Title: Effects of prior voluntary hyperventilation on the 3-min all-out cycling test in men

Authors: Michael A. Johnson[1], Graham R. Sharpe[1], Robert S. Needham[1], Neil C. Williams[1]

Institutional affiliations: [1]Exercise and Health Research Group, Sport, Health and Performance Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK

Corresponding author

Dr Michael A Johnson

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

Telephone: +44 (0)115 8483362

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

ABSTRACT

Introduction The ergogenic effects of respiratory alkalosis induced by prior voluntary hyperventilation (VH) are controversial. This study examined the effects of prior VH on derived parameters from the 3-min all-out cycling test (3MT). **Methods** Eleven men ($\dot{V}O_{2max}$ = 46 ± 8 mL·kg^{-1}·min^{-1}) performed a 3MT preceded by 15-min of rest (CONT) or voluntary hyperventilation ($\dot{V}_E$ = 38 ± 5 L·min^{-1}) with $P_{ET}CO_2$ reduced to 21 ± 1 mmHg (HYP). End-test power (EP; synonymous with critical power) was calculated as the mean power output over the last 30-s of the 3MT, and the work done above EP (WEP; synonymous with W′) was calculated as the power-time integral above EP. **Results** At the start of the 3MT, capillary blood PCO_2 and [H^+] were lower in HYP (25.2 ± 3.0 mmHg, 27.1 ± 2.6 nmol·L^{-1}) than CONT (43.2 ± 2.0 mmHg, 40.0 ± 1.5 nmol·L^{-1}) (P < 0.001). At the end of the 3MT, blood PCO_2 was still lower in HYP (35.7 ± 5.4 mmHg) than CONT (40.6 ± 5.0 mmHg) (P < 0.001). WEP was 10% higher in HYP (19.4 ± 7.0 kJ) than CONT (17.6 ± 6.4 kJ) (P = 0.006), whereas EP was 5% lower in HYP (246 ± 69 W) than CONT (260 ± 74 W) (P = 0.007). The ΔWEP (J·kg^{-1}) between CONT and HYP correlated positively with the PCO_2 immediately before the 3MT in HYP (r = 0.77, P = 0.006). **Conclusion** These findings suggest that acid-base changes elicited by prior voluntary hyperventilation increase WEP but decrease EP during the all-out 3MT.

Key words: Power-duration relationship, respiratory alkalosis, critical power, hypocapnia

INTRODUCTION

The hyperbolic power-duration relationship is conventionally determined using 3-5 severe-intensity constant power exercise tests performed to task failure and lasting ~2-15-min (1,2). This relationship derives two parameters: critical power, represented by the power asymptote of the hyperbola, and W′, represented by the curvature constant. Critical power demarcates the heavy and severe exercise intensity domains and defines the highest sustainable oxidative metabolic rate, whereas W′ represents a finite amount of work that can be performed above critical power (3). Mechanistically, the physiological underpinnings of the power-duration relationship are closely associated with the oxygen uptake ($\dot{V}O_2$) kinetics during severe-intensity exercise (4). An inverse relationship exists between critical power and the time constant of the fundamental (or phase II) $\dot{V}O_2$ kinetics ($\tau\dot{V}O_2$) (5,6), and a positive relationship exists between W′ and the $\dot{V}O_2$ slow component magnitude (6). The association between W′ and the $\dot{V}O_2$ slow component magnitude suggests that W′ is intrinsically linked, in part, to fatigue-related metabolic and ionic perturbation (1,2) and the associated muscle fatigue and muscle inefficiency (7).

Valid estimates of critical power and W′ can be derived in recreationally active individuals using a 3-min all-out cycling test (3MT) (8). During the 3MT, power output peaks within ~5-s followed by an exponential decline during the subsequent ~2-min as W′ is rapidly depleted (8,9). The mean power output during the final 30-s of the 3MT (termed the end-test power, EP) provides an estimate of critical power, whereas the work done above EP (WEP) provides an estimate of W′ (8). However, whether EP and WEP are mechanistically equivalent to critical power and W′ remains uncertain (1,10). The all-out nature of the 3MT elicits maximal motor unit recruitment from the outset (11) and marked metabolic and ionic perturbation (10,12). Positive relationships have been reported between the magnitude of WEP

and the muscle [La^-] and [Cr] at the end of the 3MT (12). Moreover, the O_2 cost of exercise, reflected by the $\dot{V}O_2$ gain, increases throughout the 3MT and a positive relationship exists between WEP and the $\dot{V}O_2$ slow component magnitude (11,12). These observations suggest that the exponential decline in power output during the 3MT and the magnitude of WEP may depend, in part, on an intrinsic link between substrate level phosphorylation, fatigue-inducing intramuscular accumulation of H^+, Pi, $H_2PO_4^-$, and/or K^+, and an associated loss of muscular efficiency. Moreover, intramuscular metabolic and ionic perturbation and a progressive fall in cerebral blood flow may contribute to the progressive development of central fatigue (13,14), which may be an important determinant of EP (10,15).

Exercise-induced increases in intramuscular [H^+] primarily result from a decrease in the strong ion difference ([SID]) due to La^- accumulation and K^+ efflux (16). Intramuscular acidosis is partly resolved by the systemic circulation as it traverses active and inactive muscle, which maintains transmembrane ion concentration gradients (17). Fatigability and exercise tolerance may, therefore, be sensitive to interventions that modify intracellular and/or extracellular ion balance (1,10,18,19). For example, sodium bicarbonate ingestion increases plasma [HCO_3^-] by ~4 mmol·L^{-1} and reduces plasma [H^+] by ~6 nmol·L^{-1} (18) without affecting intramuscular [H^+] (20). The ergogenic effects of sodium bicarbonate ingestion are, however, variable, possibly due to adverse gastrointestinal side effects and/or ineffective dosing schedules (19). This may explain why the effects of sodium bicarbonate ingestion on the 3MT are inconclusive: Deb et al. (21) reported a 15% increase in WEP whereas Vanhatalo et al. (22) reported no change (EP was unchanged in both studies). Interestingly, compared to sodium bicarbonate ingestion and without gastrointestinal side effects, a more rapid and pronounced pre-exercise alkalosis can be achieved using voluntary hyperventilation, which reduces blood (23–26) and muscle (27) [H^+] by reducing blood PCO_2 (i.e. hypocapnia) and body CO_2 stores. However, the ergogenicity of respiratory alkalosis is also inconclusive. While some studies

report that respiratory alkalosis improves 30-s all-out Wingate cycling test performance (24) and repetitive cycling sprint performance (25), others report no effect on Wingate cycling test performance (28,29), small muscle mass exercise tolerance (26,30), or fatigue development during tetanic stimulation of the perfused rat hindlimb (31).

The mechanisms that may underpin an increase in exercise tolerance with induced respiratory alkalosis remain uncertain. A stimulatory effect of respiratory alkalosis on glycolysis has been observed during a 30-s all-out Wingate cycling test (24) and during the first minute of heavy-intensity (indicated by an elevated but stable blood [La^-]) constant power cycling exercise (32). Moreover, using phosphorus magnetic resonance spectroscopy, Forbes et al. (27) found that respiratory alkalosis increased the amplitude of the on-transient PCr kinetic response during moderate-intensity plantar flexion exercise, indicative of greater PCr breakdown. Collectively, these studies suggest that respiratory alkalosis may increase substrate level phosphorylation during exercise. Therefore, given the positive association between WEP and muscle [La^-] and [Cr] at the end of the 3MT (12), WEP may be increased by respiratory alkalosis induced by voluntary hyperventilation. However, Chin et al. (33) observed a 17-s increase in $\tau\dot{V}O_2$ (reflecting slower $\dot{V}O_2$ kinetics) when voluntary hyperventilation was performed before and during moderate-intensity cycling exercise. Given that critical power is inversely related to $\tau\dot{V}O_2$ (5,6), slower $\dot{V}O_2$ kinetics due to respiratory alkalosis might reduce EP. Moreover, the hypocapnia that results from voluntary hyperventilation increases cerebrovascular resistance and reduces cerebral blood flow (34,35), which may also reduce EP due to an attenuation of cortical voluntary activation (36,37) and an increase in central fatigue (10,15,38,39).

Therefore, the aim of this study was to examine the effects of prior voluntary hyperventilation on the parameters of the power-duration relationship derived using the 3MT. We hypothesised that prior voluntary hyperventilation would increase WEP, but decrease EP.

METHODS

Participants and ethical approval

Eleven healthy, non-smoking men (age: 26 ± 6 years; height: 181 ± 7 cm; body mass: 81 ± 8 kg) with normal lung function (forced vital capacity: 5.52 ± 0.83 L; forced expiratory volume in 1-s: 4.51 ± 0.77 L; peak expiratory flow: 10.3 ± 1.4 $L \cdot s^{-1}$) provided written informed consent to participate in the study. 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. The Institutional Human Ethics Committee approved all procedures, which were conducted in accordance with the Declaration of Helsinki.

Experimental design

Participants attended the laboratory on four separate occasions, at about the same time of day (±1-h), separated by at least 48-h but no more than 1 week. During visit 1, pulmonary function was assessed followed by a cycling ramp incremental test for determination of gas exchange threshold and $\dot{V}O_{2max}$. During visit 2, participants performed a 3MT which served as a familiarization trial. During visits 3 and 4, which were randomized, participants performed a 3MT without (hereafter termed CONT) and with prior voluntary hyperventilation (hereafter termed HYP).

