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Title Page

The magnitude of neuromuscular fatigue is not intensity-dependent when cycling above critical power but relates to aerobic and anaerobic capacities.

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Running Title

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New Findings

- **What is the central question of this study?**

  Is the magnitude of neuromuscular fatigue dependent upon exercise intensity above critical power (CP) when *W'* (the curvature constant of the power-duration relationship) is depleted?

- **What is the main finding and its importance?**

  The magnitude of neuromuscular fatigue is the same following two bouts of supra-CP cycling (3 vs. 12 min) when controlling for *W'* depletion, but is larger for individuals of greater anaerobic capacity following the shorter, and smaller for individuals of greater aerobic capacity following the longer exercise. These findings provide new insight into the mechanisms underpinning exercise above CP.

Abstract

The aim of the present study was to test whether the development of neuromuscular fatigue within the severe intensity domain could be linked to the depletion of the curvature constant (*W'*) of the power-duration relationship. Twelve recreationally active men completed tests to determine $\dot{V}O_{2peak}$, Critical Power (CP) and *W'*, followed by two randomly assigned constant-load supra-CP trials set to fully deplete *W'* in 3 (P-3) and 12 min (P-12). Pre- to post-exercise changes in maximal voluntary contraction (MVC), potentiated quadriceps twitch force evoked by single (Q_{pot}) and paired high- (PS100) and low-frequency (PS10) stimulations and voluntary activation (VA) were determined. Cycling above CP reduced MVC (P-3: -20 ± 10% vs. P-12: -15 ± 7%), measures associated with peripheral fatigue (Q_{pot}: -35 ± 13% vs. -31 ± 14%; PS10: -38 ± 13% vs. -37 ± 17%; PS100: -18 ± 9% vs. -13 ± 8% for P-3 and P-12, respectively) and VA (P-3: -12 ± 3% vs. P-12: -13 ± 3%) ($P < 0.05$), with no significant difference between trials ($P > 0.05$). Changes in MVC and evoked twitch forces were inversely correlated with CP and $\dot{V}O_{2peak}$ following P-12, while *W'* was significantly correlated with changes in Q_{pot} and PS10 following P-3 ($P < 0.05$). Therefore, the magnitude of neuromuscular fatigue does not depend on exercise intensity when *W'* is fully exhausted during severe intensity exercise, yet exploration of inter-individual variations suggests that mechanisms underpinning exercise tolerance within this domain differ between short- vs. long-duration exercise.

Introduction

The relationship between power output and duration of severe intensity exercise is characterised by a hyperbolic function (Moritani *et al.* 1981; Monod & Scherrer, 1965; Poole *et al.* 1988). The asymptote of this relationship (critical power, CP) separates the heavy (< CP) from the severe (> CP) exercise intensity domain and represents the highest power output that can be sustained without continuously drawing on anaerobic energy stores. The aerobic nature of CP is well evidenced through manipulation of O_2 delivery and/or utilisation (Vanhatalo *et al.* 2010; Parker Simpson *et al.* 2015; Dekerle *et al.* 2012). The curvature constant *W'* was originally described as a fixed anaerobic work capacity, mathematically equivalent to a given amount of work that can be performed above CP; according to the CP model, exercise intolerance occurs once this energy store if fully depleted (Monod & Scherrer, 1965; Moritani *et al.* 1981). Although its reliance solely on anaerobic energy stores has been questioned due to its sensitivity to interventions altering O_2 delivery (Vanhatalo *et al.* 2010; Dekerle *et al.* 2012), its primarily anaerobic nature is still widely accepted (Miura *et al.* 1999; Miura *et al.* 2000; Smith *et al.* 1998; Jenkins & Quigley, 1993).

Peripheral fatigue, i.e. a reduction in the force generating capacity induced by alterations at or distal to the neuromuscular junction, has been evidenced within the severe intensity domain whereas central fatigue, i.e. a reduction in the ability to voluntary activate motor neurons and muscle fibres (Gandevia, 2001), seems less pronounced when exercising above CP (Thomas *et al.* 2015; Thomas *et al.* 2016; Lepers *et al.* 2002; Place *et al.* 2004).

