Changes in the power-duration relationship after prolonged endurance exercise: estimation using conventional and all-out test procedures and relationship to muscle glycogen

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ABSTRACT

It is not clear how the parameters of the power-duration relationship (critical power (CP) and W′) are influenced by the performance of prolonged endurance exercise. We used severe-intensity prediction trials (conventional protocol) and the 3-min all-out test (3MT) to measure CP and W′ following 2 h of heavy-intensity cycling exercise and took muscle biopsies to investigate possible relationships with changes in muscle glycogen concentration ([glycogen]). Fourteen participants completed a rested 3MT to establish end-test power (Control-EP) and work done above EP (Control-WEP). Subsequently, on separate days, immediately following 2 h of heavy-intensity exercise, participants completed a 3MT to establish Fatigued-EP and Fatigued-WEP and three severe-intensity prediction trials to the limit of tolerance (T_{lim}) to establish Fatigued-CP and Fatigued-W'. A muscle biopsy was collected immediately before and after one of the 2-h exercise bouts. Fatigued-CP (256 ± 41 W) and Fatigued-EP (256 ± 52 W), and Fatigued-W′ (15.3 ± 5.0 kJ) and Fatigued-WEP (14.6 ± 5.3 kJ), were not different (P>0.05), but were ~11% and ~20% lower than Control-EP (287 ± 46 W) and Control-WEP (18.7 ± 4.7 kJ), respectively (P<0.05). The change in muscle [glycogen] was not significantly correlated with the changes in either EP (r = 0.19) or WEP (r = 0.07). The power-duration relationship is substantially impacted by prolonged endurance exercise. The 3MT provides valid estimates of CP and W′ following 2 h of heavy-intensity exercise but the changes in these parameters are not primarily determined by changes in muscle [glycogen].

Key words: CRITICAL POWER, FATIGUE, PERFORMANCE, METABOLISM

Introduction

The power-asymptote of the hyperbolic power-duration relationship, critical power (CP), separates the 'severe' from the 'heavy' exercise intensity domains (22, 25, 32). During exercise performed within the heavy-intensity domain (<CP), a steady-state in oxygen uptake ($\dot{V}O_2$) can be obtained and this is accompanied by stable muscle [phosphocreatine] ([PCr]), pH, [lactate] and [inorganic phosphate] ([P_i]) responses (where square brackets denote concentration), (4, 23, 32, 36). In contrast, during exercise performed within the severe-intensity domain (>CP), the development of a $\dot{V}O_2$ 'slow component' results in the attainment of maximal oxygen uptake ($\dot{V}O_{2max}$), muscle [PCr], pH, [lactate] and [P_i] exhibit non-steady state profiles (4, 23, 36), and exercise tolerance is correspondingly limited (32). The amount of work that can be performed >CP before the limit of tolerance (T_{lim}) is represented by the curvature constant (W′) of the power-duration relationship with T_{lim} being reached when W′ is fully expended (i.e. W′ = 0 kJ; 11, 25). Knowledge of CP and W′ permits accurate prediction of performance for various distances and durations of exercise (21, 22, 40).

CP and W′ are conventionally estimated by measuring T_{lim} during a series (~3-4) of constant-power (P), severe-intensity prediction trials performed on separate days, and modelling the power-duration relationship (17). Alternatively, CP and W′ can be estimated from a single 3-min all-out cycle ergometer test against fixed resistance (3MT) where, provided that $\dot{V}O_{2max}$ is attained, the mean power output over the last 30 s of the test (end-test power; EP) reflects the CP and the work done above EP (WEP) reflects the W′ (28, 37, 38). We (8) have previously shown that EP and WEP derived from the 3MT decreased by 8% and 20%, respectively, after 2 h of heavy-intensity exercise. These effects would be expected to have significant implications for performance during events lasting ≥2 h (20), and also for the prediction of such performance from exercise tests conducted in a fresh state, i.e. without

preceding fatiguing exercise (8). However, while the EP and WEP provide valid and reliable estimates of the CP and W′ when exercise tests are commenced from a rested baseline (5, 28, 37, 38; cf. 26), it is not known whether this close agreement between the parameter estimates derived from the two different protocols is maintained following the performance of long-duration endurance exercise. It is possible, for example, that the parameters of the power-duration relationship as derived from the conventional protocol (continuous constant-power prediction trials to T_{lim} of ~2-15 min duration) and the 3MT protocol (all-out exercise for 3 min) are affected differentially by factors related to the development of fatigue during long-duration endurance exercise.

It is well established that fatigue during prolonged exercise at intensities equivalent to 70-75% of $\dot{V}O_{2max}$ is associated with the attainment of low muscle [glycogen] (9, 16). It is therefore possible that the reductions of EP and WEP measured in a fatigued compared to a rested state (8) are related to changes in muscle [glycogen]. Consistent with this, it has been reported that W′ is reduced by ~20% when glycogen stores are depleted by dietary carbohydrate restriction (24). Given that CP reflects the highest sustainable oxidative metabolic rate (23, 32, 36), it is possible that the impaired endurance performance associated with glycogen depletion is reflected in a reduced CP, but this has not been formally investigated. Resolving whether a change in the power-duration relationship following 2 h of heavy-intensity exercise is related to muscle glycogen depletion would not only provide novel mechanistic insight into this phenomenon but might also inform strategies to modulate the performance impact of long-duration endurance exercise.

The purpose of this study was to determine CP and W′ derived from the conventional protocol following 2 h of heavy-intensity exercise, assess the level of agreement with EP and WEP derived from the 3MT, and evaluate the relationships between muscle glycogen depletion and changes in EP and WEP. We hypothesized that, following 2 h of heavy-

intensity exercise: 1) 'Fatigued' CP and W′ (Fatigued-CP and Fatigued-W′) estimated using the conventional protocol would be significantly lower compared to the values estimated without the performance of prior exercise; 2) Fatigued-CP and Fatigued-W′ estimated using the conventional protocol would not be different from the Fatigued-EP and Fatigued-WEP estimated using the 3MT; and, 3) the reductions in EP and WEP would be correlated with the reduction in muscle [glycogen].

Methods

Fourteen male participants (mean ± SD: age, 31 ± 10 years; height, 1.79 ± 0.06 m; body mass, 79.2 ± 6.5 kg; $\dot{V}O_{2peak}$, 54.7 ± 5.4 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered to take part in the study. The study procedures were approved by the Institutional Research Ethics Committee and participants provided written informed consent prior to participation. All exercise tests were separated by a minimum of 24 h and the >2-h exercise bouts were separated by at least 72 h. Participants were instructed to avoid alcoholic drinks and strenuous exercise 24 h prior to testing. Participants completed a diet and exercise diary 48 h prior to their first visit. These diaries were photocopied and participants were instructed to repeat the reported dietary and exercise behavior prior to each subsequent visit.