Equipment and measurements

Pulmonary function was assessed according to ATS/ERS guidelines (40) using a pneumotachograph (Pneumotrac; Vitalograph, Buckinghm, UK) calibrated with a 3 L syringe. Exercise was performed on an electromagnetically braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands) that provides accurate measurement of power output up to a cadence of 180 rpm, which was not exceeded in the present study. For each participant 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, where T_{10-90} reflects the time taken for the analyzer output to change from 10% of the final value to 90% of the final value) relative to the volume signal. Heart rate was measured using short-range telemetry (Polar FT1; Polar Electro, Kempele, Finland). Arterial oxygen saturation (SpO_2) was estimated using a pulse oximeter (Model 8600; Nonin Medical, Plymouth, MN) and an adhesive forehead reflectance sensor (Model 8000R; Nonin Medical).

Fingertip capillary blood samples (70 μL) were collected into capillary tubes containing electrolyte balanced heparin (*safe*CLINITUBES, Radiometer, Copenhagen, Denmark) and analyzed immediately for [Hb], $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Cl^-]$, $[La^-]$, PCO_2, pH and $[HCO_3^-]$ (ABL90 FLEX; Radiometer). The $[H^+]$ was derived from pH as the antilog, and ion concentrations were corrected for hemoconcentration (41). The [SID] was calculated as the sum of the strong cations minus the sum of the strong anions: $[SID] = ([Na^+] + [K^+] + [Ca^{2+}]) - ([Cl^-] + [La^-])$ (1,10,16). Changes in blood volume from baseline were calculated from changes in [Hb] (10,42).

Protocols

Maximal incremental cycling ramp test. Participants performed 3-min of unloaded cycling followed by an incremental ramp protocol (30 $W \cdot min^{-1}$) until the limit of tolerance or task failure (cadence below 60 rpm). Participants maintained their preferred cadence throughout the test. The breath-by-breath pulmonary gas exchange data were reduced to 10-s rolling averages. The gas exchange threshold was determined using the V-slope method (43) and the $\dot{V}O_{2max}$ was taken as the highest 10-s mean value (10,12).

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 to ~120-130 rpm and then elicited maximum effort from the start of the 3MT. Participants then maintained their cadence as high as possible for the duration of the 3MT. The resistance to pedalling was set using the linear mode of the cycle ergometer so that, for each participant, the attainment of their preferred cadence (recorded during the incremental ramp test) corresponded to a power output halfway between their gas exchange threshold and $\dot{V}O_{2max}$. Verbal encouragement was provided, and participants were blinded from the elapsed time to prevent pacing. EP and end-test cadence were calculated as the mean power output and cadence over the last 30-s of the 3MT, and WEP was calculated as the power-time integral above EP (8–10). Similar to previous work (15), EP and WEP were taken as estimates of CP and W′ and used to predict the time taken (Tlim) to complete a range of total work done (W) targets (50, 75, 100, 125, 150, 175, and 200 kJ) using the equation: $Tlim = (W - W')/CP$.

CONT and HYP. A standardized 18-min period, during which participants were seated on the cycle ergometer, preceded the 3MT. During CONT, participants rested for 15-min before starting the 3-min unloaded cycling phase. During HYP, an initial 3-min rest period was followed by 15-min of voluntary hyperventilation, as previously described (24). Participants received real-time visual feedback of their end-tidal carbon dioxide pressure ($P_{ET}CO_2$) and

were instructed to progressively increase tidal volume (V_T) over 2-3 min to reduce $P_{ET}CO_2$ to 20 mmHg. Spontaneous breathing was resumed 3-s before the 3MT. An audio metronome controlled respiratory frequency (f_R) at 25 breaths·min^{-1}. During the last 3-min of voluntary hyperventilation, participants simultaneously performed the 3-min unloaded cycling phase. Breath-by-breath data were averaged into 10-s rolling averages. The O_2 cost of exercise was determined using the $\dot{V}O_2$ gain ($\dot{V}O_2$/power), and the $\dot{V}O_{2max}$ was taken as the highest 10-s rolling average (10,12). Heart rate and SpO_2 were measured via visual inspection at baseline, every 3-min during the subsequent 12-min, every minute during unloaded cycling, and every 30-s during the 3MT. Blood samples were collected at baseline, immediately before and after the 3MT, and after 5-min recovery.

Statistical analysis

Normality of the data was confirmed by the Shapiro-Wilk test. Differences in $\dot{V}O_{2max}$ between the incremental ramp test, CONT and HYP were evaluated using a one-way repeated measures ANOVA. Paired samples t-tests were used to evaluate between-trial differences in mean cardiorespiratory responses during the 15-min period preceding the 3MT, cadence at the end of unloaded cycling, peak power output and the corresponding cadence during the 3MT, WEP, EP, end-test cadence, and total work done. Since 95% of the WEP is accumulated over the first 90-s of the 3MT (44), the total work done was determined at 10-s intervals during the first 90-s of the 3MT (nine time points) and analyzed using a two-way (trial-time) repeated measures ANOVA. Ventilatory and pulmonary gas exchange responses (10-s time bins; 19 time points), $\dot{V}O_2$ gain (18 time points), heart rate and SpO_2 (both seven time points) during the 3MT were analyzed using a two-way (trial-time) repeated measures ANOVA. A two-way repeated measures ANOVA was also used to analyze blood parameters (trial-time) and predicted Tlim (trial-total work done target). Significant main effects and interactions were further explored using Bonferroni's multiple comparisons test to identify between-trial differences at each

measurement time point or total work done target. To control the family-wise error rate, *P*-values for multiple comparisons were adjusted for multiplicity (45). For ANOVA, effect sizes are given as partial eta-squared (η_p^2) and interpreted as small ($\eta_p^2 = 0.01$), medium ($\eta_p^2 = 0.06$) and large ($\eta_p^2 = 0.14$) (46). For paired comparisons, effect sizes are given as Cohen's d_z and interpreted as small ($d_z = 0.2$), medium ($d_z = 0.5$) and large ($d_z = 0.8$) (46). For correlation analyses, EP and WEP were normalized to body mass. The relationship between the difference in EP (ΔEP) between CONT and HYP and the difference in WEP (ΔWEP) between CONT and HYP was evaluated using Spearman's rank correlation coefficient (ρ). All other relationships were evaluated using Pearson's product moment correlation coefficient (*r*). 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

The gas exchange threshold occurred at 2.05 ± 0.64 L·min^{-1} (177 ± 56 W), $\dot{V}O_{2max}$ was 3.77 ± 0.81 L·min^{-1} (46 ± 8 mL·kg^{-1}·min^{-1}), and peak power output was 374 ± 67 W (4.6 ± 0.7 W·kg^{-1}).

Cardiorespiratory responses during the 15-min period preceding the 3MT

Table 1 summarizes the cardiorespiratory responses during the 15-min period preceding the 3MT. During the initial 12-min of this period (i.e. prior to unloaded cycling), $P_{ET}CO_2$ was lower, whereas $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$ and heart rate were higher in HYP compared to CONT. Except for heart rate, these differences persisted during unloaded cycling.

The 3MT

Cadence at the end of unloaded cycling in CONT (125 ± 13 rpm) and HYP (123 ± 16 rpm) was not different (t_{10} = 0.36, P = 0.723, d_z = 0.10). The power profiles during the 3MT of CONT and HYP are shown in Fig. 1A. There was no difference between CONT and HYP for peak power output (t_{10} = 1.37, P = 0.201, d_z = 0.41) (Fig. 2A) or the corresponding cadence (t_{10} = 1.12, P = 0.287, d_z = 0.35) (Fig. 2B). WEP was 10% higher in HYP than CONT (mean difference: 1.8 ± 1.8 kJ, 95% CI [0.7, 3.0 kJ]; t_{10} = 3.47, P = 0.006, d_z = 1.05) (Fig. 2C), whereas EP was 5% lower in HYP than CONT (mean difference: 13 ± 13 W, 95% CI [5, 22 W]; t_{10} = 3.42, P = 0.007, d_z = 1.09) (Fig. 2D). The end-test cadence was lower in HYP (81 ± 12 rpm) than CONT (83 ± 12 rpm) (mean difference: 2 ± 2 rpm, 95% CI [1, 3 rpm]; t_{10} = 3.88, P = 0.003, d_z = 1.17). WEP in CONT correlated positively with the peak [La^-] (r = 0.86, P = 0.001), and negatively with the lowest [HCO_3^-] (r = -0.72, P = 0.012). The EP in CONT correlated positively with the GET (r = 0.77, P = 0.006) and $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) ($r$ = 0.89, P < 0.001) determined during the preliminary incremental ramp test.

For total work done during the first 90-s of the 3MT, there was a trial-time interaction, with total work done being greater in HYP than CONT at all time points from 40-90-s (Fig. 1B). Total work done during the 3MT of CONT (64.5 ± 13.1 kJ) and HYP (63.7 ± 13.2) was not different (t_{10} = 1.20, P = 0.259, d_z = 0.38). For predicted Tlim, based on CP and W′ estimates derived in CONT and HYP, there was a main effect of trial ($F_{1,10}$ = 9.03, P = 0.013, η_p^2 = 0.73) and a trial-total work done target interaction ($F_{6,60}$ = 12.85, P < 0.001, η_p^2 = 0.56). The predicted Tlim was not different between CONT and HYP for fixed work targets of 50 kJ (CONT vs. HYP: 135 ± 50 vs. 135 ± 55 s, t_{60} = 0.13, P = 1.000, d_z = 0.05) and 75 kJ (241 ± 83 vs. 246 ± 88 s, t_{60} = 1.61, P = 0.796, d_z = 0.55). In contrast, Tlim was longer for HYP than CONT for fixed work targets of 100 kJ (346 ± 119 vs. 357 ± 126 s, t_{60} = 3.24, P = 0.014, d_z = 0.80), 125 kJ (452 ± 155 vs. 468 ± 164 s, t_{60} = 4.84, P < 0.001, d_z = 0.90), 150 kJ (557 ± 192

vs. 579 ± 203 s, t_{60} = 6.55, P < 0.001 d_z = 0.95), 175 kJ (663 ± 228 vs. 690 ± 242 s, t_{60} = 8.16, P < 0.001, d_z = 0.98), and 200 kJ (768 ± 265 vs. 802 ± 282 s, t_{60} = 9.84, P < 0.001, d_z = 1.00).

