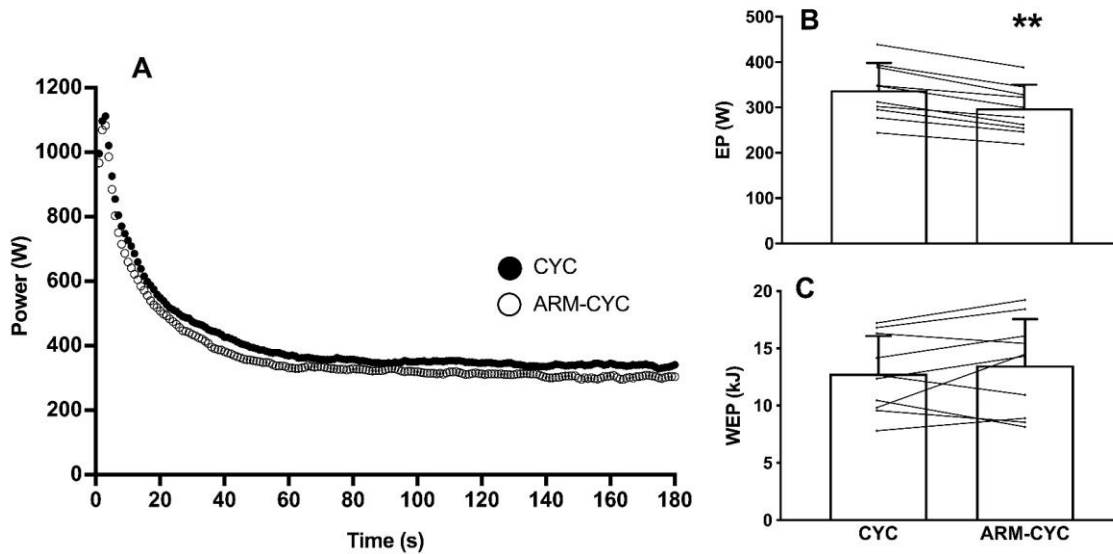
447 **REFERENCES**

- 448 1. Ferguson C, Whipp BJ, Cathcart AJ, Rossiter HB, Turner AP, Ward SA. Effects of prior
 449 very-heavy intensity exercise on indices of aerobic function and high-intensity exercise
 450 tolerance. *J Appl Physiol.* 2007;103(3):812–22.
- 451 2. Johnson MA, Sharpe GR, Brown PI. Inspiratory muscle training improves cycling time-
 452 trial performance and anaerobic work capacity but not critical power. *Eur J Appl Physiol.*
 453 2007;101(6):761–70.
- 454 3. Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM. Critical power: An
 455 important fatigue threshold in exercise physiology. *Med Sci Sports Exerc.*
 456 2016;48(11):2320–34.
- 457 4. Burnley M, Jones AM. Power-duration relationship: Physiology, fatigue, and the limits
 458 of human performance. *Eur J Sport Sci.* 2018;18(1):1–12.
- 459 5. Miura A, Kino F, Kajitani S, Sato H, Fukuba Y. The effect of oral creatine
 460 supplementation on the curvature constant parameter of the power-duration curve for
 461 cycle ergometry in humans. *Jpn J Physiol.* 1999;49(2):169–74.
- 462 6. Miura A, Sato H, Sato H, Whipp BJ, Fukuba Y. The effect of glycogen depletion on the
 463 curvature constant parameter of the power-duration curve for cycle ergometry.
 464 *Ergonomics.* 2000;43(1):133–41.
- 465 7. Green S, Dawson BT, Goodman C, Carey MF. Y-intercept of the maximal work-duration
 466 relationship and anaerobic capacity in cyclists. *Eur J Appl Physiol Occup Physiol.*
 467 1994;69(6):550–6.
- 468 8. Johnson MA, Mills DE, Brown PI, Sharpe GR. Prior upper body exercise reduces cycling
 469 work capacity but not critical power. *Med Sci Sports Exerc.* 2014;46(4):802–8.
- 470 9. Grassi B, Rossiter HB, Zoladz JA. Skeletal muscle fatigue and decreased efficiency: two
 471 sides of the same coin? *Exerc Sport Sci Rev.* 2015;43(2):75–83.
- 472 10. Vanhatalo A, Poole DC, DiMenna FJ, Bailey SJ, Jones AM. Muscle fiber recruitment and
 473 the slow component of O₂ uptake: constant work rate vs. all-out sprint exercise. *Am J*
 474 *Physiol Regul Integr Comp Physiol.* 2011;300(3):R700-707.
- 475 11. Jones AM, Vanhatalo A. The “Critical Power” concept: Applications to sports
 476 performance with a focus on intermittent high-intensity exercise. *Sports Med.*
 477 2017;47(Suppl 1):65–78.
- 478 12. Vanhatalo A, Doust JH, Burnley M. Determination of critical power using a 3-min all-
 479 out cycling test. *Med Sci Sports Exerc.* 2007;39(3):548–55.
- 480 13. Simpson LP, Jones AM, Skiba PF, Vanhatalo A, Wilkerson D. Influence of hypoxia on
 481 the power-duration relationship during high-intensity exercise. *Int J Sports Med.*
 482 2015;36(2):113–9.

