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

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ABSTRACT

Introduction Prior upper body exercise reduces the curvature constant (W′) of the hyperbolic power-duration relationship without affecting critical power. This study tested the hypothesis that prior upper body exercise reduces the work done over the end-test power (WEP; analogue of W′) during a 3-min all-out cycling test (3MT) without affecting the end-test power (EP; analogue of critical power). **Methods** Ten endurance-trained men ($\dot{V}O_{2max}$ = 62 ± 5 mL·kg^{-1}·min^{-1}) performed a 3MT without (CYC) and with (ARM-CYC) prior severe-intensity, intermittent upper body exercise. EP was calculated as the mean power output over the last 30-s of the 3MT, whereas WEP was calculated as the power-time integral above EP. **Results** At the start of the 3MT, plasma [La^-] (1.8 ± 0.4 vs. 14.1 ± 3.4 mmol·L^{-1}) and [H^+] (42.8 ± 3.1 vs. 58.6 ± 5.5 nmol·L^{-1}) were higher, whereas the strong ion difference ([SID]) (41.4 ± 2.2 vs. 30.9 ± 4.6 mmol·L^{-1}) and [HCO_3^-] (27.0 ± 1.9 vs. 16.9 ± 3.2 mmol·L^{-1}) were lower, during ARM-CYC than CYC (P < 0.010). EP was 12% lower during the 3MT of ARM-CYC (298 ± 52 W) than CYC (338 ± 60 W) (P < 0.001), whereas WEP was not different (CYC: 12.8 ± 3.3 kJ vs. ARM-CYC: 13.5 ± 4.1 kJ, P = 0.312). EP in CYC was positively correlated with the peak [H^+] (r = 0.78, P = 0008), and negatively correlated with the lowest [HCO_3^-] (r = -0.74, P = 0.015). **Conclusion** These results suggest that EP during a 3MT in endurance-trained men is sensitive to fatigue-related ionic perturbation.

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

INTRODUCTION

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

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

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

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

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

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

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

METHODS

Participants and ethical approval

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

Experimental design

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

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

Equipment and measurements

Exercise was performed using electromagnetically braked cycle (Excalibur Sport; Lode, Groningen, The Netherlands) and arm-cranking (Angio; Lode) ergometers. Participants fitted their own pedals to the cycle ergometer and the position of the seat and handlebars was replicated for all tests. Ventilatory and pulmonary gas exchange variables were measured breath-by-breath (ZAN 600USB CPX incorporating GPI V3.0 software; Nspire Health, Oberthulba, Germany). Participants wore a facemask (model 7940; Hans Rudolph, Missouri, USA) connected to a low resistance (0.51 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ at <14 $L \cdot s^{-1}$) flow sensor (ZAN variable orifice pneumotach; Nspire Health) with a combined dead space of 67 mL. The flow sensor was calibrated using a 3-L syringe. Gas concentrations were sampled (50 $mL \cdot min^{-1}$) at the mouth via a 2-m capillary line and analyzed using fast responding laser diode absorption spectroscopy sensors that were calibrated using ambient air and gases of known concentration (5% CO_2, 15% O_2, balance N_2; BOC, Guilford, UK). Volume and concentration signals were time aligned by accounting for the transit delay in the gas capillary line and the analyzer rise time (T_{10-90} <90-ms) relative to the volume signal.

Heart rate was measured using short-range telemetry (Polar RS400; Polar Electro, Kempele, Finland). Arterial oxygen saturation (SpO_2) was estimated using a pulse oximeter (Model 8500; Nonin Medical, Plymouth, MN) and an adhesive forehead reflectance sensor (Model 8000R; Nonin Medical). Arterialized venous blood (1.5-mL) was drawn from a heated dorsal hand vein using an indwelling 21-G cannula and a syringe containing dry electrolyte-balanced heparin (*safe*PICO, Radiometer, Copenhagen, Denmark). Blood was analyzed immediately (ABL90 FLEX; Radiometer) for [Hb] and pH, PCO_2, [HCO_3^-], [K^+], [Na^+], [Ca^{2+}], and [Cl^-]. 0.5-mL of each blood sample was then immediately centrifuged for 10-min at 3000*g*. The plasma

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

Cycling ramp incremental test

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

The 3MT

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

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

Reliability of the 3MT

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

CYC and ARM-CYC

After 3-min of seated rest (baseline), participants remained seated and either rested for 11.5-min (CYC) or performed severe-intensity, intermittent upper body exercise (ARM-CYC) comprising eight 1-min arm-cranking exercise intervals, interspersed by 30-s rest intervals, at an intensity of 1.5-2.0 $W \cdot kg^{-1}$ body mass (129 ± 29 W) and cadence of 100-130 rpm (8,20). Seated rest / arm-cranking was followed by a 1-min rest interval during which participants transferred to the adjacent cycle ergometer. Thereafter, 3-min of unloaded cycling commenced followed by the 3MT. Blood samples were taken at baseline, at the start and end of the 3MT, and 3- and 6-min into recovery. Heart rate and SpO_2 were measured at baseline and at the end of each arm-cranking exercise interval in ARM-CYC, or at an equivalent time point during CYC (latter data not reported). Thereafter, heart rate was measured every second during unloaded cycling and the 3MT, and SpO_2 was measured every 30-s during the 3MT. The O_2 cost of exercise was determined using the $\dot{V}O_2$ gain ($\dot{V}O_2$/power) at 10-s intervals.