Cardiorespiratory responses during the 3MT

Pulmonary gas exchange and heart rate during the 3MT are shown in Figure 3. For $\dot{V}O_2$, there was a trial-time interaction effect, with $\dot{V}O_2$ being lower in HYP than CONT at the 20-s time point (t_{180} = 4.34, P < 0.001, d_z = 0.54). The $\dot{V}O_{2max}$ during the preliminary incremental ramp test and the 3MT of CONT (3.70 ± 0.57 L·min^{-1}) and HYP (3.81 ± 0.57 L·min^{-1}) was not different ($F_{2,20}$ = 0.61, P = 0.556, η_p^2 = 0.06). For $\dot{V}CO_2$, there was a main effect of trial and a trial-time interaction effect, with $\dot{V}CO_2$ being lower in HYP than CONT from 10-150-s (t_{180} = 3.11 – 14.29, P = <0.001 – 0.042, d_z = 0.51 – 2.34). For heart rate, there was a main effect of trial and a trial-time interaction effect, with heart rate being 10 beats·min^{-1} lower in HYP than CONT at 30-s (t_{60} = 4.93, P < 0.001, d_z = 0.72). SpO_2 (n = 10) declined from the start to the end of the 3MT (pooled data: 98 ± 2% vs 92 ± 4%) (main effect of time: $F_{6,54}$ = 17.94, P < 0.001, η_p^2 = 0.67), and changes were not different between CONT and HYP (main effect of trial: $F_{1,9}$ = 2.37, P = 0.158, η_p^2 = 0.21; trial-time interaction effect: $F_{6,54}$ = 0.13, P = 0.991, η_p^2 = 0.02). Compared to the start of the 3MT, a reduction in SpO_2 was first observed at the 1.5-min time point (t_{54} = 4.61, P < 0.001, d_z = 1.12).

For the $\dot{V}O_2$ gain (Fig. 4), there was a trial-time interaction effect. The $\dot{V}O_2$ gain was ~1.0-1.3 mL·min^{-1}·W^{-1} (8-10%) higher during HYP than CONT from 90-110-s (t_{170} = 3.20 – 3.38, P = 0.016 – 0.030, d_z = 0.77 – 1.01), and at 170-s (t_{170} = 4.00, P = 0.002, d_z = 0.86) and 180-s (t_{170} = 4.41, P < 0.001, d_z = 0.84).

Ventilatory responses during the 3MT

Ventilatory responses during the 3MT are shown in Figure 5. For $\dot{V}_E$, there was a trial-time interaction effect, with $\dot{V}_E$ being 30 L·min^{-1} higher at the start of the 3MT in HYP than CONT (t_{180} = 3.70, P < 0.001, d_z = 1.12). Thereafter, from 50-70-s $\dot{V}_E$ was ~19 L·min^{-1} lower in HYP than CONT (t_{180} = 3.39 – 4.19, P = <0.001 – 0.017, d_z = 0.97 – 1.68). The lower $\dot{V}_E$ was due to a lower V_T from 30-70-s (t_{180} = 3.17 – 5.46, P = <0.001 – 0.034, d_z = 0.54 – 1.00). For the remainder of the 3MT, $\dot{V}_E$ during CONT and HYP was similar (~161 L·min^{-1}), although a relative tachypnea (~5 breaths·min^{-1} higher f_R) was observed in HYP (main effect of trial). Due to $\dot{V}_E$ being higher at the start of the 3MT in HYP than CONT, $\dot{V}_E/\dot{V}O_2$ was also higher by ~22 (t_{180} = 12.53, P < 0.001, d_z = 1.83). The lower $\dot{V}CO_2$ in HYP than CONT resulted in $\dot{V}_E/\dot{V}CO_2$ being higher in HYP than CONT from 10-50-s (t_{180} = 4.01 – 14.73, P = <0.001 – 0.002, d_z = 1.63 – 2.86). By design, the 3MT in HYP commenced with $P_{ET}CO_2$ ~16 mmHg lower than CONT. During the 3MT of HYP, $P_{ET}CO_2$ gradually increased during the initial ~60-s, after which it remained ~3-5 mmHg lower than CONT (t_{180} = 4.30 – 22.88, P = <0.001 – 0.006, d_z = 1.47 – 3.53).

Blood volume and acid-base balance

Baseline [Hb] and acid-base variables were not different between CONT and HYP and therefore these data were pooled (Table 2). A comparable hemoconcentration was observed during the 3MT of CONT and HYP, which reflected a 7-8% fall in blood volume (Table 2). A one-way repeated measures ANOVA with Bonferroni's multiple comparisons test revealed that [La^-] during HYP increased by 1.1 mmol·L^{-1} from baseline to immediately before the 3MT (t_{10} = 5.79, P = 0.001, d_z = 1.75). This increase, along with reductions in [Ca^{2+}] and [K^+], resulted in the 3MT of HYP starting with a 3.3 mmol·L^{-1} lower [SID] than CONT (t_{30} = 2.69, P = 0.046, d_z = 1.17). Based on physicochemical principles, a reduction in [SID] (an independent variable) would, by itself, cause an increase in [H^+] and a reduction in [HCO_3^-] (dependent variables)

(1,16). However, such changes were offset by the ~15 mmHg reduction in PCO_2 during voluntary hyperventilation. This reduction in PCO_2 resulted in the 3MT of HYP commencing with a 12.9 nmol·L^{-1} lower [H^+] than CONT (t_{30} = 12.60, P < 0.001, d_z = 5.16). At the end of the 3MT, [La^-], [SID] and [H^+] were not different between CONT and HYP, whereas PCO_2 was 4.9 mmHg lower in HYP (t_{30} = 4.97, P < 0.001, d_z = 1.27).

ΔWEP and ΔEP correlates

The ΔWEP (J·kg^{-1}) between CONT and HYP was not correlated with the ΔEP (W·kg^{-1}) between CONT and HYP (ρ = -0.39, P = 0.235). The ΔWEP between CONT and HYP was positively correlated with, in HYP, the PCO_2 immediately before the 3MT (r = 0.77, P = 0.006) (Fig 6A), the Δ[H^+] from baseline to immediately before the 3MT (i.e. the reduction in [H^+] due to voluntary hyperventilation) (r = 0.63, P = 0.034) (Fig 6B), and the Δ[H^+] from immediately before the 3MT to 5-min after the 3MT (r = 0.72, P = 0.012) (Fig. 6C). The ΔEP between CONT and HYP was not correlated with any between-trial differences in acid-base balance immediately before the 3MT, $\dot{V}O_2$ at the 20-s time point, heart rate at the 30-s time point, or $\dot{V}O_2$ gain from 170-180-s.

DISCUSSION

Main findings

In agreement with our hypothesis, the main finding of the present study was that prior voluntary hyperventilation increased WEP, but decreased EP, during the 3MT. Although overall 3MT performance, i.e. total work done, was not affected by prior voluntary hyperventilation, the total work done from 40-90-s of the 3MT was greater in HYP than CONT. These novel results suggest that acid-base alterations caused by prior voluntary hyperventilation increase WEP, but decrease EP.

Effects of voluntary hyperventilation on baseline physiology

The voluntary hyperventilation protocol used in the present study mimicked that used previously (24) and caused a comparable reduction (~11.0 $nmol \cdot L^{-1}$) in blood $[H^+]$. The 1.1 $mmol \cdot L^{-1}$ increase in blood $[La^-]$ with voluntary hyperventilation also corroborates previous studies (28,32) and may be explained by reduced lactate clearance by active and inactive tissues (47) and/or stimulation of glycolysis in erythrocytes (48) and brain tissue (49). The effects of voluntary hyperventilation on resting intramuscular ion balance are largely unknown. In the isolated perfused rat hindlimb, respiratory alkalosis (perfusate PCO_2 = 26.6 mmHg, $[H^+]$ = 27.2 $nmol \cdot L$) increases intramuscular $[Na^+]$, $[Cl^-]$, and $[La^-]$ (31), whereas voluntary hyperventilation in humans ($P_{ET}CO_2$ = 17 mmHg) reduces intramuscular $[H^+]$ without affecting intramuscular $[La^-]$ (27,32). In the present study, it is estimated that the 11.0 $nmol \cdot L^{-1}$ reduction in blood $[H^+]$ with voluntary hyperventilation corresponded to a 20 $nmol \cdot L^{-1}$ reduction in intramuscular $[H^+]$ from an assumed baseline of 100 $nmol \cdot L^{-1}$ (50). The increased $\dot{V}O_2$ during voluntary hyperventilation can be attributed to O_2 utilisation by respiratory muscles (51) rather than resting skeletal muscle (27). Moreover, it is very unlikely that the work of breathing during voluntary hyperventilation was sufficient to cause respiratory muscle fatigue (52). Therefore, the increased WEP and reduced EP with prior voluntary hyperventilation are likely explained by changes in fatigability caused by alterations in acid-base balance.