Experimental procedures

All exercise tests were conducted using the same electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). During all tests, except the 3MT, the participants cycled at a self-selected cadence. Cadence was not controlled during the 2-h exercise tests but participants were asked to maintain it to within ± 5 rpm during subsequent constant-power output tests. The ergometer seat and handlebars were adjusted for comfort during the first visit and settings were recorded and replicated for all subsequent visits. Participants attended

the laboratory on eight occasions. During the first visit, participants performed a 30W/min ramp incremental exercise test for the determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET). Initially, participants performed 3 min of 'unloaded' baseline cycling, after which the power output was increased by 30 W/min until T_{lim}, which was recorded once the cadence fell by >5 rpm below the participant's self-selected cadence. $\dot{V}O_{2peak}$ was determined as the highest 30-s rolling mean measured during the ramp incremental test. The GET was estimated using the methods described by Beaver et al. (1). $\dot{V}O_{2peak}$ and GET were used to calculate the resistance for the 3MTs and to normalize the power output during the 2-h heavy-intensity exercise bouts. The fixed resistance for the 3MTs was calculated using the equation: linear factor = power/(preferred cadence)^2 where power output was 50%Δ (i.e., GET plus 50% of the difference between the power outputs at GET and $\dot{V}O_{2peak}$) and preferred cadence was the cadence selected (rpm) during the ramp incremental test. The linear factor was 0.040 ± 0.005 (range 0.035 - 0.050) W/rpm^2. The linear factor ensures that a particular cadence will produce a known power output. In the calculation of power outputs to be used during exercise tests, account was taken of the lag in $\dot{V}O_2$ relative to power output during ramp exercise (42).

On visits 2 (familiarization) and 3 (control visit), a single 3MT was completed. Participants started by performing a 3-min 'unloaded' baseline period. Then, 5 s before the all-out sprint commenced, the participants were asked to increase cadence to 110-120 rpm. For the entirety of the 3MT, participants were asked to cycle as quickly as possible. Strong verbal encouragement was given throughout the test but no information was provided on time elapsed. Control-EP was estimated as the mean power output over the last 30 s of the test and Control-WEP was defined as the work done above EP (22, 37). During the 2 h heavy-intensity exercise bout, participants cycled at 25%Δ1 (i.e., GET plus 25% of the difference

between the work rate at GET and Control-EP). Pilot testing indicated that this power output (25%Δ1) was challenging but sustainable for 2 h.

During visit 4, participants completed 2 h of heavy-intensity exercise followed by a 3MT (Fatigued-3MT). Prior to the start of the exercise test, participants provided a resting muscle biopsy sample (described below). The exercise protocol started with cycling at 20 W for 3 min, after which the power output abruptly increased to 25%Δ1. Participants were instructed to maintain their preferred pedal cadence for the whole 2 h. They were allowed to consume water *ad libitum*. A clock indicating time remaining was visible during the 2 h exercise bout and participants were allowed to listen to music, but both the clock and the music were withdrawn 1 min prior to the start of the Fatigued-3MT. An end-exercise muscle biopsy was taken at 120 min and the Fatigued-3MT commenced at 121 min. The Fatigued-3MT was administered as described for the Control-3MT. Pulmonary gas exchange data were recorded at the following time points: -3-15 min, 25-30 min, 55-60 min, 85-90 min, 115-120 min and continuously throughout the Fatigued-3MT. A blood sample was taken every 30 min during the 2-h heavy-intensity exercise bout for the analysis of blood [lactate], blood [glucose] and plasma potassium ($[K^+]$). Heart rate (HR) and cadence were obtained continuously over the entire exercise testing period. Fatigued-EP and Fatigued-WEP was estimated from the Fatigued-3MT using the same procedures as for the Control-3MT.

During visits 5-7, participants performed the same 2-h heavy-intensity exercise bout as in visit 4 but this was followed immediately by a severe-intensity, constant-power output prediction trial which was continued until T_{lim}. The purpose of completing these prediction trials was to determine the power-duration parameters in a fatigued state (Fatigued-CP and Fatigued-W′) using the conventional protocol (e.g., Ref. 3). The power outputs for the three severe-intensity exercise bouts were calculated from the Fatigued-3MT (visit 4) to provide T_{lim} values ranging between approximately 2 min and 15 min (a short, intermediate and long

trial). During the prediction trials, participants were not informed of the power output applied or the time elapsed but were instructed to cycle for as long as possible. T_{lim} was recorded when participants could not maintain their preferred cadence for >5 s. Breath-by-breath pulmonary gas exchange data were obtained from 5 min before the end of the 2 h heavy-intensity exercise bout until T_{lim} during the severe-intensity prediction trials. A capillary blood sample for the determination of blood [lactate] was taken from the fingertip at the following time points during the trials: -5 min, 2 min, 4 min, 8 min and every 4 min thereafter until T_{lim}, and at T_{lim}. Linear regression using the work-time ($W = CPt + W'$) and 1/time ($P = W' (1/t) + CP$) models, as well as the hyperbolic model ($T_{lim} = W' / (P - CP)$), were used to obtain 3 sets of Fatigued-CP and Fatigued-W′ parameters from the prediction trials. The best individual fit of the 3 models was used for further analyses (3, 4).

On visit 8, participants completed a final 2-h heavy-intensity exercise bout, identical to visit 4-7, but followed immediately by a constant-power output test at 15 W below Fatigued-CP (<Fatigued-CP). This bout was completed to test the assumption that exercise <Fatigued-CP would result in physiological responses consistent with exercise in the heavy-intensity domain (5, 23, 32). The exercise bout lasted until T_{lim} or for 30 min, whichever occurred sooner. Breath-by-breath pulmonary gas exchange data were recorded continuously from 115 min of the 2 h heavy-intensity exercise bout until the cessation of the protocol. Blood [lactate] was measured at the same time points as in visits 5-7.