Statistical analyses

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

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

RESULTS

Incremental cycling ramp test

Peak power output was 419 ± 73 W (5.7 ± 0.5 $W \cdot kg^{-1}$), and $\dot{V}O_{2max}$ was 4.53 ± 0.65 $L \cdot min^{-1}$ (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 were therefore classified as 'trained' ($\dot{V}O_{2max}$ between 55.0 – 64.9 $mL \cdot kg^{-1} \cdot min^{-1}$) ($n = 6$) or 'well-trained' ($\dot{V}O_{2max}$ between 65.0 – 71.0 $mL \cdot kg^{-1} \cdot min^{-1}$) ($n = 4$) (29).

Reliability of the 3MT

There was no difference between the reliability trials for EP (328 ± 53 vs. 337 ± 57 W; t_9 = 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). For EP and WEP respectively, there was a day-to-day CV of 4% and 12%, measurement error of 14 W and 2.1 kJ, reproducibility of 40 W and 5.9 kJ, and a smallest meaningful change of 20 W and 2.9 kJ.

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

Peak power output (t_9 = 2.02, P = 0.074, d_z = 0.62), the corresponding cadence (t_9 = 2.03, P = 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 3MT were not different between CYC and ARM-CYC (Fig. 1), although a medium effect size was observed for peak power output. EP was 12% lower in ARM-CYC than CYC (mean difference = 40 ± 13 W, 95% CI [31, 49 W]; t_9 = 2.94, P < 0.001, d_z = 3.07), whereas WEP was not different (t_9 = 1.07, P = 0.312, d_z = 0.34) (Fig. 1). The total work done was 9% lower 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).

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

Pulmonary gas exchange, heart rate and SpO_2 responses are shown in Figure 2. For $\dot{V}O_2$, there was a trial × time interaction effect due to $\dot{V}O_2$ being ~0.26 L·min^{-1} (5-7%) lower during ARM-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 – 1.16). There was a main effect of test on $\dot{V}O_{2max}$ ($F_{2,18}$ = 9.20, P < 0.001, η_p^2 = 0.51). The $\dot{V}O_{2max}$ during the cycling ramp incremental test and the 3MT of CYC (4.41 ± 0.62 L·min^{-1}) was not different (P = 0.549), whereas both were 5-8% higher than $\dot{V}O_{2max}$ during the 3MT of ARM-CYC (4.17 ± 0.64 L·min^{-1}) (P = 0.002 and 0.017, respectively). The relative reduction in $\dot{V}O_{2max}$ during ARM-CYC vs. CYC was negatively correlated with the between-trial difference in peak [H^+] (r = -0.86, P = 0.001). For the $\dot{V}O_2$ gain (Fig. 3), there was a trial ×

time interaction effect. The $\dot{V}O_2$ gain was 0.9 mL·min^{-1}·W^{-1} (7%) higher during ARM-CYC 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 L·min^{-1} higher at the start of the 3MT in ARM-CYC than CYC (t_9 = 2.77, P = 0.022, d_z = 0.88). Thereafter, except for the first 30-s of the 3MT, $\dot{V}CO_2$ was ~0.78 L·min^{-1} (~15%) lower during ARM-CYC than CYC (t_9 = 3.55 – 6.11, P = <0.001 – 0.006, d_z = 0.98 – 1.92). Heart rate was ~9 beats·min^{-1} (~6%) higher throughout the 3MT of ARM-CYC than CYC (t_9 = 2.34 – 11.01, P = <0.001 – 0.044, d_z = 0.73 – 3.54). SpO_2 fell during the 3MT and was ~2% lower during ARM-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 = 0.76).

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

Ventilatory response are shown in Figure 4. For $\dot{V}_E$, the trial × time interaction effect was due to an ~19 L·min^{-1} higher $\dot{V}_E$ during ARM-CYC than CYC at the start (t_9 = 3.89, P = 0.003, d_z = 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 between CYC and ARM-CYC, although a relative tachypnea (i.e. lower V_T and higher f_R) was observed in ARM-CYC. Due to $\dot{V}_E$ being higher at the start of the 3MT of ARM-CYC than 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 $\dot{V}_E/\dot{V}O_2$ during ARM-CYC than CYC from 110-180-s of the 3MT (t_9 = 2.35 – 6.19, P = <0.001 – 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 the 3MT of ARM-CYC resulted in $\dot{V}_E/\dot{V}CO_2$ being higher during ARM-CYC than CYC (t_9 ≥ 3.27 – 8.60, P = <0.001 – 0.009, d_z = 1.02 – 2.44). $P_{ET}CO_2$ was ~6 mmHg (~17%) lower throughout the 3MT of ARM-CYC than CYC (t_9 = 3.40 – 9.26, P = <0.001 – 0.008, d_z = 0.96 – 2.74).