The development of peripheral fatigue during exercise above CP has been associated with substantial intramuscular metabolic disturbances (Burnley *et al.*, 2010; Jones *et al.*, 2008; Allen *et al.*, 2008b). Similar changes in muscle metabolic response (i.e. low pH and [PCr] and high [La]) have been reported following continuous and intermittent whole-body exercise performed at different intensities above CP (Black *et al.* 2017; Chidnok *et al.*, 2013) and plantar flexion exercise performed to exhaustion at different fractions of inspired O_2 (Hogan *et al.*, 1999). Interestingly, similar changes in evoked twitch forces (~35%) have also been reported immediately following supra-CP exercise across a wide range of severe exercise intensities, which was described as a “critical threshold“ of peripheral fatigue (Hureau *et al.* 2016; Thomas *et al.* 2015; Johnson *et al.* 2015; Amann *et al.* 2011; 2009; Romer *et al.* 2007; Amann & Dempsey, 2008). Accordingly, Burnley *et al.* (2012) found similar levels of peripheral fatigue (i.e. reductions in potentiated twitch force; Q_{pot}) following single limb exercise and suggested for critical torque to represent a critical threshold for neuromuscular fatigue development. In contrast, Thomas *et al.* (2016) reported different levels of peripheral fatigue following whole-body exercise within the severe intensity domain performed to task failure. More specifically, Thomas *et al.* (2016) reported similar reductions in MVC following supra-CP constant-load cycling (-15 to -18%), but with more predominant peripheral alterations when cycling in the upper part of the severe intensity domain. Central fatigue was conversely more predominant when cycling nearer or within the upper boundary of the heavy intensity domain. Unfortunately, in this study, the full depletion of *W'* was not controlled and therefore, an earlier termination of the voluntary task, due to behavioural effects before reaching 'true' physiological limits, could have confounded the results. An improved study design controlling for the use of *W'* and removing the potential confounding effect of participants' decision making processes associated with performance may be warranted. In addition, the use of a more sophisticated neuromuscular assessment, i.e. application of paired low-frequency (PS10) and high-frequency (PS100) stimulations, may provide further insight into the mechanisms underlying peripheral fatigue (Verges *et al.*, 2009).

The CP concept constitutes a potent framework for the investigation of exercise tolerance in the severe intensity domain (Burnley *et al.* 2016; Poole *et al.* 2016; Grassi *et al.* 2015; Murgatroyd *et al.* 2011). Its integration with electromyographic and mechanical measures of neuromuscular fatigue offers great potential for a better understanding of the limits of tolerance within the severe intensity domain (Burnley *et al.* 2012). The aim of the present study was therefore to investigate the aetiology of neuromuscular fatigue following severe cycling exercise leading to full and controlled depletion of *W'*, thus at two different power outputs above CP calculated to exhaust 100% of *W'* in 3 and 12 min. We hypothesized that exercise above CP would lead to reductions in MVC, and the development of peripheral (e.g. Q_{pot}, PS10, PS100) and central fatigue (VA), without differences between the 3 min and 12 min trials. In addition, inter-individual variations in the development of neuromuscular fatigue were further explored against classic determinants of aerobic (CP and $\dot{V}O_{2peak}$) and anaerobic capacities (*W'*).

Methods

Ethical approval

Written informed consent was obtained from each participant. The study was approved by the University of Brighton Research Ethics & Governance Committee (ethics approval reference number: 1116) and conformed to the standards set by the latest *Declaration of Helsinki,* except for registration in a database.

Participants

Twelve recreationally active males (mean ± SD: age, 23.4 ± 4.1 years; body mass 77.3 ± 10.6 kg; peak O_2 consumption ($\dot{V}O_{2peak}$), 51.6 ± 9.4 ml.min^{-1}.kg^{-1}, peak power output (P_{peak}), 337 ± 46 W) volunteered for this study. All participants were young healthy individuals who were familiar with cycle ergometry and the exercise procedures used in our laboratory.