Effects of prior voluntary hyperventilation on peak power output and WEP

Prior voluntary hyperventilation did not affect peak power output during the 3MT, which is consistent with previous studies (24,25,28,29). This is probably because hypocapnia and respiratory alkalosis do not affect baseline intramuscular [PCr] or maximal rates of PCr degradation (27,32). Conversely, the 10% increase in WEP with prior voluntary hyperventilation suggests that WEP is sensitive to changes in acid-base balance. Interestingly,

the 10% increase in WEP after prior voluntary hyperventilation is less than the 15% increase observed after sodium bicarbonate ingestion (21), although the latter is not a consistent finding (22). This is intriguing because in the present study the reduced baseline blood [H^+] with voluntary hyperventilation (-11.0 nmol·L^{-1}) was two-fold greater than after sodium bicarbonate ingestion (21). Moreover, voluntary hyperventilation, but not sodium bicarbonate ingestion, also reduces intramuscular [H^+] (20,27). Surprisingly, however, the ΔWEP between CONT and HYP correlated positively with the Δ[H^+] from baseline to immediately before the 3MT in HYP (Fig. 6B), i.e. the greater the reduction in [H^+] with voluntary hyperventilation, the smaller the improvement in WEP. This is obscure given the close association between acidosis and peripheral fatigue (13), the contribution of H^+ accumulation to group III/IV muscle afferent-mediated inhibition of motoneuronal output (13), and the view that reduced [H^+] is a primary mechanism by which sodium bicarbonate ingestion improves exercise tolerance (19). However, an important distinction is that voluntary hyperventilation, but not sodium bicarbonate ingestion, causes hypocapnia, which reduces cerebral blood flow (34,35). This may exacerbate central fatigue (38,39) and therefore moderate the positive effects of prior voluntary hyperventilation on power output and WEP. In support, the ΔWEP between CONT and HYP also correlated positively with the PCO_2 immediately before the 3MT in HYP (Fig. 6A), i.e. the lower the PCO_2 after voluntary hyperventilation, which due to physicochemical principles concomitantly reduces [H^+] (16), the smaller the improvement in WEP. Therefore, the extent to which prior voluntary hyperventilation increases WEP may be partly determined by the net effect of two opposing mechanisms, namely alkalosis (beneficial) and hypocapnia (detrimental). The interplay between the net effect of these opposing mechanisms and the task-specific nature of performance fatigability may explain some of the controversy surrounding the effects of voluntary hyperventilation on exercise performance (24–26,28–30).

Putative mechanisms underpinning the increase in WEP with prior voluntary hyperventilation

In the present study, $\dot{V}O_2$ was lower during HYP than CONT at the 20-s time point of the 3MT. This is consistent with previous studies showing that respiratory alkalosis increases the anaerobic contribution to exercise and reduces the aerobic contribution (23,27,29,32). Moreover, the lower $\dot{V}O_2$ during HYP than CONT was within the fundamental phase of the $\dot{V}O_2$ on-kinetics, which may suggest that $\dot{V}O_2$ kinetics were slower with prior voluntary hyperventilation. This is consistent with the findings of Chin et al. (33) who reported an increased $\tau\dot{V}O_2$ when voluntary hyperventilation was performed before and during moderate-intensity cycling exercise. Slower $\dot{V}O_2$ kinetics with prior voluntary hyperventilation may result, in part, from metabolic inertia due to delayed activation of the mitochondrial pyruvate dehydrogenase complex (32) and/or slower convective and diffusive oxygen delivery (23). The lower $\dot{V}O_2$ during HYP than CONT at 20-s was commensurate with a lower heart rate at 30-s, which may have indeed compromised convective oxygen delivery if not compensated by an increased cardiac stroke volume. Previous studies suggest that respiratory alkalosis may increase the anaerobic contribution to exercise by enhancing glycolytic flux (24,27,32), possibly due to greater stimulation of phosphofructokinase (32). A greater glycolytic flux due to prior voluntary hyperventilation may therefore explain, in part, why WEP and the total work done from 40-90-s of the 3MT were greater in HYP than CONT. However, the relationship between muscle [glycogen], glycolytic flux, fatigability, and WEP is complex and not fully understood. Indeed, it has been shown that although muscle [glycogen] falls by ~35% during the first 90-s of the 3MT, it is not different from baseline at the end of the 3MT (12). Moreover, a 22% reduction in WEP after 2-h of heavy intensity cycling exercise did not correlate with the reduction (~-65%) in baseline muscle [glycogen] (53). Therefore, power output and WEP during the 3MT are possibly not limited by anaerobic energy resupply (54), but by progressive

impairment of skeletal muscle function due to fatigue-inducing ionic perturbation (54). Accordingly, although prior voluntary hyperventilation may increase WEP partly by increasing glycolytic flux, this may be secondary to an attenuation of intramuscular ionic perturbation.

Although objective measurements of fatigue were not taken in the present study, fatigue during the all-out 3MT is manifest explicitly by the fall in power output. The greater work done from 40-90-s of the 3MT in HYP than CONT therefore suggests reduced fatigability with prior voluntary hyperventilation. Three observations support that prior voluntary hyperventilation may have attenuated intramuscular ionic perturbation during the first half of the 3MT: (I) the lower $\dot{V}O_2$ at the 20-s time point during HYP compared to CONT is consistent with prior voluntary hyperventilation affecting muscle bioenergetics (7); (II) the lower V_T (from 40-60-s) and corresponding lower heart rate (at 30-s) during HYP compared to CONT may be explained by reduced stimulation of metabolically sensitive skeletal muscle afferents due to less intramuscular metabolic / ionic perturbation (55,56); and (III) hypocapnia, *per se*, due to hypoxia-induced hyperventilation has been shown to attenuate peripheral fatigue during isometric knee extensor exercise (38). The specific mechanisms by which prior voluntary hyperventilation attenuates peripheral fatigue during exercise are uncertain but may include: (I) reduced baseline intramuscular $[H^+]$ that attenuates the temporal rise in intramuscular $[H^+]$; (II) increased La^- efflux from contracting muscle, which would reduce the rise in intramuscular $[La^-]$ (31) and thereby attenuate the fall in [SID] and concomitant rise in intramuscular $[H^+]$; and/or (III) reduced K^+ release (31), which would preserve membrane excitability (17) and attenuate the fall in [SID] and concomitant rise in intramuscular $[H^+]$. Moreover, the ΔWEP between CONT and HYP was positively correlated with the $\Delta[H^+]$ from immediately before to 5-min after the 3MT in HYP, which suggests that WEP is partly related to the capacity for H^+ accumulation.

Although the 3MT in HYP commenced with a lower blood [H^+] than CONT, the fall in SpO_2 during the 3MT, which was first observed at the 1.5-min time point, was not different between trials. This may suggest that prior voluntary hyperventilation did not affect the development of exercise-induced hypoxemia associated with an acidosis-mediated right shift in the oxyhemoglobin dissociation curve. However, the initial between-trial difference in blood [H^+] must have declined to zero during the 3MT given that [H^+] immediately after the 3MT was not different between trials, which may partly explain why the fall in SpO_2 during the second half of the 3MT was similar in CONT and HYP.

It could be argued that the increase in WEP with prior voluntary hyperventilation is a methodological artefact resulting from an inflated power-time integral due to the reduced EP. Interdependence between WEP and EP has been reported previously: a hypoxia-induced decrease in EP was inversely related to a concomitant increase in WEP (57), whereas a training-induced increase in EP was inversely related to a concomitant decrease in WEP (58). In contrast, acetaminophen ingestion increased EP without affecting WEP (15), whereas prior upper body exercise reduced EP without affecting WEP (10). Collectively, these studies suggest that the interdependence between WEP and EP may depend on the experimental intervention. In the present study, the greater total work done from 40-90-s of the 3MT in HYP than CONT, together with the lack of correlation between ΔEP and ΔWEP, suggests that the increase in WEP with prior voluntary hyperventilation was not a methodological artefact resulting exclusively from the decrease in EP. However, given the uncertainty regarding the mechanistic equivalence of WEP and W′ (1,10), it remains uncertain whether the increase in WEP with prior voluntary hyperventilation reflects, mechanistically, an increase in W′, which to resolve would require conventional determination of the power-duration relationship.

The effects of prior voluntary hyperventilation on EP

The present study is the first to examine the effects of prior voluntary hyperventilation on all-out exercise lasting >30-s. Interestingly, although prior voluntary hyperventilation increased WEP and the total work done over 40-90-s of the 3MT, this was at the expense of a reduced EP. The reduced EP during HYP offset the increase in WEP and, therefore, overall performance (i.e. total work done) was unaffected. Prior voluntary hyperventilation is the first acute intervention shown to increase WEP at the expense of EP. An explanation for why prior voluntary hyperventilation, but not sodium bicarbonate ingestion (21,22), reduces EP may reside in the detrimental effects of hypocapnia on cerebral blood flow and central fatigue (38,39). At rest, cerebral CO_2 reactivity (i.e. the percentage fall in cerebral blood flow per mmHg fall in arterial PCO_2) is 1-3% (59). Therefore, it is estimated that the ~15.2 mmHg reduction in PCO_2 during voluntary hyperventilation resulted in the 3MT of HYP commencing with an ~15-46% lower cerebral blood flow than CONT. This is similar to the 33-44% reduction in cerebral blood flow previously observed during voluntary hyperventilation with PCO_2 reduced to 20-28 mmHg (34,35). Moreover, PCO_2 is the primary regulator of cerebral perfusion during exercise (59), and exercise *per se* increases cerebral CO_2 reactivity to 4-5% (39). Therefore, it is estimated that the ~5 mmHg lower PCO_2 at the end of the 3MT of HYP compared to CONT corresponded to a 20-25% lower cerebral perfusion, which may have exacerbated central fatigue and contributed to the reduced EP. This notion is indirectly supported by the observation that f_R, which is modulated by fast inputs acting centrally (56), was higher during HYP than CONT.