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

During the 3MT of CYC blood volume fell by ~8%, as reflected by the 1.4 ± 0.3 g·dL^{-1} increase in [Hb] from baseline (Table 1). The 3MT of ARM-CYC commenced with a pre-existing ~8% reduction in blood volume from baseline, as reflected by the 1.3 ± 0.2 g·dL^{-1} increase in [Hb]. A further reduction in blood volume (~12% from baseline) was observed at the end of the 3MT in ARM-CYC, as reflected by a further 0.8 ± 0.2 g·dL^{-1} increase in [Hb]. The greater [Hb] at 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 of ARM-CYC than CYC reflects a lower blood volume during ARM-CYC.

Plasma acid-base variables at baseline were not different between CYC and ARM-CYC and therefore these data were pooled (Table 1). The [La^-], [Na^+], [Cl^-], and [PPr^-] were higher at the start and end of the 3MT of ARM-CYC than CYC. The net increase in [La^-] during the 3MT was smaller during ARM-CYC (7.8 ± 4.0 mmol·L^{-1}) than CYC (13.2 ± 3.5 mmol·L^{-1}) (t_9 = 4.51, P = 0.001, d_z = 1.42). The between-trial differences in ion concentrations and [PPr^-] resulted in between-trial differences for the independent acid-base variables [SID] and [A_{tot}]. Specifically, [SID] was lower during ARM-CYC than CYC at the start (~-10.5 mmol·L^{-1}) and end (~-5.2 mmol·L^{-1}) of the 3MT, whereas [A_{tot}] was higher during ARM-CYC than CYC at the start (~2.3 mmol·L^{-1}) and end (~1.4 mmol·L^{-1}) of the 3MT. PCO_2 was ~12.0-13.0 mmHg lower during ARM-CYC than CYC at the start and end of the 3MT. The between-trial differences in the independent acid-base variables resulted in between-trial differences in the dependent acid-base variables [H^+] and [HCO_3^-]. Specifically, [H^+] was higher and [HCO_3^-] was lower during ARM-CYC than CYC at the start (~15.8 nmol·L^{-1} and ~-10.1 mmol·L^{-1}) and end (~10.4 nmol·L^{-1} and ~-4.0 mmol·L^{-1}) of the 3MT. The net increase in [H^+] during the 3MT was smaller during ARM-CYC (22.6 ± 10.2 nmol·L^{-1}) than CYC (28.0 ± 10.6 nmol·L^{-1}) (t_9 = 3.43, 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

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

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

The findings of the present study, along with those of Johnson et al. (8), show that prior exercise using non-homologous muscles reduces EP and W′ without affecting CP or WEP. Interestingly, prior exercise using homologous muscles reduces both W′ and WEP without affecting CP or EP (1,17,18). Previous studies preceded the 3MT with 4-min of severe intensity cycling 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·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·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.

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

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

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

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

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

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

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

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

Conclusion

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

Acknowledgements

None

Conflict of Interest

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

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

REFERENCES

1. Ferguson C, Whipp BJ, Cathcart AJ, Rossiter HB, Turner AP, Ward SA. Effects of prior very-heavy intensity exercise on indices of aerobic function and high-intensity exercise tolerance. *J Appl Physiol.* 2007;103(3):812–22.

2. Johnson MA, Sharpe GR, Brown PI. Inspiratory muscle training improves cycling time-trial performance and anaerobic work capacity but not critical power. *Eur J Appl Physiol.* 2007;101(6):761–70.

3. Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM. Critical power: An important fatigue threshold in exercise physiology. *Med Sci Sports Exerc.* 2016;48(11):2320–34.

4. Burnley M, Jones AM. Power-duration relationship: Physiology, fatigue, and the limits of human performance. *Eur J Sport Sci.* 2018;18(1):1–12.

5. Miura A, Kino F, Kajitani S, Sato H, Fukuba Y. The effect of oral creatine supplementation on the curvature constant parameter of the power-duration curve for cycle ergometry in humans. *Jpn J Physiol.* 1999;49(2):169–74.

6. Miura A, Sato H, Sato H, Whipp BJ, Fukuba Y. The effect of glycogen depletion on the curvature constant parameter of the power-duration curve for cycle ergometry. *Ergonomics.* 2000;43(1):133–41.

7. Green S, Dawson BT, Goodman C, Carey MF. Y-intercept of the maximal work-duration relationship and anaerobic capacity in cyclists. *Eur J Appl Physiol Occup Physiol.* 1994;69(6):550–6.

8. Johnson MA, Mills DE, Brown PI, Sharpe GR. Prior upper body exercise reduces cycling work capacity but not critical power. *Med Sci Sports Exerc.* 2014;46(4):802–8.

9. Grassi B, Rossiter HB, Zoladz JA. Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? *Exerc Sport Sci Rev.* 2015;43(2):75–83.