Pulmonary gas exchange and heart rate

Pulmonary gas exchange was measured breath-by-breath and bin-averaged over 10-s periods. Participants wore an oro-nasal mask (Hans Rudolf 7450 Series V2^{TM} Mask, CareFusion, Germany). The inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Vyntus, CareFusion, Germany) via a capillary line connected to the

mask. The analyzer was calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, MO). The volume and concentration signals were time-aligned by accounting for the delay in capillary gas transit and analyzer rise time relative to the volume signal. The baseline $\dot{V}O_2$ during all tests was defined as the mean value over the final minute of the 3-min period of unloaded pedalling. Fat and carbohydrate oxidation rates were calculated from $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) using stoichiometric equations with the assumption that protein oxidation during exercise did not change (19).

Carbohydrate oxidation ($g \cdot min^{-1}$) = [4.21 ($\dot{V}CO_2$) – 2.692 ($\dot{V}O_2$)]

Fat oxidation ($g \cdot min^{-1}$) = [1.695 ($\dot{V}O_2$) – 1.701 ($\dot{V}CO_2$)]

HR was recorded every 5 s during all visits (Garmin FR70, Garmin Ltd, Schaffhausen, Switzerland).

Muscle biopsies

Muscle samples were obtained from an incision from the medial region of the *m. vastus lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle biopsy technique under suction (2) . Muscle samples were taken at rest and immediately following 2 h of heavy-intensity exercise during visit 4. The post-exercise muscle biopsies were taken while participants remained on the cycle ergometer and snap frozen in liquid N_2 within ≤10 s of the completion of the exercise bout. Biopsy samples were stored at -80 °C for subsequent analysis.

Muscle glycogen concentration

Muscle samples were freeze-dried prior to dissection from connective tissue, fat and blood. Approximately 2 mg of dry weight muscle tissue was hydrolyzed in 500 µl of 1 M

hydrochloric acid at 100°C for 3 h to release glycosyl units and immediately measured using an automated glucose analyser to determine muscle [glycogen] (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH), (33). The precision of this method of analysis within this physiological range (0.05 to 0.55 mmol/l) was checked by measuring the glucose concentration across a range of solutions made up using glucose diluted in hydrochloric acid; the measured vs. expected values lay on the line of identity with an R^2 of 0.99.

Blood analyses

During visit 4, blood samples were obtained from a cannula (Insyte-W; Becton Dickinson, Madrid, Spain) inserted in an antecubital vein. Samples were drawn at rest and at specific times during the 2-h heavy-intensity exercise bout. Blood samples were collected into a lithium-heparin vacutainer (Becton-Dickinson, New Jersey, USA). 200 µL of blood was immediately extracted and haemolyzed in 200 µL of Triton X-100 Solution (Triton X-100, Amresco, Salon, OH) and blood [glucose] and [lactate] were measured (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was centrifuged at 4000 rpm for 10 min at 4°C. The plasma was extracted and frozen at -80°C and subsequently analysed for [K^+] using Stat Profile pHOx Ultra (Nova Biomedical, Waltham, MA, USA). All fingertip blood samples (~25 µl) (visit 5-8) were collected into capillary tubes and analysed promptly for blood [lactate] using an automated lactate analyser (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH).

Statistical analysis

Errors associated with mathematical modelling of the CP and W′ parameters from prediction trial data were quantified as standard error, and expressed relative to the parameter estimate (coefficient of variation, CV%) for each individual. One-way ANOVA with repeated

measurements were used to assess differences in Control-EP, Fatigued-EP and Fatigued-CP; Control-WEP, Fatigued-WEP and Fatigued-W′; and $\dot{V}O_{2peak}$ during the ramp test, Control-3MT and Fatigued-3MT. A one-way ANOVA with repeated measurements was also used to assess differences in $\dot{V}O_{2peak}$ alongside HR_{max} between the ramp test, <Fatigued-CP, short, intermediate and long duration severe-intensity prediction trials, as well as differences in respiratory gas exchange variables, blood [lactate] and blood [glucose] during all visits. Differences in total work done as well as peak power output measured during the Control-3MT and Fatigued-3MTs were assessed using paired samples t-tests. Agreement between the power-duration parameters derived from different protocols was assessed using intra-class correlation coefficients and the Bland-Altman analysis. The difference in muscle [glycogen] between rest and following 2 h of heavy-intensity exercise was assessed using a paired samples t-test. Relationships between absolute muscle [glycogen], and changes in muscle [glycogen], over the 2-h heavy-intensity exercise test and the changes in EP and WEP were assessed using Pearson product moment correlation coefficients. Statistical significance was accepted when $P<0.05$. Data are reported as mean ± SD.

Results

The $\dot{V}O_{2peak}$ in the ramp incremental test was 4.31 ± 0.35 $L \cdot min^{-1}$, and the peak power output was 368 ± 48 W. During the 2-h heavy-intensity exercise bout, the relative intensity increased from 10-15 min (65 ± 6 % $\dot{V}O_{2peak}$) to 115-120 min (72 ± 4 % $\dot{V}O_{2peak}$; $P<0.05$) and the respiratory exchange ratio decreased from 10-15 min (0.86 ± 0.05) to 115-120 min (0.79 ± 0.05; $P<0.05$). There was a decrease in carbohydrate oxidation (10-15 min: 1.77 ± 0.73 g/min, 115-120 min: 1.17 ± 0.76 g/min) and an increase in fat oxidation (10-15 min: 0.68 ± 0.29 g/min, 115-120 min: 1.08 ± 0.30 g/min) during the 2-h exercise bout ($P<0.05$). HR

increased over time during the 2-h exercise bout (P<0.05). Blood [lactate] and blood [glucose] did not change during the 2-h exercise bout; plasma [K^+] was elevated above the resting value at all time points (P<0.05) during the 2-h exercise bout but remained stable beyond 30 min (Table 1). HR throughout the 2-h exercise bouts and end-exercise blood [lactate] were not different between visits 4-8. Body mass fell by ~0.9 kg during the prolonged exercise tests with no difference between visits.