- 483 14. Bartram JC, Thewlis D, Martin DT, Norton KI. Predicting critical power in elite cyclists:
484 Questioning the validity of the 3-minute all-out test. *Int J Sports Physiol Perform.*
485 2017;12(6):783–7.
- 486 15. Wright J, Bruce-Low S, Jobson SA. The 3-minute all-out cycling test is sensitive to
487 changes in cadence using the Lode Excalibur Sport ergometer. *J Sports Sci.*
488 2019;37(2):156–62.
- 489 16. Vanhatalo A, Doust JH, Burnley M. A 3-min all-out cycling test is sensitive to a change
490 in critical power. *Med Sci Sports Exerc.* 2008;40(9):1693–9.
- 491 17. Parker Simpson L, Jones AM, Vanhatalo A, Wilkerson DP. Influence of initial metabolic
492 rate on the power-duration relationship for all-out exercise. *Eur J Appl Physiol.*
493 2012;112(7):2467–73.
- 494 18. Vanhatalo A, Jones AM. Influence of prior sprint exercise on the parameters of the “all-
495 out critical power test” in men. *Exp Physiol.* 2009;94(2):255–63.
- 496 19. Clark IE, Vanhatalo A, Thompson C, Wylie LJ, Bailey SJ, Kirby BS, Wilkins BW, Jones
497 AM. Changes in the power-duration relationship following prolonged exercise:
498 estimation using conventional and all-out protocols and relationship with muscle
499 glycogen. *Am J Physiol Regul Integr Comp Physiol.* 2019;317(1):R59–67.
- 500 20. Johnson MA, Sharpe GR, Williams NC, Hannah R. Locomotor muscle fatigue is not
501 critically regulated after prior upper body exercise. *J Appl Physiol.* 2015;119(7):840–50.
- 502 21. Bangsbo J, Madsen K, Kiens B, Richter EA. Effect of muscle acidity on muscle
503 metabolism and fatigue during intense exercise in man. *J Physiol.* 1996;495(Pt 2):587–
504 96.
- 505 22. Thomas K, Goodall S, Howatson G. Performance fatigability is not regulated to a
506 peripheral critical threshold. *Exerc Sport Sci Rev.* 2018;46(4):240–6.
- 507 23. Salam H, Marcora SM, Hopker JG. The effect of mental fatigue on critical power during
508 cycling exercise. *Eur J Appl Physiol.* 2018;118(1):85–92.
- 509 24. Martin K, Thompson KG, Keegan R, Ball N, Rattray B. Mental fatigue does not affect
510 maximal anaerobic exercise performance. *Eur J Appl Physiol.* 2015;115(4):715–25.
- 511 25. Vanhatalo A, Jones AM. Influence of creatine supplementation on the parameters of the
512 “all-out critical power test.” *J Exerc Sci Fit.* 2009;7(1):9–17.
- 513 26. Stickland MK, Lindinger MI, Olfert IM, Heigenhauser GJF, Hopkins SR. Pulmonary gas
514 exchange and acid-base balance during exercise. *Compr Physiol.* 2013;3(2):693–739.
- 515 27. Harrison MH. Effects on thermal stress and exercise on blood volume in humans. *Physiol*
516 *Rev.* 1985;65(1):149–209.
- 517 28. Vanhatalo A, Black MI, DiMenna FJ, Blackwell JR, Schmidt JF, Thompson C, Wylie LJ,
518 Mohr M, Bangsbo J, Krstrup P, Jones AM. The mechanistic bases of the power-time
519 relationship: muscle metabolic responses and relationships to muscle fibre type. *J Physiol.*
520 2016;594(15):4407–23.