Study design

The participants reported to the laboratory on eight different occasions over a 3- to 5-week period. The tests included a ramp incremental test for the determination of $\dot{V}O_{2peak}$, a familiarisation to the experimental protocol, four to five constant-load trials performed to task failure for the determination of CP and *W'* and subsequently, two randomised visits to assess neuromuscular fatigue before and 1 min following constant-load cycling above CP. Ventilatory and pulmonary gas exchange was measured using a breath-by-breath open-circuit system (MediSoft Ergocard®, Sorinnes, Belgium). Due to technical issues a different breath-by-breath system (Metalyzer Sport, Cortex Biophysik, Leipzig, Germany) was used for three participants. All tests were performed on an electromagnetically-braked, computer-controlled cycle ergometer (SRM High Performance Ergometer with 8 strain gauges; Schoberer Rad Meßtechnik, Jülich, Germany) and pedals were fitted with standard toe clips. Seat height, handle bar height and distance from seat to the handlebar were adjusted and recorded to replicate the set-up for each participant for the duration of the study. Each session was preceded by a warm-up protocol, consisting of 3 min rest, 5 min baseline pedalling at 50 W, 3 min rest and 4 min baseline pedalling at 20 W. Participants were instructed to maintain a cadence of 80 rev.min^{-1} during all sessions and to stay seated throughout cycling. Task failure was defined as a drop in cadence twice below 75 rev.min^{-1} for more than 5 s despite strong verbal encouragement. All tests were performed at the same time of day (± 2 h) to control for the effect of diurnal variation (Atkinson & Reilly, 1996) and separated by a minimum of 24 h. The two randomly assigned main trials (visit 7 and 8) were separated by a minimum of 48 h. Participants were instructed to report to the laboratory in a fully rested and well hydrated state, to avoid vigorous activity within the previous 24 h and to refrain from alcohol (24 h) and caffeine consumption (12 h) prior to testing.

Incremental test and familiarisation

Power for the maximal ramp incremental test was initially set to 50-125 W depending on individual fitness level and increased by 5 W every 12 s until task failure. At task failure, the power was reduced to 20 W for 5 min of baseline pedalling, followed by an increase to 105%

P_{peak} performed to task failure in order to confirm if a true $\dot{V}O_{2max}$ was reached (Rossiter *et al.* 2006). $\dot{V}O_{2peak}$ was defined as the highest 15 s moving average. Peak power was calculated as the highest 15 s moving average during the ramp test.

During a second visit, participants were familiarised with constant-load trials performed to task failure, neuromuscular function assessment (NMFA) and a quick transition from the cycle ergometer to the isometric rig.

Determination of CP and *W'*

The participants completed a semi-randomised series of four to five constant-load tests to elicit task failure within ~3 and 15 min (Poole *et al.*, 1988; Hill, 1993). Participants were blinded for elapsed time, power output and heart rate throughout testing and not informed about any other measure except cadence.

For each participant, three different models (equation 1-3) were used to obtain estimates of both CP and *W′* (least-squares regression model):

Non-linear power (P) vs. time to task failure (t_{lim}):

$$t_{lim} = W' / (P - CP) \qquad (1)$$

Linear work (W) vs. time to task failure (t_{lim}):

$$W = CP \cdot t_{lim} + W' \qquad (2)$$

Power (P) vs. inverse-time to task failure ($1/t_{lim}$):

$$P = (1/t_{lim}) \cdot W' + CP \qquad (3)$$

Standard error (SE) for CP and *W'* derived from the three regression models were compared and the model that best fit the data for each participant (lowest SE) was selected. An additional fifth trial was performed if these SE were above 2% and 10% of CP and *W'*, respectively (Dekerle *et al.* 2015; Murgatroyd *et al.* 2011). The 95% CI for the CP estimate was calculated to ensure that powers for the main trials were confidently above CP.

Experimental trials

Power output was predicted for each participant from interpolation of the power - time relationship and set to induce full depletion of *W'* within 12 min (P-12) and 3 min (P-3). Neuromuscular function assessment was performed before and 1 min post-exercise. Ventilation and pulmonary gas exchange were recorded continuously throughout cycling exercise.

Neuromuscular function assessment

Participants were seated on a custom-built isometric chair adjusted to enable hip and knee joint angles of 90° (Becker & Awiszus, 2001). Upper body movement was minimised via two cross-shoulder straps. EMG activity of the vastus lateralis (VL) was recorded using surface electrodes (Kendall H59P, Coviden, Massachusettes, USA). Electrodes were positioned based on the SENIAM recommendations (Hermens *et al.* 1999). The reference electrode was fixed to the right patella. All electrodes were marked with indelible ink to ensure consistent electrode placement between sessions. All raw EMG data was amplified (gain x1000), digitized at 4 kH and filtered using a digital band-pass filter with high cut-off frequency of 2 kHz and a low cut-off frequency of 20 kHz. All data was recorded and processed off-line using a data acquisition system (PowerLab 26T with LabChart 7, ADInstrument Ltd, Oxford, UK).