10. Vanhatalo A, Poole DC, DiMenna FJ, Bailey SJ, Jones AM. Muscle fiber recruitment and the slow component of O_2 uptake: constant work rate vs. all-out sprint exercise. *Am J Physiol Regul Integr Comp Physiol.* 2011;300(3):R700-707.

11. Jones AM, Vanhatalo A. The "Critical Power" concept: Applications to sports performance with a focus on intermittent high-intensity exercise. *Sports Med.* 2017;47(Suppl 1):65–78.

12. Vanhatalo A, Doust JH, Burnley M. Determination of critical power using a 3-min all-out cycling test. *Med Sci Sports Exerc.* 2007;39(3):548–55.

13. Simpson LP, Jones AM, Skiba PF, Vanhatalo A, Wilkerson D. Influence of hypoxia on the power-duration relationship during high-intensity exercise. *Int J Sports Med.* 2015;36(2):113–9.

14. Bartram JC, Thewlis D, Martin DT, Norton KI. Predicting critical power in elite cyclists: Questioning the validity of the 3-minute all-out test. *Int J Sports Physiol Perform*. 2017;12(6):783–7.

15. Wright J, Bruce-Low S, Jobson SA. The 3-minute all-out cycling test is sensitive to changes in cadence using the Lode Excalibur Sport ergometer. *J Sports Sci.* 2019;37(2):156–62.

16. Vanhatalo A, Doust JH, Burnley M. A 3-min all-out cycling test is sensitive to a change in critical power. *Med Sci Sports Exerc*. 2008;40(9):1693–9.

17. Parker Simpson L, Jones AM, Vanhatalo A, Wilkerson DP. Influence of initial metabolic rate on the power-duration relationship for all-out exercise. *Eur J Appl Physiol.* 2012;112(7):2467–73.

18. Vanhatalo A, Jones AM. Influence of prior sprint exercise on the parameters of the "all-out critical power test" in men. *Exp Physiol.* 2009;94(2):255–63.

19. Clark IE, Vanhatalo A, Thompson C, Wylie LJ, Bailey SJ, Kirby BS, Wilkins BW, Jones AM. Changes in the power-duration relationship following prolonged exercise: estimation using conventional and all-out protocols and relationship with muscle glycogen. *Am J Physiol Regul Integr Comp Physiol.* 2019;317(1):R59–67.

20. Johnson MA, Sharpe GR, Williams NC, Hannah R. Locomotor muscle fatigue is not critically regulated after prior upper body exercise. *J Appl Physiol.* 2015;119(7):840–50.

21. Bangsbo J, Madsen K, Kiens B, Richter EA. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol.* 1996;495(Pt 2):587–96.

22. Thomas K, Goodall S, Howatson G. Performance fatigability is not regulated to a peripheral critical threshold. *Exerc Sport Sci Rev*. 2018;46(4):240–6.

23. Salam H, Marcora SM, Hopker JG. The effect of mental fatigue on critical power during cycling exercise. *Eur J Appl Physiol*. 2018;118(1):85–92.

24. Martin K, Thompson KG, Keegan R, Ball N, Rattray B. Mental fatigue does not affect maximal anaerobic exercise performance. *Eur J Appl Physiol.* 2015;115(4):715–25.

25. Vanhatalo A, Jones AM. Influence of creatine supplementation on the parameters of the "all-out critical power test." *J Exerc Sci Fit.* 2009;7(1):9–17.

26. Stickland MK, Lindinger MI, Olfert IM, Heigenhauser GJF, Hopkins SR. Pulmonary gas exchange and acid-base balance during exercise. *Compr Physiol.* 2013;3(2):693–739.

27. Harrison MH. Effects on thermal stress and exercise on blood volume in humans. *Physiol Rev.* 1985;65(1):149–209.

28. Vanhatalo A, Black MI, DiMenna FJ, Blackwell JR, Schmidt JF, Thompson C, Wylie LJ, Mohr M, Bangsbo J, Krustrup P, Jones AM. The mechanistic bases of the power-time relationship: muscle metabolic responses and relationships to muscle fibre type. *J Physiol.* 2016;594(15):4407–23.

29. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to classify subject groups in sport-science research. *Int J Sports Physiol Perform*. 2013;8(2):111–22.

30. McClave SA, LeBlanc M, Hawkins SA. Sustainability of critical power determined by a 3-minute all-out test in elite cyclists. *J Strength Cond Res*. 2011;25(11):3093–8.

31. Mainwood GW, Worsley-Brown P. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J Physiol.* 1975;250(1):1–22.

32. Davies NW. Modulation of ATP-sensitive K+ channels in skeletal muscle by intracellular protons. *Nature*. 1990;343(6256):375–7.

33. Kent-Braun JA, Fitts RH, Christie A. Skeletal muscle fatigue. *Compr Physiol.* 2012;2(2):997–1044.

34. Fernandez-del-Olmo M, Rodriguez FA, Marquez G, Iglesias X, Marina M, Benitez A, Vallejo L, Acero RM. Isometric knee extensor fatigue following a Wingate test: peripheral and central mechanisms. *Scand J Med Sci Sports*. 2013;23(1):57–65.