- 521 29. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to
522 classify subject groups in sport-science research. *Int J Sports Physiol Perform.*
523 2013;8(2):111–22.
- 524 30. McClave SA, LeBlanc M, Hawkins SA. Sustainability of critical power determined by a
525 3-minute all-out test in elite cyclists. *J Strength Cond Res.* 2011;25(11):3093–8.
- 526 31. Mainwood GW, Worsley-Brown P. The effects of extracellular pH and buffer
527 concentration on the efflux of lactate from frog sartorius muscle. *J Physiol.*
528 1975;250(1):1–22.
- 529 32. Davies NW. Modulation of ATP-sensitive K⁺ channels in skeletal muscle by intracellular
530 protons. *Nature.* 1990;343(6256):375–7.
- 531 33. Kent-Braun JA, Fitts RH, Christie A. Skeletal muscle fatigue. *Compr Physiol.*
532 2012;2(2):997–1044.
- 533 34. Fernandez-del-Olmo M, Rodriguez FA, Marquez G, Iglesias X, Marina M, Benitez A,
534 Vallejo L, Acero RM. Isometric knee extensor fatigue following a Wingate test:
535 peripheral and central mechanisms. *Scand J Med Sci Sports.* 2013;23(1):57–65.
- 536 35. Decorte N, Lafaix PA, Millet GY, Wuyam B, Verges S. Central and peripheral fatigue
537 kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports.*
538 2012;22(3):381–91.
- 539 36. Amann M, Sidhu SK, Weavil JC, Mangum TS, Venturelli M. Autonomic responses to
540 exercise: group III/IV muscle afferents and fatigue. *Auton Neurosci.* 2015;188:19–23.
- 541 37. Bogdanis GC, Nevill ME, Lakomy HK. Effects of previous dynamic arm exercise on
542 power output during repeated maximal sprint cycling. *J Sports Sci.* 1994;12(4):363–70.
- 543 38. Grant MC, Robergs R, Baird MF, Baker JS. The effect of prior upper body exercise on
544 subsequent wingate performance. *Biomed Res Int.* 2014;329328.
- 545 39. Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE. Acidosis inhibits
546 oxidative phosphorylation in contracting human skeletal muscle *in vivo*. *J Physiol.*
547 2003;553(Pt 2):589–99.
- 548 40. Gifford JR, Garten RS, Nelson AD, Trinity JD, Layec G, Witman MAH, et al.
549 Symmorphosis and skeletal muscle $\dot{V}O_{2max}$: *in vivo* and *in vitro* measures reveal differing
550 constraints in the exercise-trained and untrained human. *J Physiol.* 2016;594(6):1741–51.
- 551 41. Morales-Alamo D, Losa-Reyna J, Torres-Peralta R, Martin-Rincon M, Perez-Valera M,
552 Curtelin D, Ponce-González JG, Santana A, Calbet JAL. What limits performance during
553 whole-body incremental exercise to exhaustion in humans? *J Physiol.*
554 2015;593(20):4631–48.