The standard error (and CV%) for the Fatigued-CP parameter estimate from the 'best fit' model was 3 ± 3 W (1.1 ± 1.1%), and the standard error (and CV%) for Fatigued-W′ for the 'best fit' model was 1.3 ± 1.2 kJ (8.9 ± 9.0%). The 'best fit' model was provided by the 1/time model for 7 subjects and by the hyperbolic model for the other 7 subjects. Fatigued-EP (256 ± 52 W) and Fatigued-CP (256 ± 41 W) were not different from one another (95% confidence limits 13, -13 W; P = 0.94) but were ~11% lower than Control-EP (287 ± 46 W; P<0.005; Fig. 1A). The intra-class correlation coefficient for Fatigued-CP and Fatigued-EP was r = 0.91 (P<0.001), and the standard error of estimate was 17 W (7%) (Fig. 2A, B). Fatigued-WEP (14.6 ± 5.3 kJ) and Fatigued-W′ (15.3 ± 5.0 kJ) were not different from one another (95% confidence limits 3.6, -2.3 kJ; P=0.65) but were 22% and 17% lower, respectively, compared to Control-WEP (18.7 ± 4.7 kJ; P<0.05; Fig. 1B). The intra-class correlation coefficient for Fatigued-WEP and Fatigued-W′ was r = 0.52 (P = 0.59), and the standard error of estimate was 4.4 kJ (29%) (Fig. 2C, D). The changes in EP and WEP observed over the 2-h exercise bout were not significantly correlated (r = -0.18; P=0.54). The peak power output was not different between the Fatigued-3MT (1083 ± 246 W) and the Control-3MT (1037 ± 389 W; P=0.48). Total work done was ~14% lower during the Fatigued-3MT (60.7 ± 12.6 kJ) compared to the Control-3MT (70.2 ± 9.6 kJ; P<0.001).

Muscle [glycogen] decreased by ~65% over the 2-h heavy-intensity exercise bout (Pre: 639 ± 235 mmol/kg d.w, Post: 226 ± 194 mmol/kg d.w; Fig. 3A; P<0.001). There was no

289 significant correlation between the decline in muscle [glycogen] (413 ± 116 mmol/kg d.w)
290 and the difference between Control-EP and Fatigued-EP (30 ± 27 W; r = 0.19; P=0.52).
291 Moreover, the decline in muscle [glycogen] was not significantly correlated with the
292 difference between Control-WEP and Fatigued-WEP (4.1 ± 3.3 kJ; r = 0.07; P=0.80).

293 The constant power outputs for the short, intermediate and long severe-intensity prediction
294 trials and the <Fatigued-CP test were 336 ± 60 W, 302 ± 52 W, 281 ± 46 W and 241 ± 41 W,
295 respectively. The T_{lim} for the short (199 ± 55 s), intermediate (362 ± 92 s) and long (668 ±
296 119 s) severe-intensity prediction trials were within the desired range. Five (of 14)
297 participants were able to complete the target of 30 min during the <Fatigued-CP test; the T_{lim}
298 for the remaining 9 participants was 1193 ± 295 s. There was an increase in $\dot{V}O_2$ from the
299 end of the 2-h heavy-intensity exercise bout to the end of all the severe-intensity prediction
300 trials as well as to the end of the <Fatigued-CP bout (P<0.001; Fig. 4). There were no
301 differences in $\dot{V}O_{2peak}$ measured in the ramp incremental test (4.31 ± 0.35 L·min^{-1}) and the
302 short (4.37 ± 0.41 L·min^{-1}), intermediate (4.32 ± 0.31 L·min^{-1}) and long (4.36 ± 0.38 L·min^{-1})
303 severe-intensity prediction trials. $\dot{V}O_{2peak}$ in the <Fatigued-CP test (3.99 ± 0.45 L·min^{-1}) was
304 lower than $\dot{V}O_{2peak}$ during the ramp incremental test and the short, intermediate and long
305 prediction trials (P<0.05; Fig. 4). There were no differences in $\dot{V}O_{2peak}$ between the Control-
306 3MT (4.32 ± 0.32 L·min^{-1}), the Fatigued-3MT (4.42 ± 0.30 L·min^{-1}) and the ramp
307 incremental test. HR_{max} obtained during the ramp incremental test (178 ± 8 b·min^{-1}) was not
308 different from end-exercise HR in the short (178 ± 10 b·min^{-1}), intermediate (178 ± 9 b·min^{-1})
309 and long (177 ± 10 b·min^{-1}) prediction trials or the <Fatigued-CP test (171 ± 13 b·min^{-1}).

310 Blood [lactate] increased from the end of the 2-h heavy-intensity exercise bout to T_{lim} in all
311 four subsequent exercise tests (P<0.005). End-exercise blood [lactate] was lower (P<0.05)
312 during the <Fatigued-CP exercise test (3.8 ± 2.7 mM) compared to the short (5.6 ± 1.8 mM),

intermediate (6.4 ± 3.1 mM) and long (6.4 ± 2.9 mM) severe-intensity prediction trials but was not different between the three severe-intensity prediction trials.

Discussion

This is the first study to investigate changes in the parameters of the power-duration relationship (CP and W′) after prolonged endurance exercise estimated using both the conventional protocol and the 3MT. Consistent with our experimental hypotheses, following 2 h of heavy-intensity exercise: 1) the Fatigued-CP and Fatigued-W′ measured using the conventional protocol were significantly lower (by 11% and 20%, respectively) compared to the values estimated in the absence of prior exercise; and 2) the Fatigued-EP measured using the Fatigued-3MT provided and accurate (SEE 7%) estimate of the Fatigued-CP established using the conventional protocol, while the agreement between Fatigued-WEP and Fatigued-W′ was limited (SEE 29%). However, contrary to our third hypothesis, there were no significant correlations between muscle glycogen depletion and the change in either EP or WEP following 2 h of heavy-intensity exercise. The results of this study provide evidence that the parameters of the power-duration relationship are profoundly altered by prolonged endurance exercise, with implications for the prediction of performance during such exercise based on parameters measured in a rested state. Understanding dynamic changes in these parameters may provide insight into the nature of fatigue development during such exercise and enable the development of interventions to enhance human performance.

The EP and WEP declined when estimated following 2 h of heavy-intensity exercise compared to a rested state. Compared to Control-EP, there was a 10% reduction in Fatigued-CP and an 11% reduction in Fatigued-EP with no significant difference between Fatigued-CP and Fatigued-EP. Similarly, compared to Control-WEP, there was a 17% reduction in Fatigued-W′ and a 22% reduction in Fatigued-WEP. There was no significant difference

between Fatigued-W′ and Fatigued-WEP, but it is important to note that Fatigued-W′ and Fatigued-WEP were not significantly correlated and showed limited agreement with an SEE of 4.4 kJ or 29%. These findings indicate, for the first time, that the CP parameter of the power-duration relationship estimated with the 3MT was not different from that estimated using the conventional protocol after 2 h of heavy-intensity exercise, and confirm our previous findings that such exercise leads to substantial reductions in CP and W′ of ~10% and ~20%, respectively (8). We (8) previously reported that the power profile during the Fatigued-3MT was highly reproducible. In the present study, the close agreement between Fatigued-EP and Fatigued-CP following 2 h of heavy-intensity exercise provides confidence in the sensitivity and practicality of the Fatigued-3MT to accurately evaluate changes in CP during prolonged, fatiguing, endurance exercise. It should be noted, however, that the Fatigued-3MT provided a much more accurate estimate of Fatigued-CP (7% error) than of Fatigued-W′ (29%). This observation is consistent with greater test-retest variability of the WEP and W′ compared to EP and CP, respectively, in the rested state (37, 38). It is important to recognize that, when determined using conventional procedures, the test-retest standard error of the estimate for W= (~14%) is generally higher than for CP (~4 – 8%) (12, 27).