Single and paired square-wave electrical stimulations (200 µs pulse width) were delivered via adhesive surface electrodes to the femoral nerve (ValuTrode, Axelgaard, Fallbrook, USA) using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK). Therefore, the cathode was positioned in the femoral triangle and the anode midway between the iliac crest and the greater trochanter. Stimulation threshold was determined by delivering two single stimuli separated by 5 s to the femoral nerve and current was increased progressively (+ 20 mA) starting at 10 mA until no further increase in M-wave peak-to-peak amplitude and resting twitch force was evoked. Stimulation intensity was set at 130% to ensure full spatial motor-unit recruitment. The determination of stimulation threshold was conducted prior to each first NMFA of every subsequent trial.

Prior to the first NMFA of each visit, participants performed a standardised isometric warm-up with their right knee extensors which consisted of ten 3 s isometric contractions with progressively increasing contraction intensity and maximal efforts during the last three contractions (3s on – 7 s off) (adapted from Girard *et al.* 2013). Additional MVCs were performed if coefficient of variation (CV) over three MVCs was ≥ 5%. Neuromuscular function assessment involved the completion of five isometric 3 s MVCs separated by 20 s rest. Paired stimuli at 100 Hz (PS100) were delivered during and 2 s after the last three contractions, followed by paired stimuli at 10 Hz (PS10) and a single stimulus (Q_{pot}). The time window between exercise termination and the start of the first MVC for NMFA was standardised to 60 s for every participant and every session to avoid different magnitudes of neuromuscular function recovery due to different time windows between test termination and the start of NMFA. Real-time visual feedback was displayed throughout each effort as recommended by Gandevia (2001).

Peak MVC was defined as the greatest 0.5 s mean force produced prior to electrical stimulation and reported as the mean of five MVCs. Potentiated twitch force was measured as the peak twitch force minus the onset force of the twitch evoked in response to supramaximal stimulation. Low-frequency fatigue (LFF) was determined as the ratio between twitch forces evoked by low- and high-frequency paired stimuli (PS10:PS100). Within-twitch measures (i.e. contraction time, CT; maximal rate of force development, MRFD; maximal rate of relaxation, MRR; and half-relaxation time, HRT) were derived from each resting twitch. One participant was excluded from the data analysis for MRR, HRT and MRFD after being identified as outlier (values were greater than two standard deviations from the mean). Voluntary activation was calculated using the interpolated paired stimulation technique (Merton, 1954). The peak-to-peak amplitude (PPA) was measured as the absolute difference of the maximum and minimum point of the biphasic M-wave and the M-wave area was determined as the integral of the absolute value of the M-wave. One participant was excluded from the data analysis for M-wave parameters due to technical issues. For twitch forces, within-twitch parameters, VA and M-wave properties, the mean of three was reported for each NMFA.

Statistical analysis

All data was analysed using a standardised statistical package (SPSS version 22 for Windows, IBM Corporation, New York, USA) and reported as mean ± SD, unless stated otherwise. Each data set was assessed for normal distribution using the Shapiro-Wilk´s test and sphericity was checked using the Mauchly´s test. Two-way repeated measures ANOVA on the factors "condition" (P-3 vs. P-12) and "time" (pre- vs. post-exercise) were used to test for differences in neuromuscular, physiological and perceptual-measures. Post-hoc analysis was performed following a significant main or interaction effect using Bonferroni post-hoc adjusted pairwise comparisons. Paired-samples *t*-tests were used to compare the $\dot{V}O_{2peak}$ achieved during the ramp incremental test and the verification trial. Relationships were investigated using Pearson´s product-moment correlations or partial correlations. The level of significance was set at $P < 0.05$. Partial eta squared (η_p^2) was calculated for main and interaction effects (Cohen, 1988).

Results

Incremental test and determination of CP and *W'*

P_{peak} was 337 ± 46 W. There was no significant difference in $\dot{V}O_{2peak}$ achieved during the fast ramp test (51.6 ± 9.4 ml.min^{-1}.kg^{-1}) compared to the subsequent verification trial (48.8 ± 7.9 ml.min^{-1}.kg^{-1}) ($t_{(11)} = 2.17$; $P = 0.053$). Six out of 12 participants achieved a $\dot{V}O_{2max}$ (rise in $\dot{V}O_2$ of less than 2.1 mL.kg^{-1}.min^{-1}; Taylor et al., 1955). CP and *W'* were 220 ± 46 W (65.1 ±

8.1% P_{peak}) and 19.9 ± 6.0 kJ with associated standard errors of 2.7 ± 1.3 W and 1.2 ± 0.6 kJ. Mean power outputs for P-3 and P-12 were 329 ± 47 W (98 ± 4% P_{peak}) and 248 ± 45 W (73 ± 6% P_{peak}), respectively.