Studies have shown that the conventionally determined critical power is inversely related to $\tau\dot{V}O_2$ (5,6), and that $\tau\dot{V}O_2$ is increased when voluntary hyperventilation is performed before and during moderate-intensity cycling exercise (33). Moreover, the data of Murgatroyd et al. (6) suggest that meaningful changes in CP (~10 W) can result from relatively small (~1-2 s) changes in $\tau\dot{V}O_2$. Therefore, if, compared to CONT, the lower $\dot{V}O_2$ at the 20-s time point

of the 3MT in HYP reflects slower $\dot{V}O_2$ kinetics with prior voluntary hyperventilation, this may have contributed to the reduced EP. Moreover, compared to CONT, the lower EP in HYP was also associated with a higher $\dot{V}O_2$ gain during the last 20-s of the 3MT. Given that a reduction in muscular efficiency is intrinsically linked to the mechanisms of muscle fatigue (7), the higher $\dot{V}O_2$ gain in HYP may have resulted from greater intramuscular metabolic and/or ionic perturbation towards the end of the 3MT. Although this notion remains speculative, Forbes et al. (27) used phosphorus magnetic resonance spectroscopy to examine changes in intracellular [H^+] and [Pi] during 6-min of moderate intensity plantar flexion exercise with voluntary hyperventilation performed before and during exercise. Compared to the control condition, voluntary hyperventilation resulted in a higher intracellular [H^+] and [Pi] in the last 2-3-min of exercise. It is therefore possible that, in the present study, prior voluntary hyperventilation exacerbated the intramuscular metabolic and/or ionic perturbation towards the end of the 3MT, which increased muscle fatigue and the $\dot{V}O_2$ gain, thereby lowering the EP.

Practical applications

In the present study, the total work done over 40-90-s of the 3MT was greater in HYP than CONT, which is consistent with a previous study reporting greater work done during a 30-s all-out Wingate test preceded by the same voluntary hyperventilation protocol (24). Since 95% of the WEP is accumulated over the first 90-s of the 3MT (44), our findings therefore suggest that an increase in WEP with prior voluntary hyperventilation may improve short-duration all-out exercise performance. However, the increase in WEP with prior voluntary hyperventilation was at the expense of a decrease in EP. This may have implications for severe-intensity exercise performance that depends on the interplay between critical power and W′, which depends on exercise intensity and duration (60). We therefore used estimates of critical power and W′ derived in CONT and HYP to predict time-trial performance, i.e. predicted Tlim for fixed work targets ranging from 50-200 kJ. Interestingly, whilst Tlim was not different between CONT

and HYP for fixed work targets of 50 kJ and 75 kJ (~2-4 min), Tlim was ~3-4% longer for HYP than CONT for fixed work targets ranging from 100-200 kJ (~6-13 min). Collectively, our findings suggest that the effects of prior voluntary hyperventilation on exercise performance may depend on exercise intensity and duration. However, further study should determine whether prior voluntary hyperventilation affects critical power and W′ determined conventionally using constant power exercise tests confined to the severe domain, which is important because changes in the power-duration parameters with some interventions may depend on the test protocol (1,10,58). Furthermore, the present study was undertaken on males and due to sex differences in fatigability (61) the results may not extend to females.

Lack of access to blood gas and $P_{ET}CO_2$ measurements may limit the use of prior voluntary hyperventilation in training and competition, although Leithäuser et al. (24) suggest that the protocol can be trained and individualized under controlled laboratory conditions and subsequently applied in the field. Moreover, hypocapnia may impair cognitive function (62) and induce paraesthesia and tetany (63), which may be undesirable in some circumstances. Careful consideration of the task specific determinants of performance fatigability, along with the potential side-effects, is therefore essential to establish the likelihood that prior voluntary hyperventilation will improve exercise performance.

Conclusion

In summary, the present study demonstrates that voluntary hyperventilation prior to the all-out 3MT increases WEP, but reduces EP. Although the increase in WEP may improve short-duration (≤ 90-s) all-out exercise performance, the reduced EP may reduce severe-intensity exercise performance. The mechanisms by which prior voluntary hyperventilation affect WEP and EP remain unknown but may be mediated by the degree of hypocapnia incurred along with changes in muscle bioenergetics and fatigue etiology.

Acknowledgements

Funding disclosures: None

Author conflicts of interest: 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. 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.

2. 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.

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. Oxygen uptake kinetics as a determinant of sports performance. *Eur J Sport Sci*. 2007;7(2):63–79.

5. Goulding RP, Roche DM, Marwood S. Effect of hyperoxia on critical power and VO_2 kinetics during upright cycling. *Med Sci Sports Exerc*. 2020;52(5):1041–9.

6. Murgatroyd SR, Ferguson C, Ward SA, Whipp BJ, Rossiter HB. Pulmonary O_2 uptake kinetics as a determinant of high-intensity exercise tolerance in humans. *J Appl Physiol*. 2011;110(6):1598–606.

7. 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.

8. 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.

9. Burnley M, Doust JH, Vanhatalo A. A 3-min all-out test to determine peak oxygen uptake and the maximal steady state. *Med Sci Sports Exerc*. 2006;38(11):1995–2003.

10. Johnson MA, Williams NC, Graham AW, Ingram LAL, Cooper SB, Sharpe GR. Effects of prior upper body exercise on the 3-min all-out cycling test in men. *Med Sci Sports Exerc*. 2020;52(11):2402-11.

11. 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.

12. Vanhatalo A, Black MI, DiMenna FJ, et al. The mechanistic bases of the power-time relationship: muscle metabolic responses and relationships to muscle fibre type. *J Physiol*. 2016;594(15):4407–23.

13. Blain GM, Mangum TS, Sidhu SK, et al. Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *J Physiol*. 2016;594(18):5303–15.

14. Curtelin D, Morales-Alamo D, Torres-Peralta R, et al. Cerebral blood flow, frontal lobe oxygenation and intra-arterial blood pressure during sprint exercise in normoxia and severe acute hypoxia in humans. *J Cereb Blood Flow Metab*. 2018;38(1):136–50.

15. Morgan PT, Vanhatalo A, Bowtell JL, Jones AM, Bailey SJ. Acetaminophen ingestion improves muscle activation and performance during a 3-min all-out cycling test. *Appl Physiol Nutr Metab*. 2019;44(4):434–42.

16. 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.

17. Lindinger MI, McKelvie RS, Heigenhauser GJ. K^+ and Lac^- distribution in humans during and after high-intensity exercise: role in muscle fatigue attenuation? *J Appl Physiol*. 1995;78(3):765–77.

18. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports Med*. 2011;41(10):801–14.

19. Lancha Junior AH, Painelli V de S, Saunders B, Artioli GG. Nutritional strategies to modulate intracellular and extracellular buffering capacity during high-intensity exercise. *Sports Med*. 2015;45 Suppl 1:S71-81.

20. Raymer GH, Marsh GD, Kowalchuk JM, Thompson RT. Metabolic effects of induced alkalosis during progressive forearm exercise to fatigue. *J Appl Physiol*. 2004;96(6):2050–6.

21. Deb SK, Gough LA, Sparks SA, McNaughton LR. Determinants of curvature constant (W′) of the power duration relationship under normoxia and hypoxia: the effect of pre-exercise alkalosis. *Eur J Appl Physiol*. 2017;117(5):901–12.

22. Vanhatalo A, McNaughton LR, Siegler J, Jones AM. Effect of induced alkalosis on the power-duration relationship of "all-out" exercise. *Med Sci Sports Exerc*. 2010;42(3):563–70.

23. Chin LMK, Heigenhauser GJF, Paterson DH, Kowalchuk JM. Effect of voluntary hyperventilation with supplemental CO_2 on pulmonary O_2 uptake and leg blood flow kinetics during moderate-intensity exercise. *Exp Physiol*. 2013;98(12):1668–82.

24. Leithäuser RM, Böning D, Hütler M, Beneke R. Enhancement on Wingate anaerobic test performance with hyperventilation. *Int J Sports Physiol Perform*. 2016;11(7):627–34.

25. Sakamoto A, Naito H, Chow C-M. Hyperventilation as a strategy for improved repeated sprint performance. *J Strength Cond Res*. 2014;28(4):1119–26.

26. Sakamoto A, Naito H, Chow CM. Hyperventilation-induced respiratory alkalosis falls short of countering fatigue during repeated maximal isokinetic contractions. *Eur J Appl Physiol*. 2015;115(7):1453–65.

27. Forbes SC, Kowalchuk JM, Thompson RT, Marsh GD. Effects of hyperventilation on phosphocreatine kinetics and muscle deoxygenation during moderate-intensity plantar flexion exercise. *J Appl Physiol.* 2007;102(4):1565–73.

28. Fujii N, Tsuchiya S-I, Tsuji B, Watanabe K, Sasaki Y, Nishiyasu T. Effect of voluntary hypocapnic hyperventilation on the metabolic response during Wingate anaerobic test. *Eur J Appl Physiol.* 2015;115(9):1967–74.

29. Dobashi K, Fujii N, Watanabe K, et al. Effect of voluntary hypocapnic hyperventilation or moderate hypoxia on metabolic and heart rate responses during high-intensity intermittent exercise. *Eur J Appl Physiol*. 2017;117(8):1573–83.

30. Hilbert M, Shushakov V, Maassen N. The influence of respiratory acid-base changes on muscle performance and excitability of the sarcolemma during strenuous intermittent hand grip exercise. *J Appl Physiol*. 2012;112(4):571–9.