35. Decorte N, Lafaix PA, Millet GY, Wuyam B, Verges S. Central and peripheral fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports.* 2012;22(3):381–91.

36. Amann M, Sidhu SK, Weavil JC, Mangum TS, Venturelli M. Autonomic responses to exercise: group III/IV muscle afferents and fatigue. *Auton Neurosci.* 2015;188:19–23.

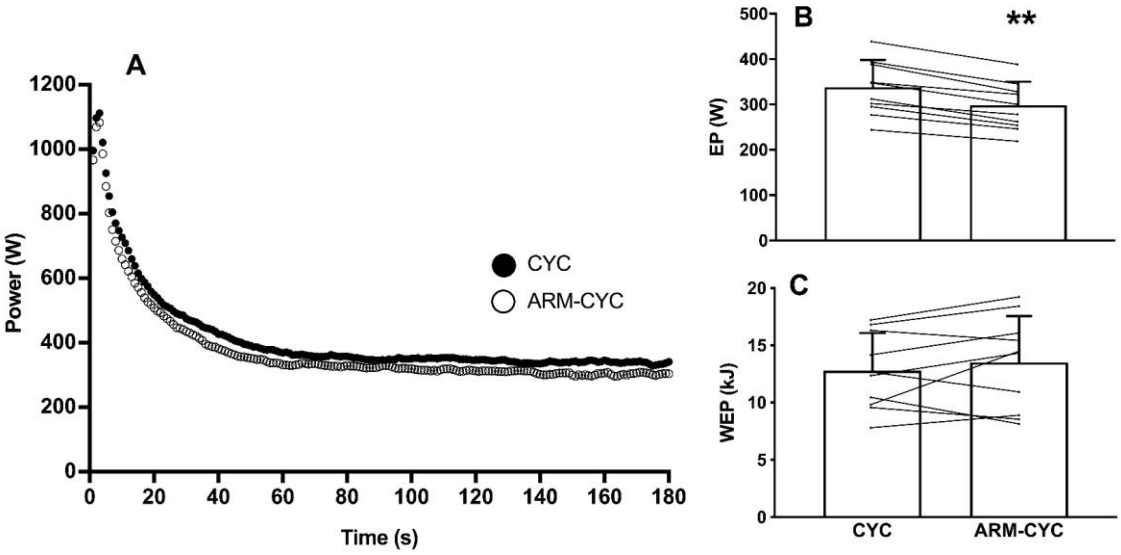
37. Bogdanis GC, Nevill ME, Lakomy HK. Effects of previous dynamic arm exercise on power output during repeated maximal sprint cycling. *J Sports Sci.* 1994;12(4):363–70.

38. Grant MC, Robergs R, Baird MF, Baker JS. The effect of prior upper body exercise on subsequent wingate performance. *Biomed Res Int.* 2014;329328.

39. Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. *J Physiol*. 2003;553(Pt 2):589–99.

40. Gifford JR, Garten RS, Nelson AD, Trinity JD, Layec G, Witman MAH, et al. Symmorphosis and skeletal muscle $\dot{V}O_{2max}$: *in vivo* and *in vitro* measures reveal differing constraints in the exercise-trained and untrained human. *J Physiol.* 2016;594(6):1741–51.

41. Morales-Alamo D, Losa-Reyna J, Torres-Peralta R, Martin-Rincon M, Perez-Valera M, Curtelin D, Ponce-González JG, Santana A, Calbet JAL. What limits performance during whole-body incremental exercise to exhaustion in humans? *J Physiol*. 2015;593(20):4631–48.