Experimental trials

Maximal voluntary force. MVC decreased significantly from pre- to post-exercise by 20 ± 10% and 15 ± 7% for P-3 and P-12, respectively ($F_{(1,11)} = 35.23$; $P < 0.001$; $\eta_p^2 = 0.76$), with no significant main effect for condition ($F_{(1,11)} = 0.34$, $P = 0.57$, $\eta_p^2 = 0.03$) and no interaction effect ($F_{(1,11)} = 3.64$; $P = 0.08$; $\eta_p^2 = 0.25$) (Fig. 1A and Table 1).

Potentiated twitch force and doublet twitch forces. Potentiated twitch force, PS10, PS100 and PS10:100 were significantly reduced by 35 ± 13%, 38 ± 13%, 18 ± 9% and 26 ± 11% following P-3 and by 31 ± 14%, 37 ± 17%, 13 ± 8% and 27 ± 15% following P-12 (Q_{pot}: $F_{(1,11)} = 95.96$; $P < 0.001$; $\eta_p^2 = 0.90$; PS10: $F_{(1,11)} = 109.30$; $P < 0.001$; $\eta_p^2 = 0.91$; PS100: $F_{(1,11)} = 52.64$; $P < 0.001$; $\eta_p^2 = 0.83$, PS10:100: $F_{(1,11)} = 71.33$; $P < 0.001$; $\eta_p^2 = 0.87$) (Fig. 1 and Table 1). There was no significant main effect for condition ($P > 0.05$; Fig. 1 and Table 1) and no interaction effect ($P > 0.05$).