31. Lindinger MI, Heigenhauser GJ, Spriet LL. Effects of alkalosis on muscle ions at rest and with intense exercise. *Can J Physiol Pharmacol*. 1990;68(7):820–9.

32. LeBlanc PJ, Parolin ML, Jones NL, Heigenhauser GJF. Effects of respiratory alkalosis on human skeletal muscle metabolism at the onset of submaximal exercise. *J Physiol*. 2002;544(Pt 1):303–13.

33. Chin LMK, Leigh RJ, Heigenhauser GJF, Rossiter HB, Paterson DH, Kowalchuk JM. Hyperventilation-induced hypocapnic alkalosis slows the adaptation of pulmonary O_2 uptake during the transition to moderate-intensity exercise. *J Physiol*. 2007;583(Pt 1):351–64.

34. Kety SS, Schmidt CF. The effects of active and passive hyperventilation on cerebral blood flow, cerebral oxygen consumption, cardiac output, and blood pressure of normal young men. *J Clin Invest*. 1946;25(1):107–19.

35. Willie CK, Macleod DB, Shaw AD, et al. Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol*. 2012;590(14):3261–75.

36. Hartley GL, Watson CL, Ainslie PN, et al. Corticospinal excitability is associated with hypocapnia but not changes in cerebral blood flow. *J Physiol*. 2016;594(12):3423–37.

37. Ross EZ, Cotter JD, Wilson L, Fan J-L, Lucas SJE, Ainslie PN. Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *J Appl Physiol.* 2012;112(5):748–58.

38. Rupp T, Mallouf TLR, Perrey S, Wuyam B, Millet GY, Verges S. CO_2 clamping, peripheral and central fatigue during hypoxic knee extensions in men. *Med Sci Sports Exerc*. 2015;47(12):2513–24.

39. Nybo L, Rasmussen P. Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev*. 2007;35(3):110–8.

40. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J.* 2005;26(2):319–38.

41. Matomäki P, Kainulainen H, Kyröläinen H. Corrected whole blood biomarkers - the equation of Dill and Costill revisited. *Physiol Rep*. 2018;6(12):e13749.

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

43. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*. 1986;60(6):2020–7.

44. Vanhatalo A, Doust JH, Burnley M. Robustness of a 3 min all-out cycling test to manipulations of power profile and cadence in humans. *Exp Physiol*. 2008;93(3):383–90.

45. Wright SP. Adjusted P-values for simultaneous inference. *Biometrics*. 1992;48(4):1005–13.

46. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988.

47. Druml W, Grimm G, Laggner AN, Lenz K, Schneeweiss B. Lactic acid kinetics in respiratory alkalosis. *Crit Care Med*. 1991;19(9):1120–4.

48. Zborowska-Sluis DT, Dossetor JB. Hyperlactatemia of hyperventilation. *J Appl Physiol*. 1967;22(4):746–55.

49. Kazemi H, Valenca LM, Shannon DC. Brain and cerebrospinal fluid lactate concentration in respiratory acidosis and alkalosis. *Respir Physiol*. 1969;6(2):178–86.

50. Heisler N. Intracellular pH of isolated rat diaphragm muscle with metabolic and respiratory changes of extracellular pH. *Respir Physiol*. 1975;23(2):243–55.

51. Dominelli PB, Render JN, Molgat-Seon Y, Foster GE, Romer LM, Sheel AW. Oxygen cost of exercise hyperpnoea is greater in women compared with men. *J Physiol*. 2015;593(8):1965–79.

52. Babcock MA, Pegelow DF, McClaran SR, Suman OE, Dempsey JA. Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue. *J Appl Physiol*. 1995;78(5):1710–9.

53. Clark IE, Vanhatalo A, Thompson C, et al. 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.

54. Bundle MW, Weyand PG. Sprint exercise performance: does metabolic power matter? *Exerc Sport Sci Rev*. 2012;40(3):174–82.

55. Fisher JP. Autonomic control of the heart during exercise in humans: role of skeletal muscle afferents. *Exp Physiol*. 2014;99(2):300–5.

56. Nicolò A, Girardi M, Bazzucchi I, Felici F, Sacchetti M. Respiratory frequency and tidal volume during exercise: differential control and unbalanced interdependence. *Physiol Rep*. 2018;6(21):e13908.

57. 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.

58. 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.

59. Smith KJ, Ainslie PN. Regulation of cerebral blood flow and metabolism during exercise. *Exp Physiol*. 2017;102(11):1356–71.

60. Jones AM, Vanhatalo A, Burnley M, Morton RH, Poole DC. Critical power: implications for determination of VO_{2max} and exercise tolerance. *Med Sci Sports Exerc*. 2010;42(10):1876–90.

61. Ansdell P, Škarabot J, Atkinson E, et al. Sex differences in fatigability following exercise normalised to the power-duration relationship. *J Physiol*. 2020;598(24):5717-37.

62. Friend AT, Balanos GM, Lucas SJE. Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function. *Exp Physiol*. 2019;104(10):1482–93.

63. Macefield G, Burke D. Paraesthesiae and tetany induced by voluntary hyperventilation. Increased excitability of human cutaneous and motor axons. *Brain*. 1991;114 (Pt 1B):527–40.

703 **TABLE 1** Cardiorespiratory responses during the 15-min period preceding the 3MT. Values
704 are mean ± SD.

	0-12-min		12-15-min (unloaded cycling)	
	CONT	HYP	CONT	HYP
$\dot{V}_E$ (L·min^{-1})	12 ± 4	38 ± 5**	25 ± 8	59 ± 14**
V_T (L)	0.78 ± 0.16	1.56 ± 0.19**	1.38 ± 0.38	2.39 ± 0.55**
f_R (breaths·min^{-1})	15 ± 4	24 ± 1**	18 ± 4	24 ± 1**
$\dot{V}O_2$ (L·min^{-1})	0.38 ± 0.11	0.52 ± 0.10**	0.94 ± 0.25	1.11 ± 0.28*
$P_{ET}CO_2$ (mmHg)	35 ± 2	21 ± 1**	39 ± 1	21 ± 1**
Heart rate (beats·min^{-1})	80 ± 16	101 ± 17**	98 ± 20	100 ± 17

705 $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, respiratory frequency; $\dot{V}O_2$, pulmonary oxygen
706 uptake; $P_{ET}CO_2$, end-tidal CO_2. Different from CONT: *P < 0.050, **P < 0.010.

707

708

709 **TABLE 2** [Hb], changes in blood volume (ΔBV) from baseline, and blood acid-base variables.
710 Values are mean ± SD.