The relative intensity over the 2 h of heavy-intensity exercise increased from ~65 to ~72% $\dot{V}O_{2peak}$ which is in accordance with our previous findings (8). This 'drift' in $\dot{V}O_2$, which is mechanistically distinct from the $\dot{V}O_2$ slow component (22), reflects, in part, the reduction in RER due to increased reliance on fat compared to carbohydrate oxidation. Alongside this, in the present study we found that muscle [glycogen] was reduced by ~65% during the 2-h exercise bout. When muscle [glycogen] reaches low values, the reliance on fat oxidation is increased to sustain exercise, especially when carbohydrate supplements are not provided (9), and exercise performance is typically impaired (17). However, we found no significant correlations between muscle glycogen depletion during the 2-h heavy-intensity exercise bout

and the decrease in EP or WEP. Our findings therefore suggest that muscle glycogen depletion did not occur in parallel with changes in the parameters of the power-duration relationship following 2 h of heavy-intensity exercise. It should be noted, however, that the relationship between absolute muscle [glycogen], measured at a discrete site in the *m. vastus lateralis*, and the rate of energy supply from carbohydrate to support whole-body oxidative metabolism (i.e., CP) is unclear and may not be directly proportional.

The physiological basis for the changes observed in EP after 2 h of heavy-intensity exercise is likely multifactorial. Peripheral factors, such as changes in high-energy phosphates and pH, would seem to be unlikely candidates given that exercise of similar duration and intensity does not appreciably perturb the intramuscular milieu (4, 33) and blood [lactate] remained low and stable across time in the present study. An alteration in neuromuscular excitability would also seem an unlikely explanation (28) given that plasma [K^+] was stable over the final 90 min of the 2-h heavy-intensity exercise bout. Acute changes in mitochondrial function, such as increased uncoupling, during endurance exercise would reduce power output for a given $\dot{V}O_2$ and could explain a lower EP. However, while the expression of uncoupling protein 3 has been reported to be increased in rat skeletal muscle following 2-h of endurance exercise (20), similar effects have not been consistently demonstrated in humans (10, 35). It is known that critical torque (the analogue of CP) measured during knee extension exercise represents a critical threshold for neuromuscular fatigue development (6) such that so-called central fatigue makes a greater contribution to fatigue development and exercise intolerance in the heavy-intensity domain compared to the severe-intensity domain (4, 6). Consistent with this, Thomas et al. (32) reported a greater degree of central fatigue, as determined by greater reductions in voluntary activation measured by motor nerve and cortical stimulation, during self-paced cycle exercise requiring >30 min duration compared to shorter exercise bouts. It is possible, therefore, that the development of central fatigue during the 2-h heavy-intensity

388 exercise bout in the present study influenced the subsequent severe-intensity prediction trials
389 and the 3MT, limiting exercise performance and reducing CP and EP.

390 Other possible contributory factors to the reduction in EP following 2-h heavy-intensity
391 exercise include the development of muscle damage, respiratory muscle fatigue, and
392 challenges to thermoregulation. The submaximal cycling exercise performed in the present
393 study has no eccentric component and is therefore unlikely to result in significant muscle
394 damage (31). It is possible, however, that muscle damage incurred during prolonged exercise
395 in other modalities which have a greater eccentric muscle action, such as running, results in
396 greater changes in the speed-time relationship than we report herein for cycling. While
397 respiratory muscle fatigue can develop during prolonged endurance exercise, despite
398 relatively low rates of ventilation, effects on performance are controversial and unlikely to be
399 appreciable (15). We did not measure core temperature or sweat rate in the present study but
400 participants were allowed to consume water *ad libitum* such that the reduction in body mass
401 over the prolonged exercise bout was ~0.9 kg. In more extreme environmental conditions
402 (high heat and/or humidity, or indeed at altitude), or when opportunities for fluid replacement
403 are limited, it is possible that the deleterious effects of prolonged exercise on the power-time
404 relationship may be amplified.

405 We asked participants to complete an exercise bout at 15 W below Fatigued-CP to test the
406 assumption that exercise performed <Fatigued-CP would produce physiological responses
407 consistent with exercise in the heavy-intensity domain, as is the case when CP is determined
408 without prior fatiguing exercise (4, 5, 32). We found that exercise <Fatigued-CP could not be
409 sustained for 30 min by all participants following 2 h of heavy-intensity cycling despite the
410 attainment of a physiological steady-state. Indeed, only five out of the 14 participants were
411 able to complete 30 min of exercise <Fatigued-CP. The remaining nine participants were

unable to complete 30 min of exercise <Fatigued-EP despite steady-state $\dot{V}O_2$ and blood [lactate] profiles being evident in eight of them. Given that the participants displayed physiological responses which were indicative of heavy-intensity exercise in the <Fatigued-CP test, it may be considered surprising that the majority of them could not complete 30 min of exercise. However, this is likely the result of muscle glycogen depletion. Muscle [glycogen] was decreased in all participants during the 2-h exercise bout, albeit with substantial inter-subject variability. Interestingly, muscle [glycogen] was 37 ± 46 mmol/kg d.w. after the 2-h heavy-intensity exercise bout in participants who reached T_{lim} in <20 min in the <Fatigued-CP test, compared to 277 ± 187 mmol/kg d.w. in the participants who completed >20 min of exercise. Moreover, the participants who completed <20 min exercise in the <Fatigued-CP test exhibited a larger decrease in CP (59 ± 16 W) than participants who completed >20 min exercise (20 ± 22 W). It might be speculated that a low muscle [glycogen] at the start of the <Fatigued-CP test restricted carbohydrate supply and 'rate-limited' oxidative metabolism such that the external power output could not be maintained.