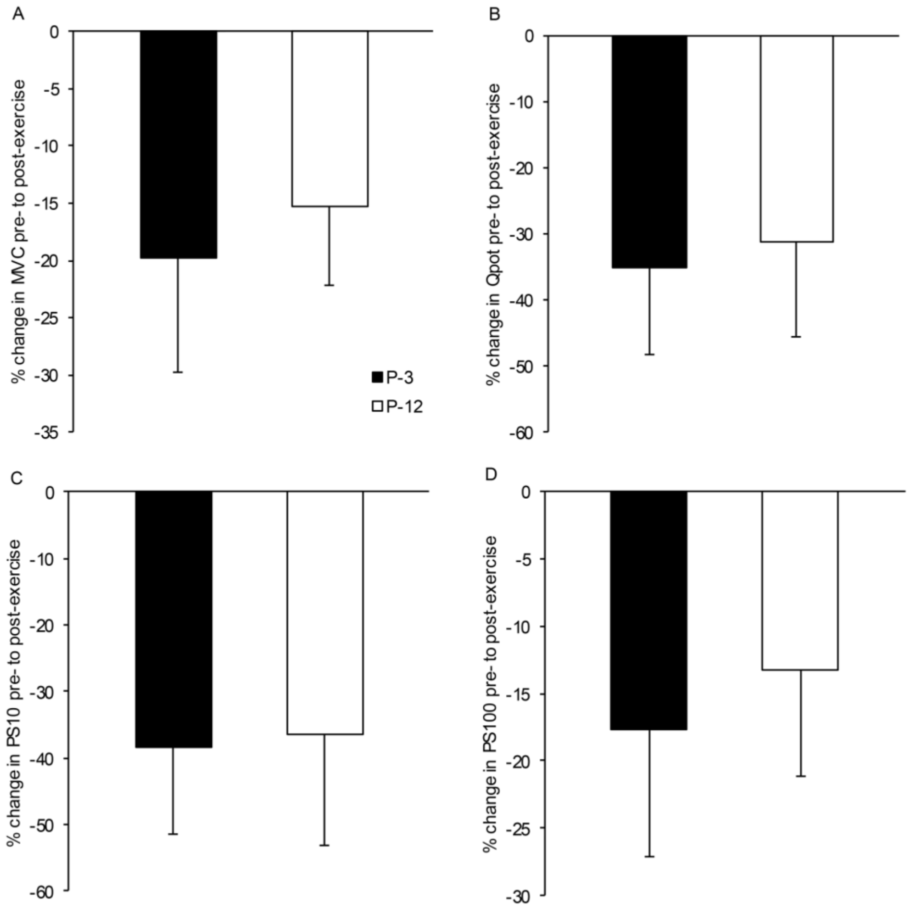

Figure 1. Pre- to post-trial percentage change in maximal voluntary contraction (MVC; A), potentiated twitch force (Qpot; B), low-frequency (10 Hz) doublet force (PS10; C) and high-frequency (100 Hz) doublet force (PS100; D) for 3 (P-3) and 12 min (P-12).

M-wave properties. M-wave PPA and M-wave area showed no significant main effect for time ($F_{(1,10)}$ = 0.47; P = 0.51; P = 0.06; η_p^2 = 0.05) or condition (P = 0.16; P = 0.45; Table 1) and no interaction effect ($F_{(1,10)}$ = 1.92; P = 0.20; P = 0.07; η_p^2 = 0.16).

Voluntary activation. VA decreased significantly pre- to post-exercise by 11% and 12% for P-3 and P-12 ($F_{(1,11)}$ = 53.51; P < 0.001; η_p^2 = 0.83), with no main effect for condition (Table 1) and no interaction effect ($F_{(1,11)}$ = 0.13; P = 0.73; η_p^2 = 0.01).

Table 1. Neuromuscular measures at pre-exercise (PRE) and following exhaustive constant-load cycling (POST) for 3 (P-3) and 12 min (P-12).

	P-3		P-12	
Parameter	PRE	POST	PRE	POST
Neuromuscular fatigue				
MVC (N)	573 ± 128	451 ± 72*	563 ± 110	473 ± 77*
Peripheral fatigue				
Q_{pot} (N)	156 ± 23	100 ± 19*	150 ± 18	104 ± 26*
PS10 (N)	224 ± 44	137 ± 36*	212 ± 36	136 ± 46*
PS100 (N)	228 ± 25	186 ± 18*	222 ± 26	192 ± 25*
PS10:PS100	0.98 ± 0.13	0.73 ± 0.17*	0.96 ± 0.11	0.70 ± 0.17*
CT (ms)	77 ± 12	70 ± 8*	74 ± 8	67 ± 7*
MRFD# ($N \cdot ms^{-1}$)	4.92 ± 0.98	2.61 ± 0.70*	4.96 ± 1.00	3.23 ± 1.39*
MRR# ($N \cdot ms^{-1}$)	-1.77 ± 0.47	-1.07 ± 0.28*	-1.74 ± 0.46	-1.36 ± 0.36*†
HRT# (ms)	75.5 ± 13.2	78.2 ± 18.1	74.8 ± 10.8	61.2 ± 14.6†
Central fatigue				
VA (%)	88 ± 5	77 ± 15*	88 ± 8	76 ± 9*
Surface EMG				
M-wave PPA# (mV)	9.3 ± 2.5	9.2 ± 3.9	8.7 ± 2.4	8.9 ± 2.0
M-wave area# ($\mu V \cdot s^{-1}$)	30.5 ± 12.9	34.9 ± 14.9	29.8 ± 11.4	30.3 ± 12.3

Data are presented as mean ± SD (n = 12). Abbreviations: MVC, maximal voluntary contraction; Q_{pot}, potentiated twitch force; PS10, low-frequency (10 Hz) doublet force; PS100, high-frequency (100 Hz) doublet force; CT, contraction time; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; HRT, half-relaxation time; M-wave PPA, M-wave peak-to-peak area; VA, voluntary activation; *P < 0.05 *versus* PRE, †P < 0.05 *versus* P-3 at POST; #n = 11.

Bivariate correlations between changes in neuromuscular function and measures of aerobic and anaerobic capacities.