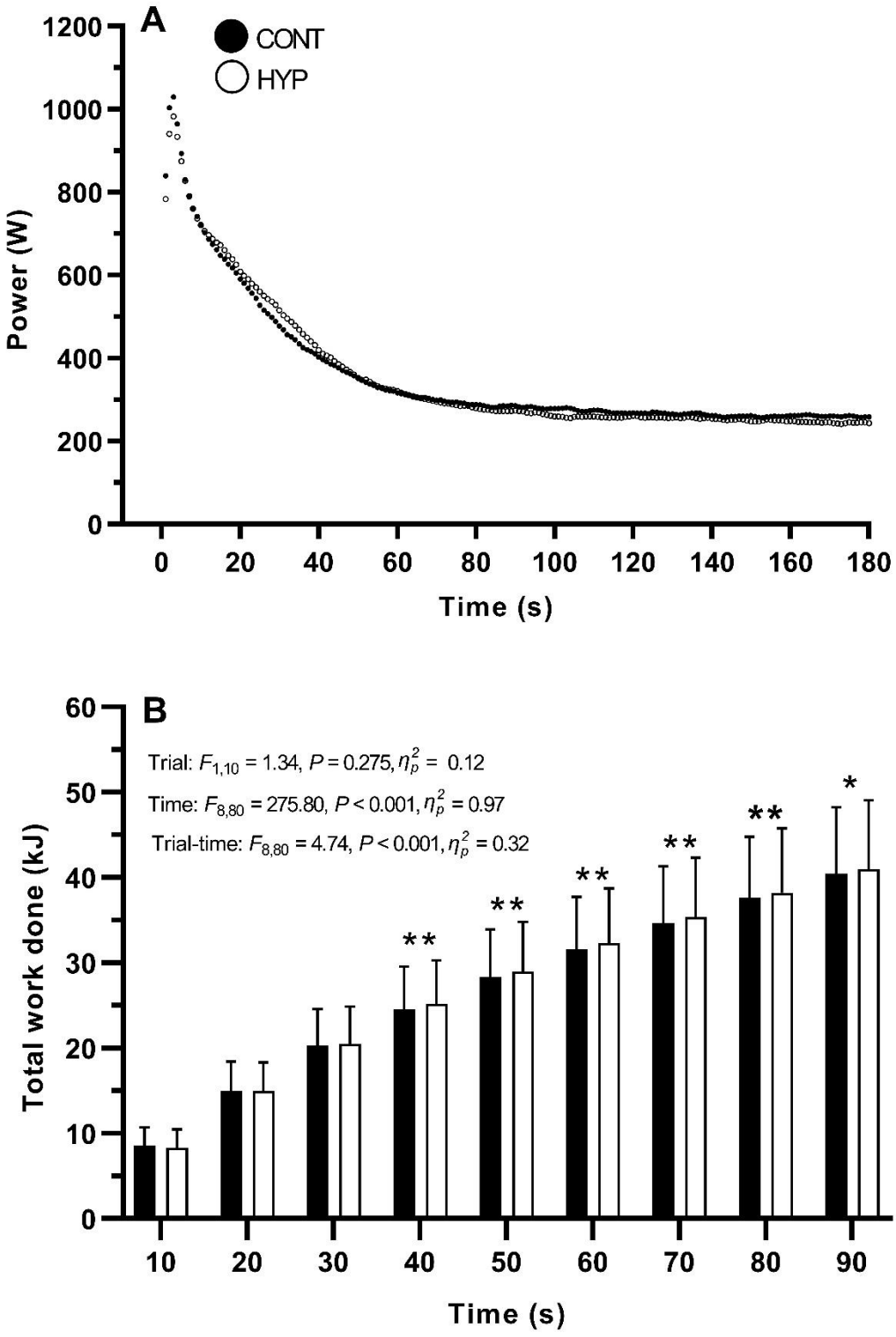
		Immediately before 3MT		Immediately after 3MT		5-min recovery	
	Baseline	CONT	HYP	CONT	HYP	CONT	HYP
[Hb] (g·dL^{-1})[b]	16.1 ± 1.0	16.1 ± 1.1	16.4 ± 1.1	17.4 ± 1.1	17.6 ± 1.1	17.2 ± 1.5	17.3 ± 1.1
ΔBV (%)		-0.1 ± 3.3	-1.6 ± 3.1	-7.2 ± 3.2	-8.2 ± 3.5	-6.2 ± 4.9	-6.5 ± 3.2
[La$^-$] (mmol·L^{-1})[a,b]	0.9 ± 0.7	1.2 ± 0.3	1.9 ± 0.7	15.7 ± 3.2	16.9 ± 3.4	18.2 ± 3.5	18.9 ± 3.8
[Na^+] (mmol·L^{-1})[b]	140 ± 1	140 ± 5	138 ± 4	137 ± 5	136 ± 6	143 ± 6	144 ± 5
[K^+] (mmol·L^{-1})[a,b]	4.2 ± 0.3	4.3 ± 0.3	4.0 ± 0.1*	5.1 ± 0.7	4.9 ± 0.6	3.7 ± 0.3	3.5 ± 0.2
[Ca^{2+}] (mmol·L^{-1})[c]	1.20 ± 0.03	1.22 ± 0.05	1.16 ± 0.04**	1.22 ± 0.04	1.19 ± 0.06	1.19 ± 0.05	1.18 ± 0.04
[Cl^-] (mmol·L^{-1})[b]	106 ± 1	105 ± 1	105 ± 4	101 ± 3	100 ± 4	100 ± 4	101 ± 8
Independent acid-base variables							
[SID] (mmol·L^{-1})[a,b]	39.3 ± 1.7	40.2 ± 2.6	36.8 ± 1.3*	26.8 ± 3.5	25.1 ± 3.3	29.9 ± 3.7	28.3 ± 6.7
PCO_2 (mmHg)[a,b,c]	40.4 ± 2.8	43.2 ± 2.0	25.2 ± 3.0**	40.6 ± 5.0	35.7 ± 5.4**	30.9 ± 2.6	28.6 ± 3.6
Dependent acid-base variables							
[H^+] (nmol·L^{-1})[a,b,c]	38.7 ± 1.9	40.0 ± 1.5	27.1 ± 2.6**	64.6 ± 7.2	64.1 ± 8.6	72.6 ± 10.8	74.8 ± 15.3
[HCO_3^-] (mmol·L^{-1})[b]	25.5 ± 1.0	25.4 ± 1.4	25.7 ± 1.2	13.3 ± 1.8	12.4 ± 1.7	11.2 ± 2.2	10.7 ± 2.0

711 [a] Main effect of trial (P = <0.001 – 0.049, η_p^2 = 0.33 – 0.95).

712 [b] Main effect of time (P < 0.001, η_p^2 = 0.56 – 0.98).

713 [c] Trial-time interaction effect (P = <0.001 – 0.033, η_p^2 = 0.25 – 0.88).

714 Different from equivalent CONT value: *P < 0.050, **P < 0.010.



715

716 **FIGURE 1 –** Power profiles (A) and total work done at 10-s intervals during the first 90-s of
717 the 3MT in CONT (filled bars) and HYP (open bars) (B). Data in A are mean with error bars
718 omitted to enhance clarity. Data in B are mean ± SD. Difference between trials: *P < 0.050,
719 **P < 0.010.

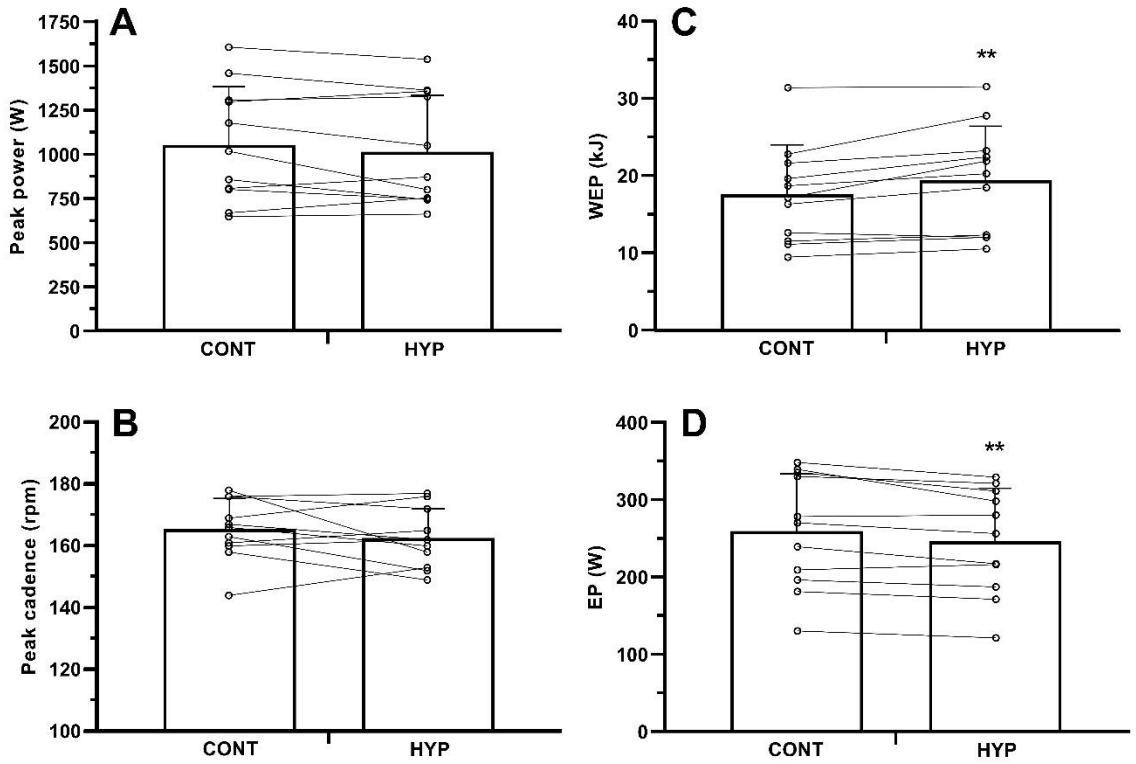
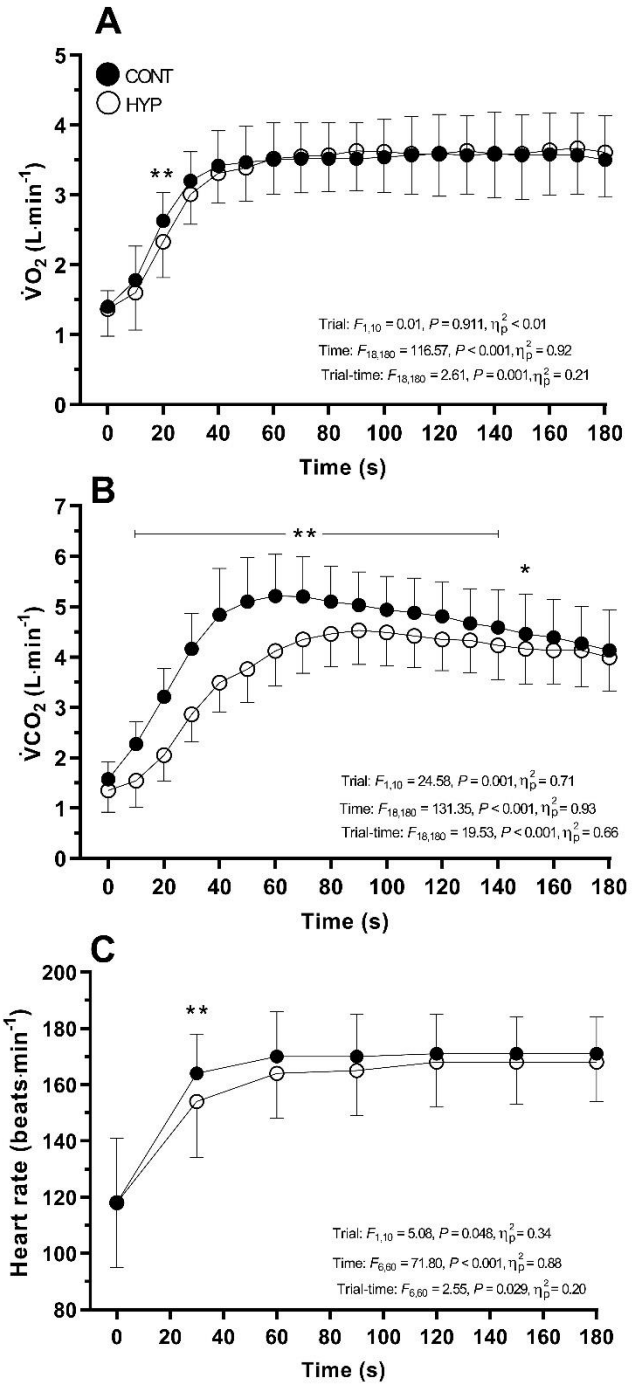