Two hours of heavy-intensity exercise resulted in a ~20% reduction in WEP, but this was not correlated with the fall in muscle [glycogen]. This result is perhaps surprising given that Miura et al. (24) reported a ~20% reduction in W′, with no change in CP, following an exercise and dietary regimen designed to result in muscle glycogen depletion. During long-duration endurance exercise there is a decrease in muscle [glycogen] in both type I and type II muscle fibres (13, 14). A low muscle [glycogen] impairs sarcoplasmic reticulum Ca^{2+} release, leading to excitation-contraction coupling failure and reduced force production (7). Considering the results of the present study alongside those of Miura et al. (24), it appears that glycogen depletion either limits energy production above CP or results in earlier/greater accumulation of metabolites for a given amount of work done above CP, with total work capacity being reduced in either case. Despite the lack of significant correlation between

changes in WEP and muscle [glycogen] in the present study, it remains possible that low muscle [glycogen] could impact WEP and W′, albeit in a more complex fashion (8, 24). It is interesting to note here that completing severe-intensity or sprint exercise immediately prior to a 3MT reduces WEP and peak power output without affecting EP (30, 39) whereas completing heavy-intensity exercise reduces WEP and EP but not peak power output (present study). This dissimilarity is presumably related to differential effects of these prior exercise protocols on muscle [PCr] and [glycogen]. It is also possible that muscle [glycogen] influences WEP and W′ differently due to differences in motor unit recruitment patterns evident in the 'all-out' 3MT compared to the constant-power, severe-intensity prediction trials employed in the conventional protocol (38, 41).

Experimental Considerations

To reduce the demand on the participants, which was already significant, we did not measure CP and W′ using conventional severe-intensity prediction trials when the subjects had not completed preceding exercise but rather relied on the 3MT to estimate these parameters. However, it is well established that the EP and WEP measured in a 3MT provides valid and reliable estimates of CP and W′ in moderately-trained subjects, provided that the test is performed against appropriately normalized fixed resistance and the $\dot{V}O_{2max}$ is attained (22, 29, 35, 37; cf. Ref. 26). A possible limitation of our study was that pre- and post-2 h exercise muscle biopsies were only obtained on one of the visits. However, participants kept a food and training diary and replicated their dietary and physical activity before each visit in order to minimize the likelihood of large differences in pre-exercise muscle [glycogen] between tests. Baseline, end-exercise and changes in HR, body mass and blood [lactate] were similar in all of the 2-h exercise bouts, providing reassurance that the physiological demands of the repeated 2-h exercise bouts were consistent. Another limitation was that the relatively large

amount of tissue required to measure [glycogen] precluded the measurement of other intramuscular substrates and metabolites (e.g. PCr, lactate), although these would not be expected to change substantially (4). Finally, it should be acknowledged that muscle biopsy samples are obtained from a small area of the active muscle mass engaged during cycle exercise such that the lack of correlation between individual changes in muscle [glycogen] and changes in EP and WEP does not exclude the possibility that muscle glycogen availability makes an important contribution to changes in the parameters of the power-duration relationship reported in the present study.

Perspectives and Significance

The power-duration relationship has significant utility in predicting performance and optimizing athletic training programs (21, 40). However, the results of the present study indicate that the values of both CP and W′ are subject to change during and following prolonged endurance exercise. These findings have important implications for the prediction of sporting performance and for optimal pacing strategy. Dynamic changes in the parameters of the power-duration relationship during fatiguing exercise could mean that a given speed/power output predicted to reside within the heavy-intensity exercise domain may, at some stage during competition, begin to elicit physiological responses characteristic of the severe-intensity domain. Performance in endurance competition therefore depends not only upon the CP and W′ measured in a 'fresh' state but also on the extent to which these parameters deteriorate during fatiguing exercise. Further research is necessary to investigate the extent to which CP and W′ are affected by fatigue development in other exercise settings, the time course over which CP and W′ decline during prolonged exercise, and the efficacy of various interventions to offset these effects. The findings of the present study indicate that the 3MT may provide a practical and expeditious approach to elucidate dynamic changes in the power-duration relationship during endurance exercise.

In conclusion, the parameters of the power-duration relationship were appreciably reduced when estimated following 2 h of heavy-intensity exercise compared to the rested state. The reductions in CP (~10%) and W′ (~20%) were similar when estimated with the conventional protocol and the 3MT, indicating that the 3MT may be used to conveniently estimate the CP and W′ under these conditions. The changes in EP and WEP following 2 h of heavy-intensity exercise were not significantly correlated with the reduction in muscle [glycogen]. Importantly, when CP is estimated in a Fatigued condition, subsequent exercise performed ostensibly below CP cannot be sustained beyond ~20 min despite the attainment of a physiological steady-state. This indicates that the 'characteristic' physiological responses elicited during <CP exercise differ when assessed following prolonged endurance exercise, an effect that may be related to low pre-test muscle [glycogen]. These results may have important implications for understanding the interaction between fatigue development and performance capacity during prolonged endurance exercise.

Acknowledgements

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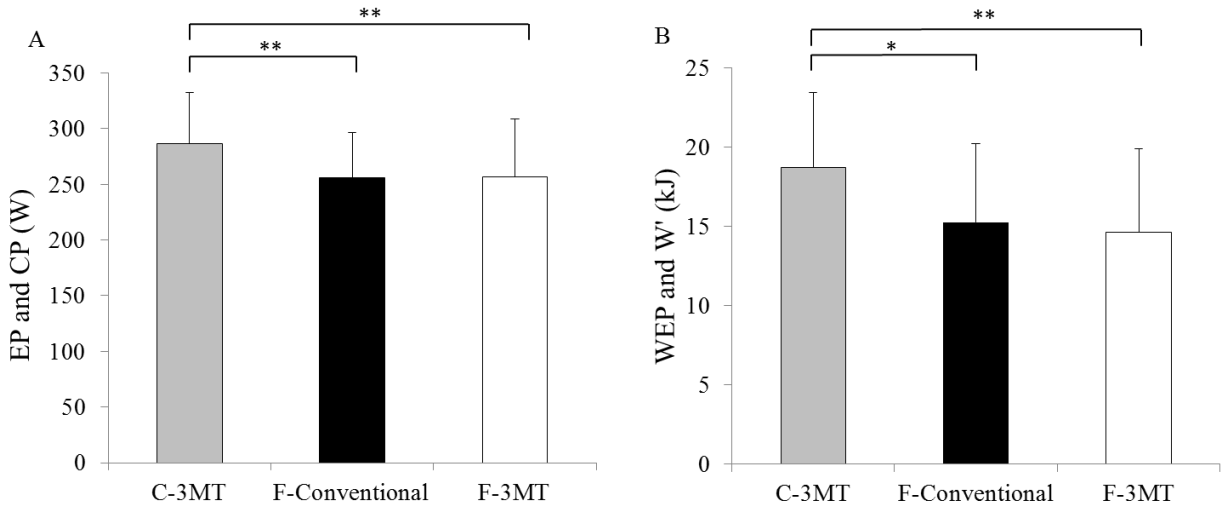
Figure Legends

Figure 1. Panel A: End-test power (EP) in a rested state (Control-3MT) and Fatigued state (Fatigued-3MT), and critical power (CP) derived from a conventional prediction trial protocol in a Fatigued state (F-Conventional). Panel B: Group mean work done above end-test power (WEP) in a rested state (Control-3MT) and Fatigued state (Fatigued-3MT), and W′ derived from a conventional prediction trial protocol in a Fatigued state (F-Conventional). * = $P<0.01$, ** = $P<0.05$.