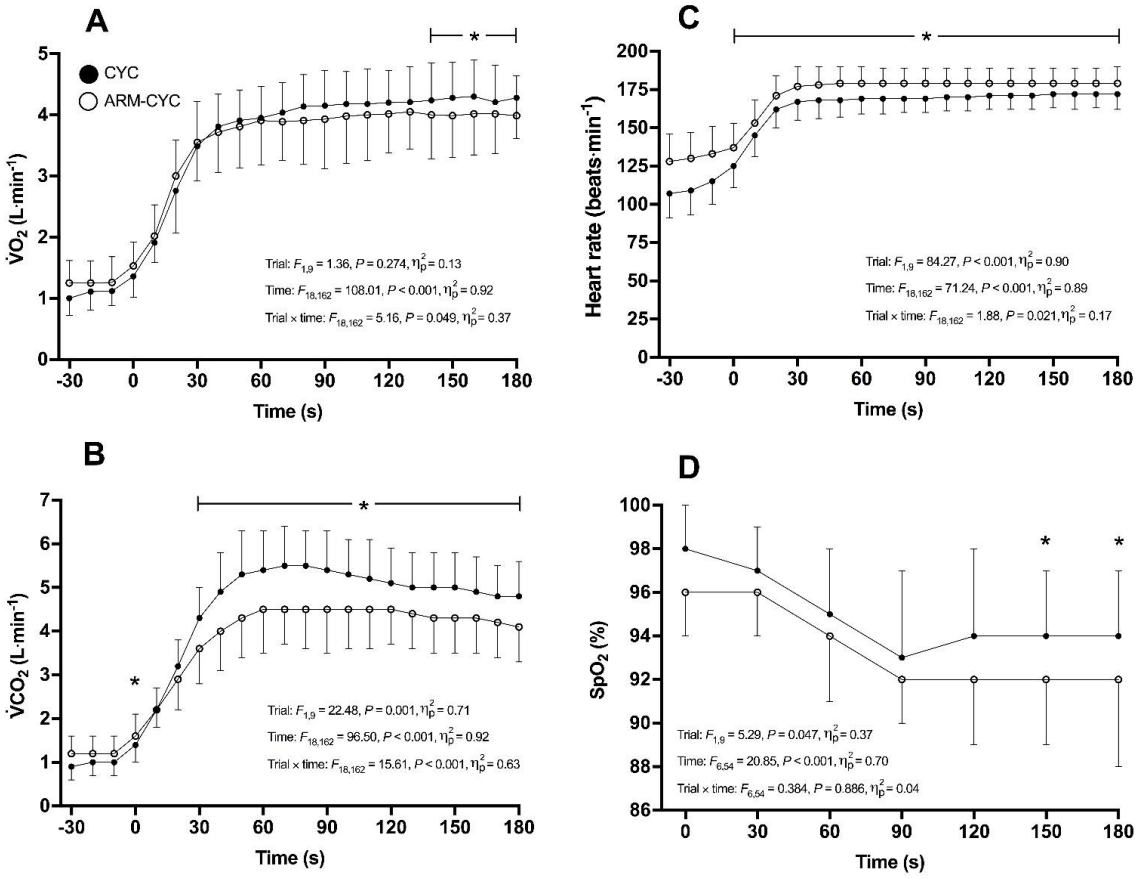


556

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 ± SD, with lines
560 representing individual participants. **Different from CYC ($P < 0.001$).

561

562



563

FIGURE 2 – Pulmonary oxygen uptake ($\dot{V}O_2$) (A), carbon dioxide production ($\dot{V}CO_2$) (B), heart rate (C), and arterial oxygen saturation by pulse oximetry (SpO_2) (D) during the 3MT of CYC and ARM-CYC. Panels A-C also show the final 30-s of unloaded cycling, with the 3MT commencing at time 0-s. Data are mean ± SD. *Difference between trials ($P < 0.05$). Capped lines with asterix denote the range of measurement points that differ between CYC and ARM-CYC.

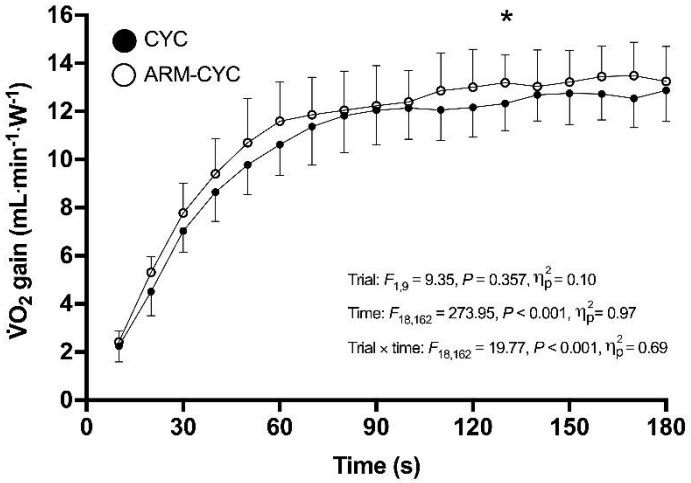


FIGURE 3 – Pulmonary oxygen uptake ($\dot{V}O_2$) gain during the 3MT of CYC and ARM-CYC. Data are mean ± SD. *Difference between trials ($P < 0.05$).