A larger *W′* was associated with larger reductions in Q_{pot} (r = 0.65; P = 0.022) and PS10 (r = 0.62; P = 0.033) for P-3 (Fig. 2). No significance was obtained for relationships between aerobic capacity (CP and $\dot{V}O_{2peak}$) and measures of neuromuscular fatigue for P-3 (P > 0.05), except an inverse relationship between CP and PS10 (r = -0.63; P = 0.028). In contract for P-12, the changes in neuromuscular function did not correlate with *W′* for P-12 (P > 0.05). However, changes in MVC were inversely related to CP (r = -0.74; P = 0.006) and $\dot{V}O_{2peak}$ (r = 0.66; P = 0.019), so that individuals with greater aerobic capacities showed smaller changes in MVC (Fig. 3). Similar relationships were found between changes in evoked twitch forces and $\dot{V}O_{2peak}$ for P-12, with smaller changes in Q_{pot} (r = 0.65; P = 0.023) and PS10 (r = 0.58; P = 0.047) for individuals of higher $\dot{V}O_{2peak}$ (Fig. 3).

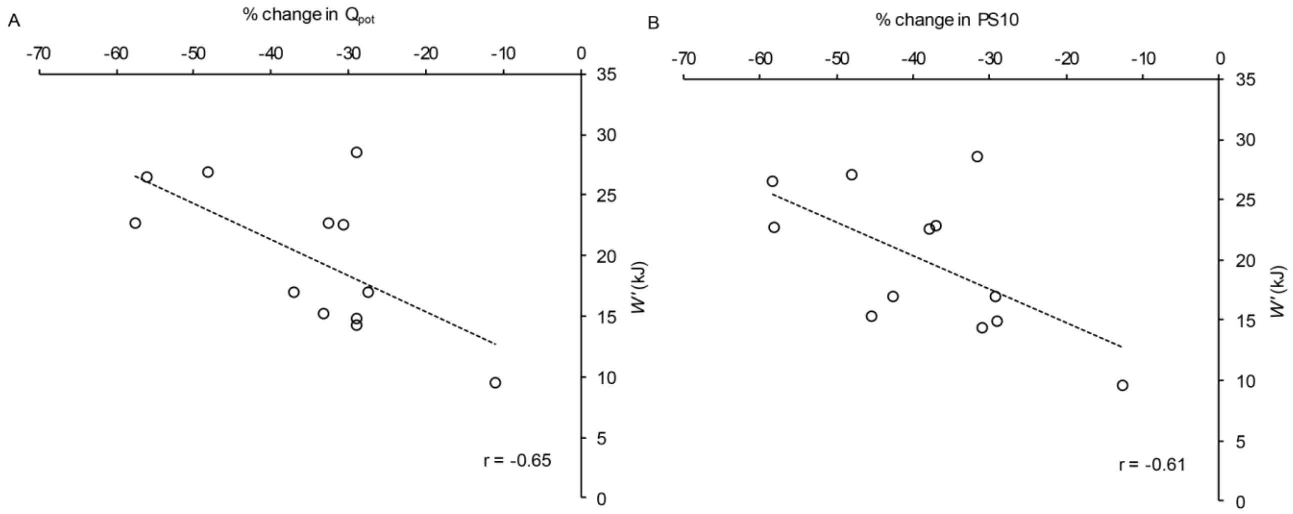

Figure 2. Correlations between *W′* and % change in maximal voluntary contraction, MVC (A) and between *W′* and % change in potentiated twitch force, Q_{pot} (B) for P-3. Pearson's correlation coefficient (r) are displayed. Correlations were significant (P < 0.05).