Figure 2. Bland-Altman plots of the relationship and limits of agreement between end-test power (Fatigued-EP) and critical power (Fatigued-CP) (panels A and B), and work done above EP (Fatigued-WEP) and W′ (Fatigued-W′) (panels C and D) after 2 h of heavy-intensity exercise. Fatigued-EP and Fatigued-WEP were estimated using a 3-min all-out test and the CP and W′ were derived from a conventional prediction trial protocol. In panels A and C the solid line is the best-fit linear regression and the dashed line is the line of identity. In panels B and D the solid horizontal line represents the mean difference between the two measurements and the dashed lines represent limits of agreement. * = $P<0.01$.

Figure 3. Muscle [glycogen] before and after 2 h of heavy-intensity exercise (panel A). There were no significant correlations between the change (Δ) in muscle [glycogen] and changes in EP (ΔEP; panel B) or WEP (ΔWEP; panel C) estimated in a 3-min all-out test at rest and after 2 h of heavy-intensity exercise. Dashed lines in panel A represent individual responses, and solid lines in panels B and C indicate linear regression.

Figure 4. Pulmonary $\dot{V}O_2$ during the short (black circles), intermediate (white circles), long (black triangles) severe-intensity prediction trials and the <Fatigued-CP trial (white triangles). The dashed horizontal line indicates $\dot{V}O_{2peak}$ measured in the ramp incremental test. Error bars (SD) are shown for end-exercise time points only to aid clarity. a = different from $\dot{V}O_2$ measured at the end of 2 h of heavy-intensity exercise (P<0.01), b = different from end-exercise $\dot{V}O_2$ measured in the <Fatigued-CP trial (P<0.01).


A
**
**
350
300
250
200
150
100
50
0
EP and CP (W)
C-3MT
F-Conventional
F-3MT
B
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*
25
20
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10
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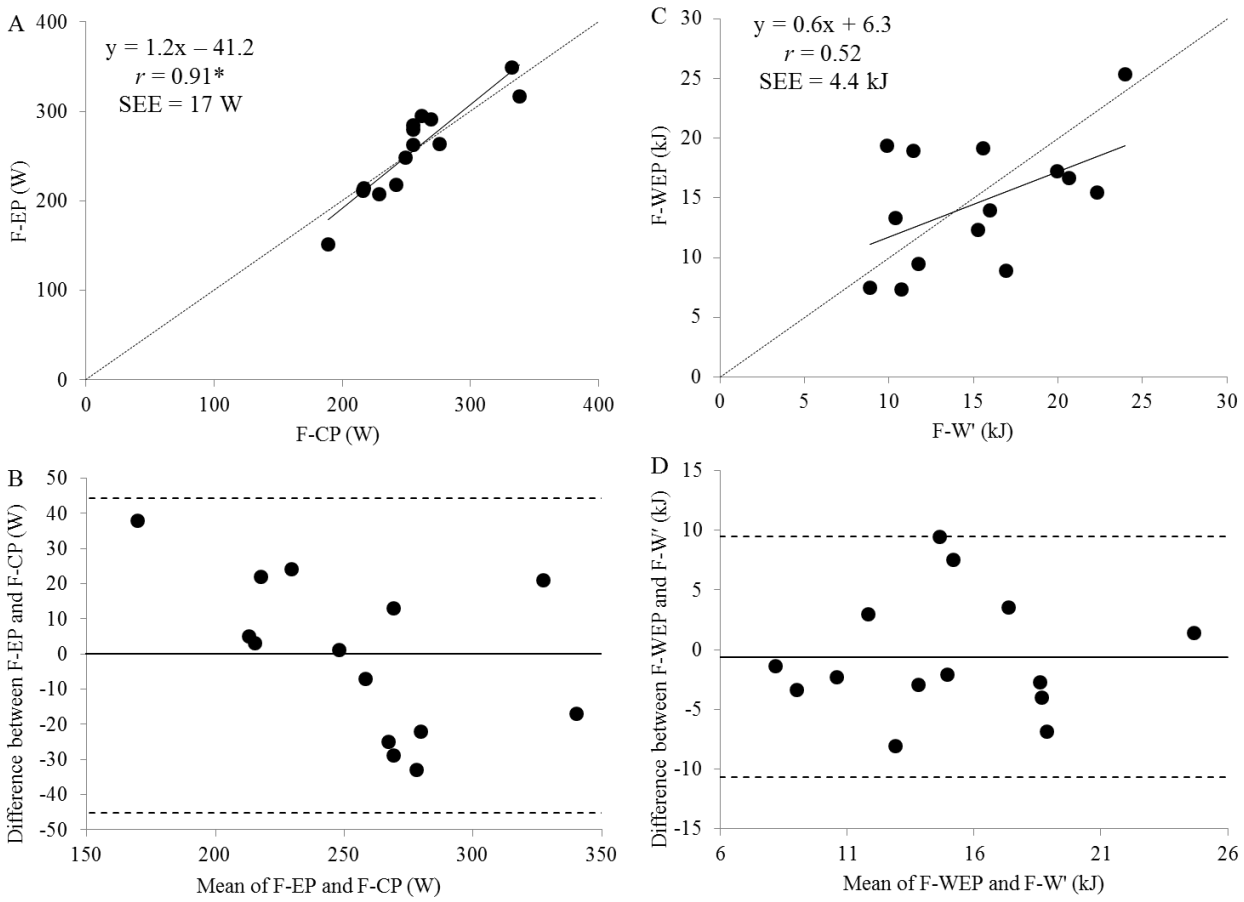
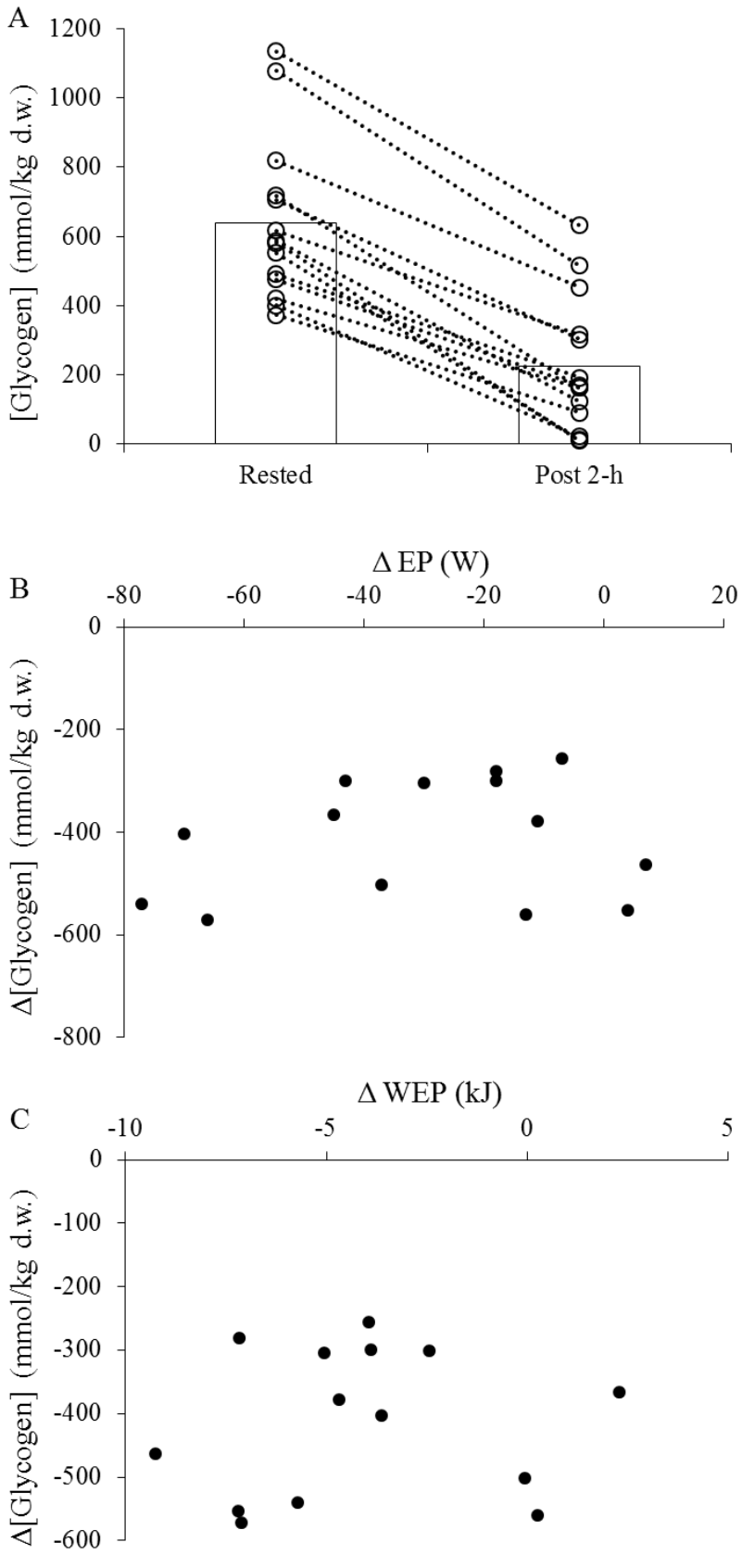
0
WEP and W' (kJ)
C-3MT
F-Conventional
F-3MT