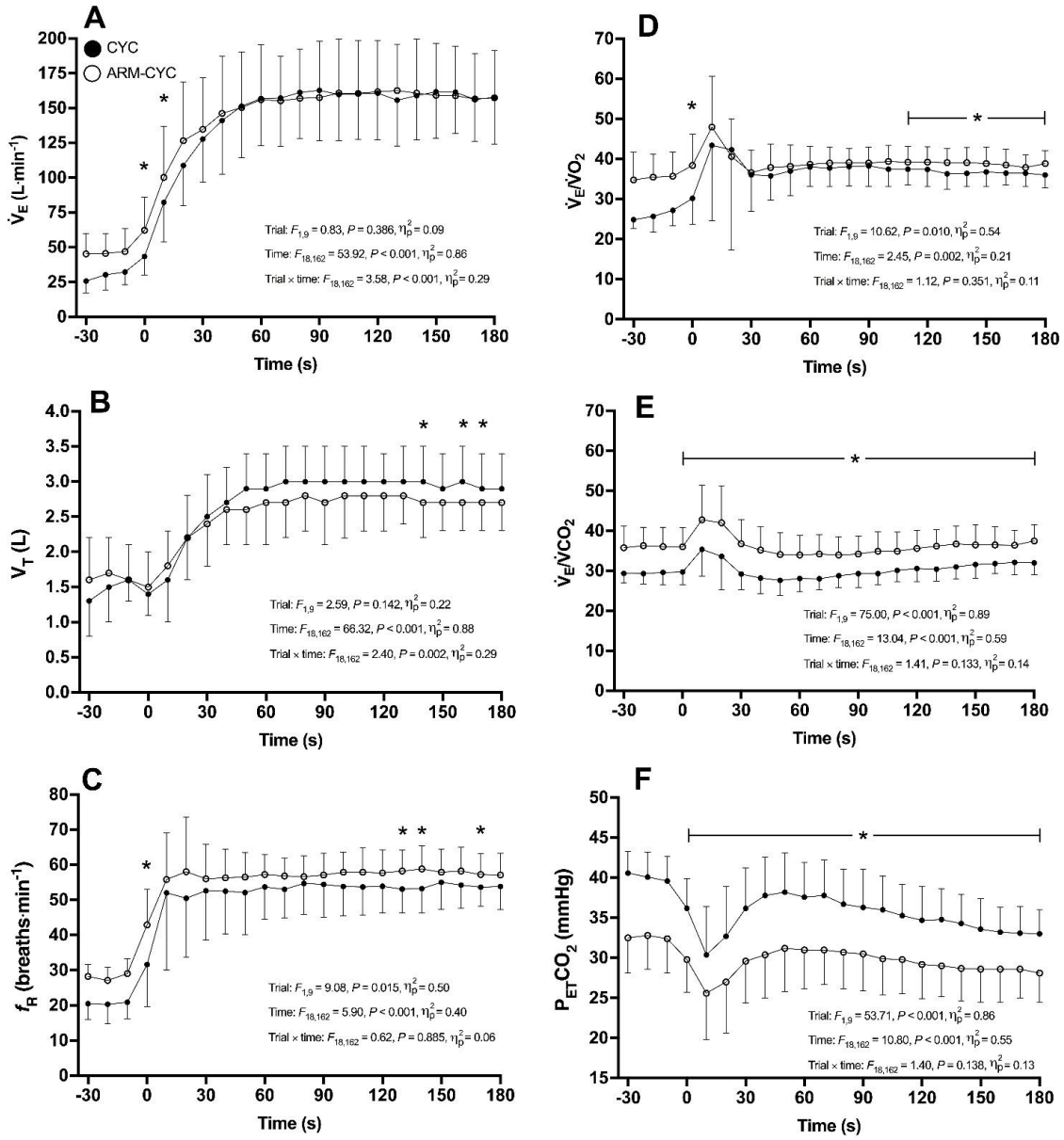
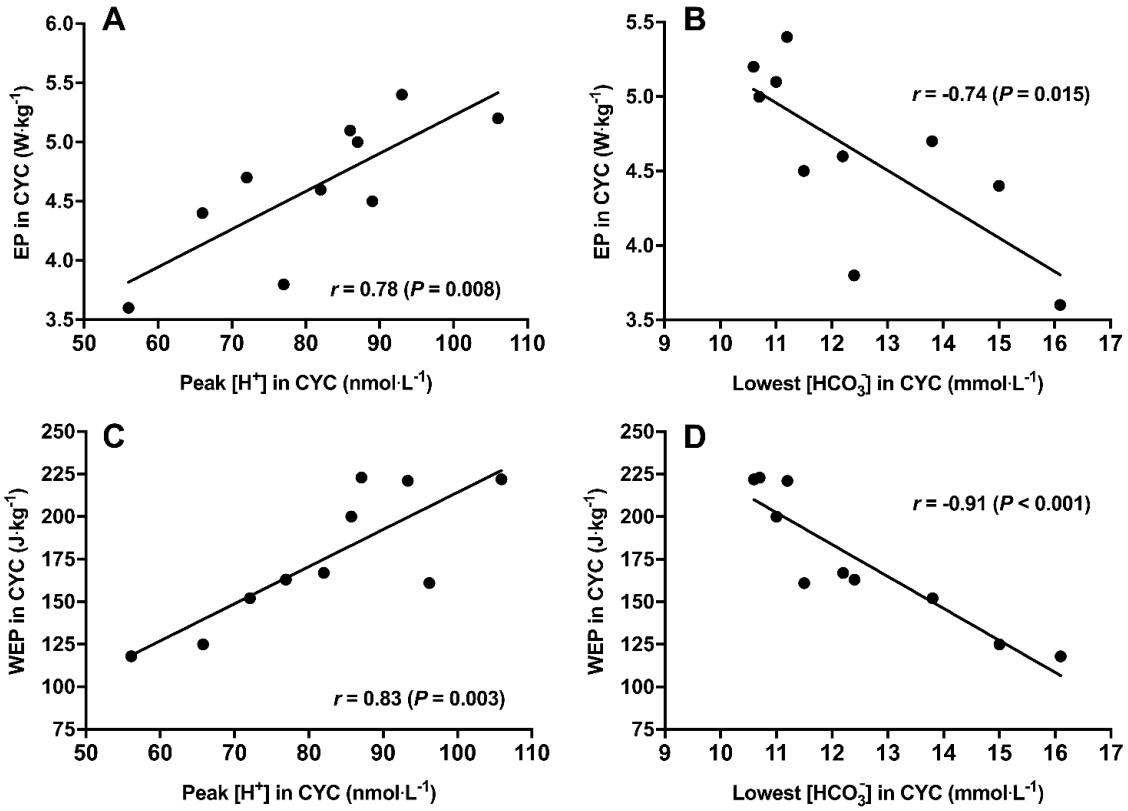