555

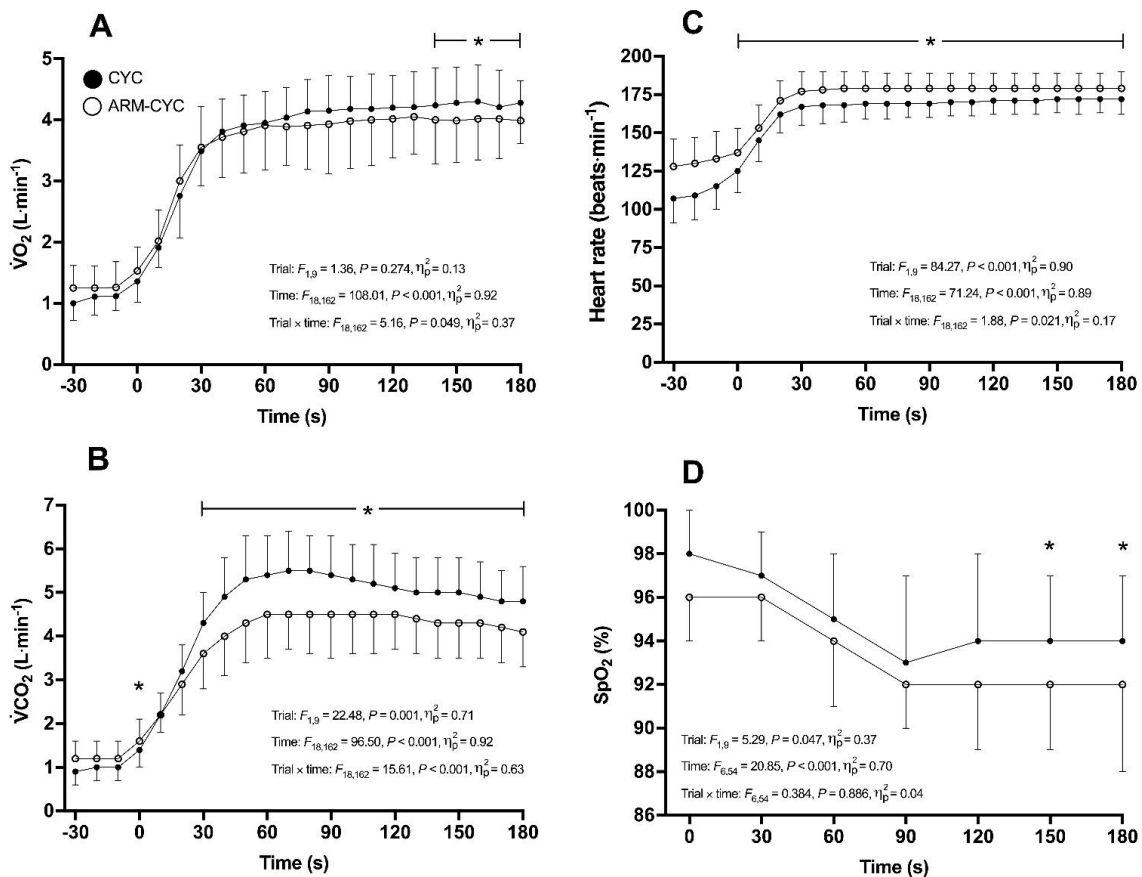


556

557 **FIGURE 1** – Power profiles (A), end-test power output (EP) (B), and work done above end-
 558 test power output (WEP) (C) during the 3MT of CYC and ARM-CYC. Data in A are mean
 559 with error bars omitted to enhance clarity. Data in B and C are mean \pm SD, with lines
 560 representing individual participants. **Different from CYC ($P < 0.001$).

561

562

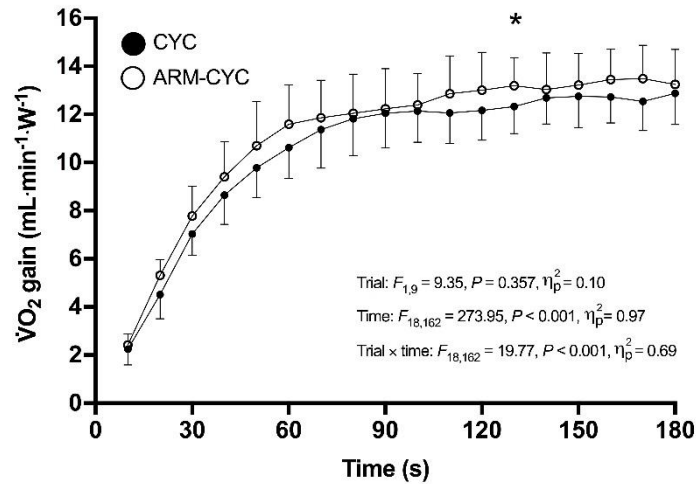


563

564 **FIGURE 2** – Pulmonary oxygen uptake ($\dot{V}O_2$) (A), carbon dioxide production ($\dot{V}CO_2$) (B),
 565 heart rate (C), and arterial oxygen saturation by pulse oximetry (SpO₂) (D) during the 3MT of
 566 CYC and ARM-CYC. Panels A-C also show the final 30-s of unloaded cycling, with the 3MT
 567 commencing at time 0-s. Data are mean \pm SD. *Difference between trials ($P < 0.05$). Capped
 568 lines with asterix denote the range of measurement points that differ between CYC and ARM-
 569 CYC.

570

571



572

573 **FIGURE 3** – Pulmonary oxygen uptake ($\dot{V}O_2$) gain during the 3MT of CYC and ARM-CYC.
 574 Data are mean \pm SD. *Difference between trials ($P < 0.05$).