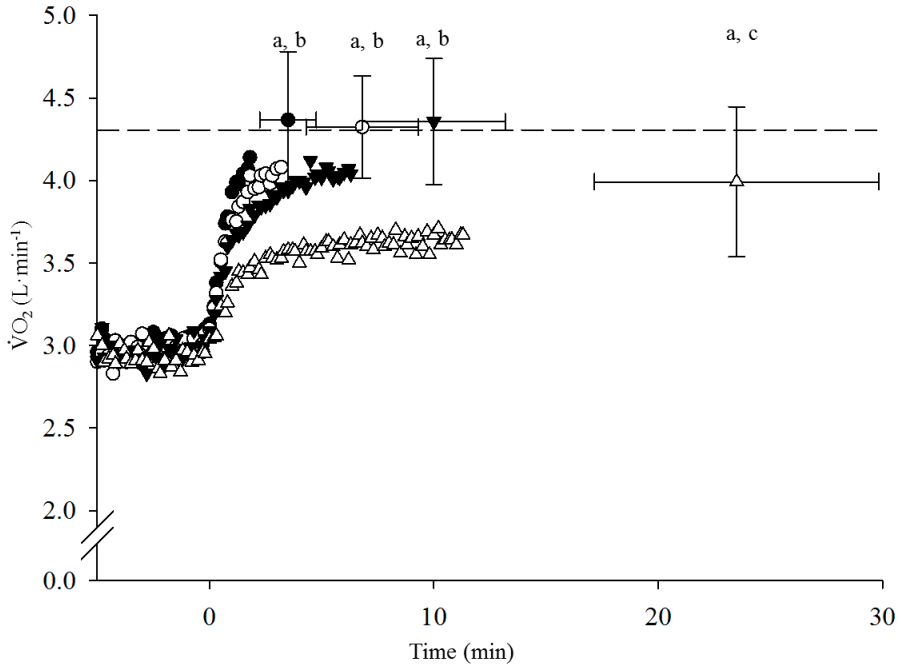
649
650


A
y = 1.2x – 41.2
r = 0.91*
SEE = 17 W
F-EP (W)
F-CP (W)
C
y = 0.6x + 6.3
r = 0.52
SEE = 4.4 kJ
F-WEP (kJ)
F-W' (kJ)
B
Difference between F-EP and F-CP (W)
Mean of F-EP and F-CP (W)
D
Difference between F-WEP and F-W' (kJ)
Mean of F-WEP and F-W' (kJ)

651
652


A
[Glycogen] (mmol/kg d.w.)
1200
1000
800
600
400
200
0
Rested
Post 2-h
B
Δ EP (W)
-80
-60
-40
-20
0
20
Δ[Glycogen] (mmol/kg d.w.)
-200
-400
-600
-800
C
Δ WEP (kJ)
-10
-5
0
5
Δ[Glycogen] (mmol/kg d.w.)
-100
-200
-300
-400
-500
-600

653



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