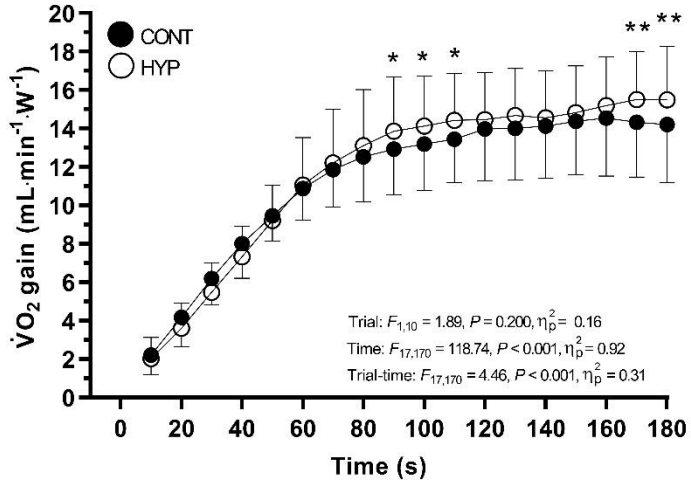
FIGURE 2 – Peak power output (A), peak cadence (B), work done above end-test power output (WEP) (C) and end-test power output (EP) (D) during the 3MT of CONT and HYP. Data are mean ± SD, with lines representing individual participants. Difference from CONT: $^{**}P < 0.010$.



738

739 **FIGURE 3 –** Pulmonary oxygen uptake ($\dot{V}O_2$) (A), carbon dioxide production ($\dot{V}CO_2$) (B)
740 and heart rate (C) during the 3MT. Data are mean ± SD. Difference between trials: *P < 0.050,
741 **P < 0.010. Capped line with asterisks denotes the range of individual 10-s time-bins at which
742 a difference exists between CONT and HYP.

743



744

745 **FIGURE 4** – Pulmonary oxygen uptake ($\dot{V}O_2$) gain during the 3MT. Data are mean ± SD.
746 Difference between trials (*P < 0.050, **P < 0.010).

747

748

749

750

751

752

753

754

755

756

757

758

759

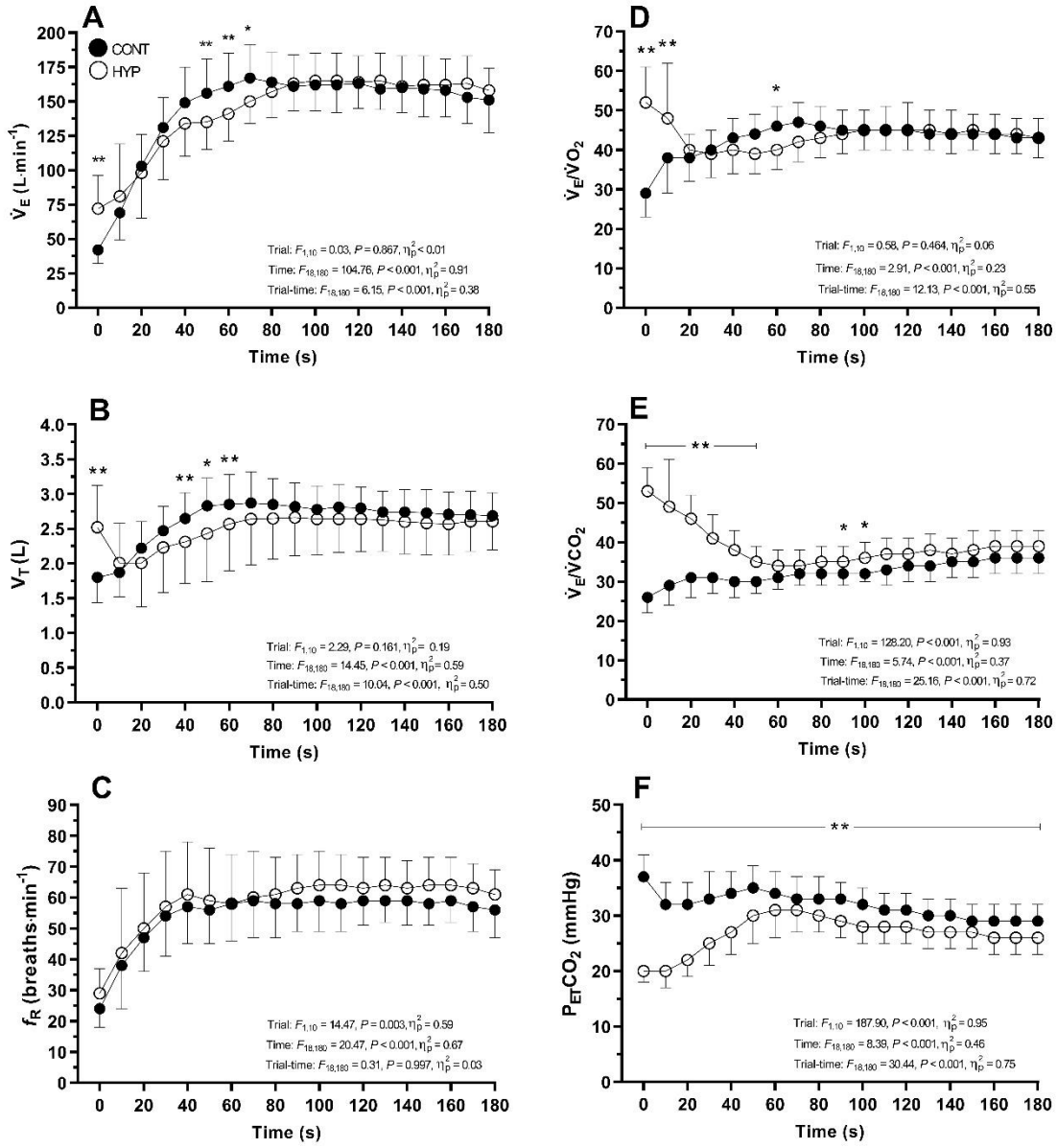
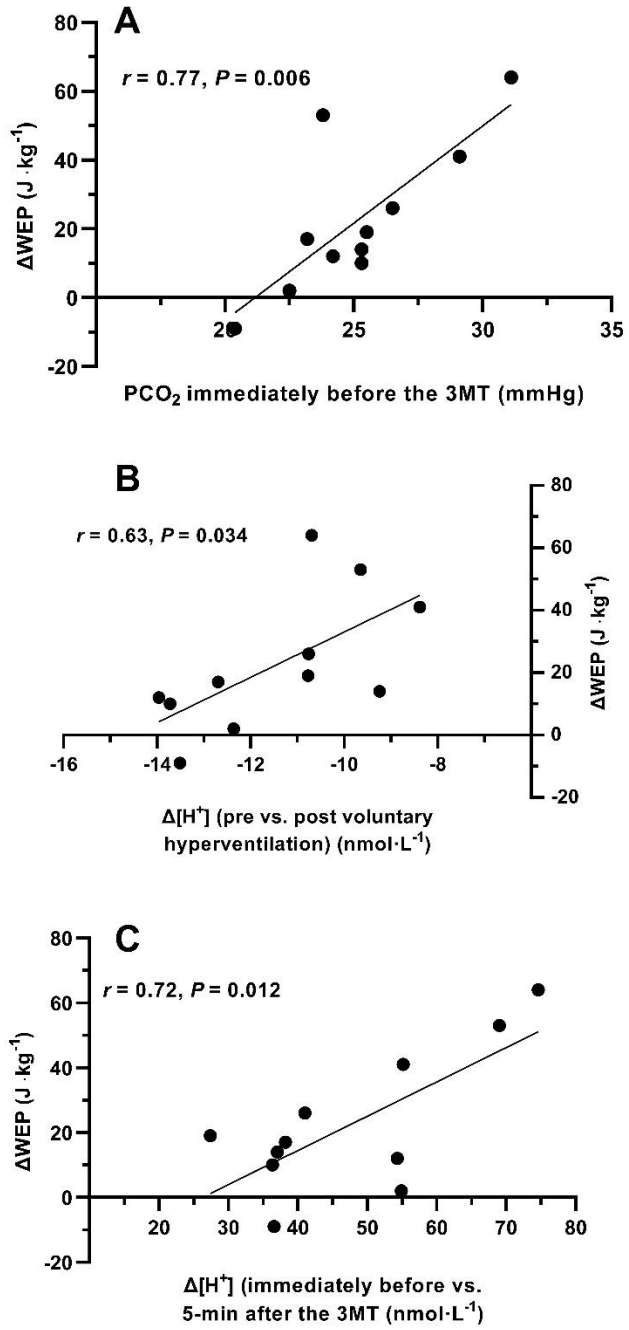


FIGURE 5 – Minute ventilation ($\dot{V}_E$) (A), tidal volume (V_T) (B), respiratory frequency (f_R) (C), ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}O_2$) (D) and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) (E), and end-tidal CO_2 ($P_{ET}CO_2$) (F) during the 3MT. Data are mean ± SD. Difference between trials (*P < 0.05, **P < 0.01). Capped line with asterisks denotes the range of individual 10-s time-bins at which a difference exists between CONT and HYP.



772

773 **FIGURE 6** – Correlations between the difference in WEP (ΔWEP) (normalized to body mass)
774 between CONT and HYP and the blood PCO_2 measured immediately before the 3MT in HYP
775 (A), the $\Delta[H^+]$ from baseline to immediately before the 3MT in HYP (B), and the $\Delta[H^+]$ from
776 immediately before the 3MT to 5-min recovery in HYP (C).

777