575

576

577

578

579

580

581

582

583

584

585

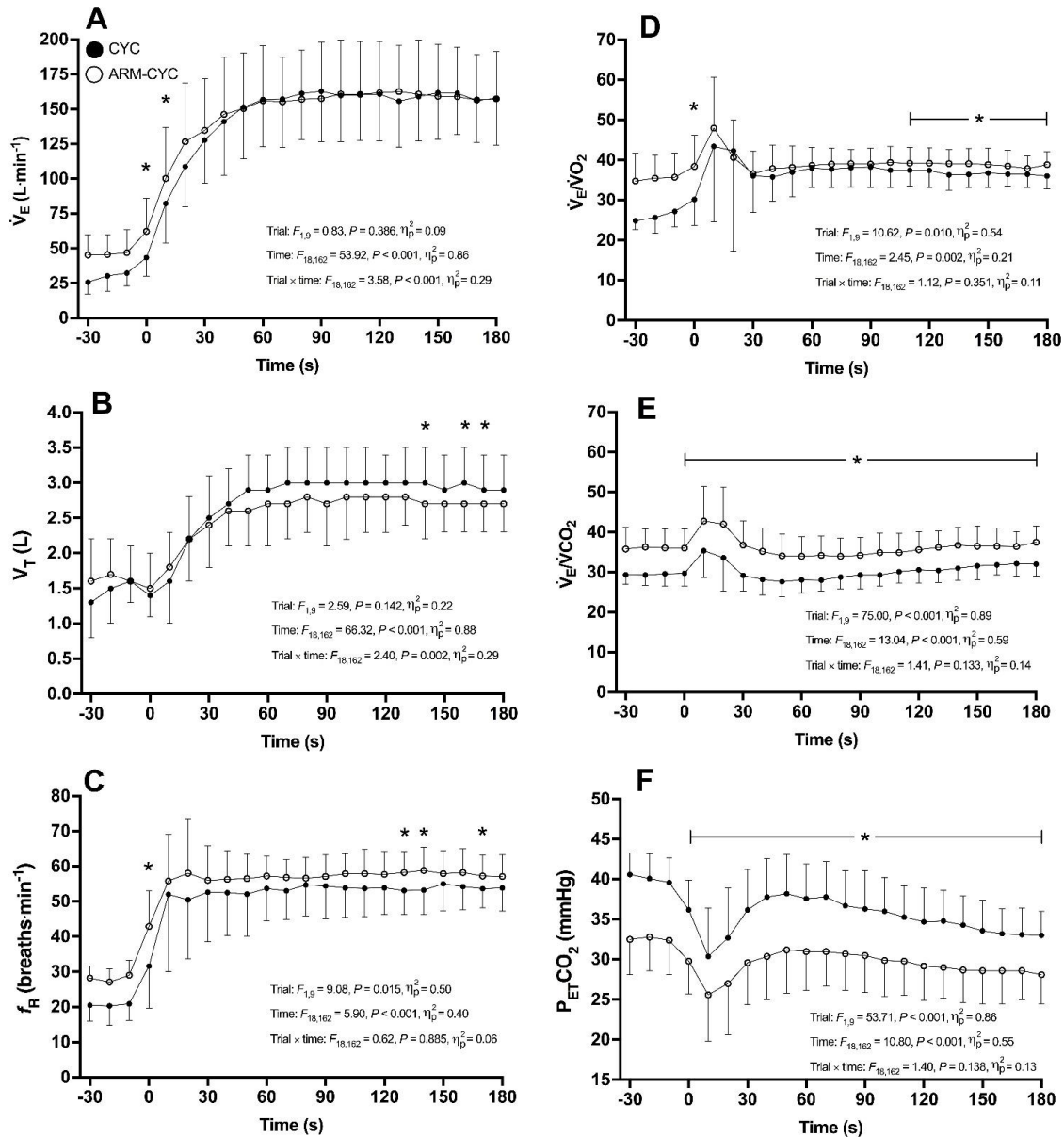
586

587

588

589

590



591

592

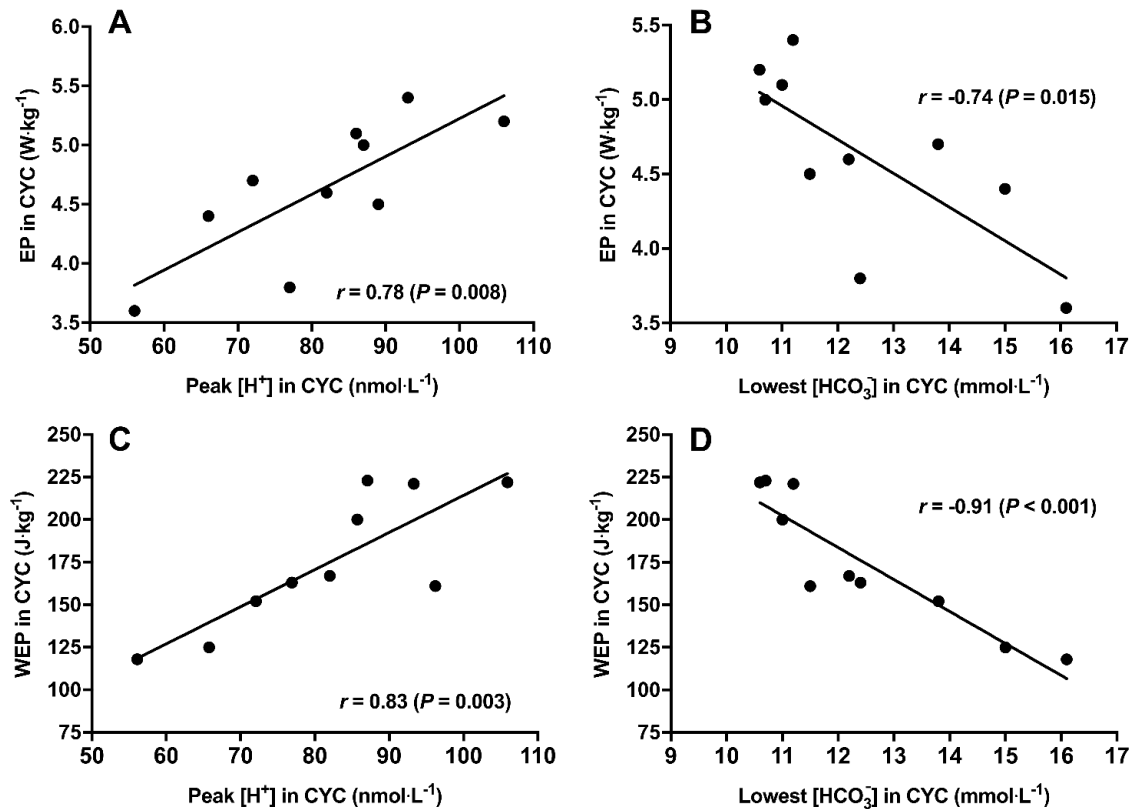
593 **FIGURE 4** – Minute ventilation (\dot{V}_E) (A), tidal volume (V_T) (B), breathing frequency (f_R) (C),
 594 ventilatory equivalents for oxygen (\dot{V}_E/\dot{V}_{O_2}) (D) and carbon dioxide (\dot{V}_E/\dot{V}_{CO_2}) (E), and end-
 595 tidal CO₂ ($P_{ET}CO_2$) (F) during the 3MT of CYC and ARM-CYC. The final 30-s of unloaded
 596 cycling is also shown, with the 3MT commencing at time 0-s. Data are mean ± SD. *Difference
 597 between trials ($P < 0.05$). Capped lines with asterisk denote the range of measurement points
 598 that differ between CYC and ARM-CYC.

599

600

601

602



603

604 **FIGURE 5** – Correlations between end-test power (EP) and peak $[H^+]$ (A), EP and lowest
 605 $[HCO_3^-]$ (B), work done above EP (WEP) and peak $[H^+]$ (C), and WEP and lowest $[HCO_3^-]$
 606 (D).

607

608 **TABLE 1** [Hb] and plasma acid-base variables at baseline (pooled data), at the start and end of the 3MT, and
 609 after 3- and 6-min recovery. Δ Blood volume represents the percentage change from baseline. Values are mean \pm
 610 SD.