FIGURE 4 – Minute ventilation ($\dot{V}_E$) (A), tidal volume (V_T) (B), breathing frequency (f_R) (C), ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}O_2$) (D) and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) (E), and end-tidal CO_2 ($P_{ET}CO_2$) (F) during the 3MT of CYC and ARM-CYC. The final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



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. ΔBlood volume represents the percentage change from baseline. Values are mean ±
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] ($g \cdot dL^{-1}$)#†‡	14.9 ± 0.7	15.2 ± 0.7	16.2 ± 0.7**	16.3 ± 0.9	17.0 ± 0.7*	16.3 ± 0.8	16.5 ± 0.8	16.1 ± 0.7	16.1 ± 0.7
ΔBlood volume (%)		-1.5 ± 1.4	-8.1 ± 1.3	-8.3 ± 1.6	-12.5 ± 1.1	-8.1 ± 1.3	-9.5 ± 1.0	-6.8 ± 1.1	-7.7 ± 1.2
Ions and [PPr^-]									
[La^-] ($mmol \cdot L^{-1}$)#†‡	1.3 ± 0.3	1.8 ± 0.4	14.1 ± 3.4**	15.0 ± 3.7	21.9 ± 4.2**	21.7 ± 4.2	23.6 ± 3.2*	20.3 ± 4.1	21.8 ± 3.3
[Na^+] ($mmol \cdot L^{-1}$)#†‡	141 ± 1	142 ± 1	145 ± 2**	147 ± 2	150 ± 2**	146 ± 2	147 ± 2	144 ± 1	145 ± 1
[K^+] ($mmol \cdot L^{-1}$)†	3.9 ± 0.2	4.1 ± 0.2	3.9 ± 0.2	5.1 ± 0.4	5.1 ± 0.5	3.8 ± 0.2	3.8 ± 0.2	3.7 ± 0.3	3.7 ± 0.2
[Ca^{2+}] ($mmol \cdot L^{-1}$)†‡	1.2 ± 0.4	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
[Cl^-] ($mmol \cdot L^{-1}$)†‡	104 ± 2	103 ± 2	105 ± 3*	106 ± 3	108 ± 2**	105 ± 2	106 ± 1	105 ± 2	105 ± 1
[PPr^-] ($g \cdot dL^{-1}$)#†‡	7.4 ± 0.4	7.4 ± 0.3	8.3 ± 0.4**	8.5 ± 0.4	9.1 ± 0.5*	8.4 ± 0.4	8.8 ± 0.5	8.2 ± 0.6	8.5 ± 0.6
Independent acid-base variables									
[SID] ($mmol \cdot L^{-1}$)#†‡	40.4 ± 1.6	41.4 ± 2.2	30.9 ± 4.6**	33.3 ± 3.2	28.0 ± 4.0**	25.5 ± 2.9	24.1 ± 3.3*	25.5 ± 3.1	24.2 ± 3.2*
[A_{tot}] ($mmol \cdot L^{-1}$)#†‡	18.2 ± 1.0	18.1 ± 0.7	20.4 ± 1.1**	20.8 ± 1.1	22.2 ± 1.2*	20.7 ± 1.1	21.5 ± 1.2	20.2 ± 1.4	20.9 ± 1.4
PCO_2 (mmHg)#†‡	42.1 ± 3.4	51.7 ± 5.9	40.1 ± 8.0**	57.6 ± 8.7	44.8 ± 10.3**	39.0 ± 4.1	34.4 ± 5.2**	38.8 ± 4.8	34.1 ± 3.3**
Dependent acid-base variables									
[H^+] ($nmol \cdot L^{-1}$)#†‡	38.0 ± 1.7	42.8 ± 3.1	58.6 ± 5.5**	70.8 ± 12.4	81.2 ± 13.5**	81.3 ± 14.2	86.5 ± 14.0*	79.2 ± 14.7	82.4 ± 14.2
[HCO_3^-] ($mmol \cdot L^{-1}$)#†‡	26.6 ± 1.3	27.0 ± 1.9	16.9 ± 3.2**	17.1 ± 2.5	13.1 ± 2.4**	12.5 ± 1.9	11.3 ± 1.9**	12.8 ± 2.3	11.7 ± 2.1**

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