	Baseline	Start of 3-min test		End of 3-min test		3-min recovery		6-min recovery	
		CYC	ARM-CYC	CYC	ARM-CYC	CYC	ARM-CYC	CYC	ARM-CYC
[Hb] (g·dL ⁻¹)#†‡	14.9 \pm 0.7	15.2 \pm 0.7	16.2 \pm 0.7**	16.3 \pm 0.9	17.0 \pm 0.7*	16.3 \pm 0.8	16.5 \pm 0.8	16.1 \pm 0.7	16.1 \pm 0.7
Δ Blood volume (%)		-1.5 \pm 1.4	-8.1 \pm 1.3	-8.3 \pm 1.6	-12.5 \pm 1.1	-8.1 \pm 1.3	-9.5 \pm 1.0	-6.8 \pm 1.1	-7.7 \pm 1.2
Ions and [PPr]									
[La ⁻] (mmol·L ⁻¹)#†‡	1.3 \pm 0.3	1.8 \pm 0.4	14.1 \pm 3.4**	15.0 \pm 3.7	21.9 \pm 4.2**	21.7 \pm 4.2	23.6 \pm 3.2*	20.3 \pm 4.1	21.8 \pm 3.3
[Na ⁺] (mmol·L ⁻¹)#†‡	141 \pm 1	142 \pm 1	145 \pm 2**	147 \pm 2	150 \pm 2**	146 \pm 2	147 \pm 2	144 \pm 1	145 \pm 1
[K ⁺] (mmol·L ⁻¹)†	3.9 \pm 0.2	4.1 \pm 0.2	3.9 \pm 0.2	5.1 \pm 0.4	5.1 \pm 0.5	3.8 \pm 0.2	3.8 \pm 0.2	3.7 \pm 0.3	3.7 \pm 0.2
[Ca ²⁺] (mmol·L ⁻¹)†‡	1.2 \pm 0.4	1.2 \pm 0.0	1.3 \pm 0.0	1.3 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1
[Cl ⁻] (mmol·L ⁻¹)†‡	104 \pm 2	103 \pm 2	105 \pm 3*	106 \pm 3	108 \pm 2**	105 \pm 2	106 \pm 1	105 \pm 2	105 \pm 1
[PPr] (g·dL ⁻¹)#†‡	7.4 \pm 0.4	7.4 \pm 0.3	8.3 \pm 0.4**	8.5 \pm 0.4	9.1 \pm 0.5*	8.4 \pm 0.4	8.8 \pm 0.5	8.2 \pm 0.6	8.5 \pm 0.6
Independent acid-base variables									
[SID] (mmol·L ⁻¹)#†‡	40.4 \pm 1.6	41.4 \pm 2.2	30.9 \pm 4.6**	33.3 \pm 3.2	28.0 \pm 4.0**	25.5 \pm 2.9	24.1 \pm 3.3*	25.5 \pm 3.1	24.2 \pm 3.2*
[A _{int}] (mmol·L ⁻¹)#†‡	18.2 \pm 1.0	18.1 \pm 0.7	20.4 \pm 1.1**	20.8 \pm 1.1	22.2 \pm 1.2*	20.7 \pm 1.1	21.5 \pm 1.2	20.2 \pm 1.4	20.9 \pm 1.4
PCO ₂ (mmHg)#†‡	42.1 \pm 3.4	51.7 \pm 5.9	40.1 \pm 8.0**	57.6 \pm 8.7	44.8 \pm 10.3**	39.0 \pm 4.1	34.4 \pm 5.2**	38.8 \pm 4.8	34.1 \pm 3.3**
Dependent acid-base variables									
[H ⁺] (nmol·L ⁻¹)#†‡	38.0 \pm 1.7	42.8 \pm 3.1	58.6 \pm 5.5**	70.8 \pm 12.4	81.2 \pm 13.5**	81.3 \pm 14.2	86.5 \pm 14.0*	79.2 \pm 14.7	82.4 \pm 14.2
[HCO ₃ ⁻] (mmol·L ⁻¹)#†‡	26.6 \pm 1.3	27.0 \pm 1.9	16.9 \pm 3.2**	17.1 \pm 2.5	13.1 \pm 2.4**	12.5 \pm 1.9	11.3 \pm 1.9**	12.8 \pm 2.3	11.7 \pm 2.1**

611 # Main effect of trial ($P < 0.001 - 0.042$, $\eta_p^2 = 0.39 - 0.90$); † main effect of time ($P < 0.001$, $\eta_p^2 = 0.70 - 0.97$);
 612 ‡ trial \times time interaction ($P \leq 0.001$, $\eta_p^2 = 0.47 - 0.93$). Different from equivalent CYC value: * $P < 0.05$, ** $P <$
 613 0.01.