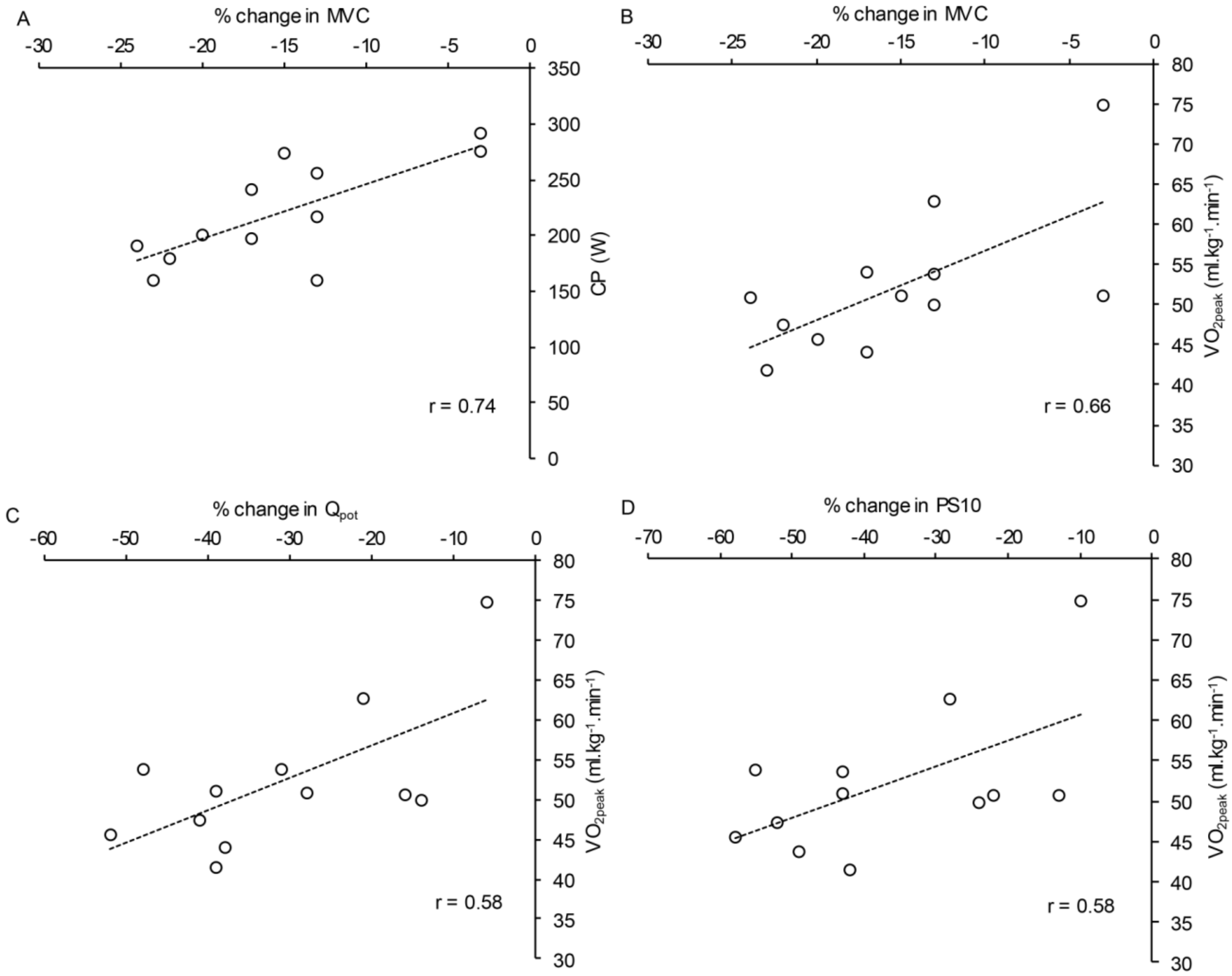

Figure 3. Correlations between CP and % change in MVC (A), VO_{2peak} and % change in MVC (B), VO_{2peak} and % change in Q_{pot} (C), VO_{2peak} and % change in PS10 (D) for P-12. Pearson's correlation coefficient (r) are displayed. All correlations were significant ($P < 0.05$)

Discussion

The present study is the first to demonstrate that neuromuscular fatigue observed following full depletion of *W'* is of similar magnitude whether supra-CP cycling exercise is performed close to the lower vs. upper boundary of the severe intensity domain. The level of peripheral fatigue in the severe intensity domain does therefore not depend on power output or exercise duration when 100% of *W'* has been exhausted above CP.

Peripheral fatigue after high-intensity cycling above CP

Peripheral fatigue has previously shown to be duration- and intensity-dependent, with greater loss of evoked twitch forces following shorter, highly intense exercise when compared to longer, low-intensity exercise (Temesi *et al.* 2017; Thomas *et al.* 2015; 2016; O'Leary *et al.* 2015; Burnley *et al.* 2012). The present study found reductions in Q_{pot} following severe cycling exercise of 30-35%, which is in line with previously reported reductions of -20 to -40% following whole-body high-intensity exercise (Dominelli *et al.* 2017; Goodall *et al*. 2015; O'Leary *et al.* 2015; Johnson *et al.* 2015; Thomas *et al.* 2015; Amann, 2011), but with no difference between P-3 and P-12.These differences between studies may be due to the design of the task (e.g. open-end vs. closed-end test, exercise mode). This has been considered in the 'sensory tolerance limit theory', refined from the 'critical threshold' concept of peripheral fatigue. The sensory tolerance limit theory described a more global negative feedback loop, taking the sum of numerous factors into account (locomotor muscles, respiratory muscles, organs and muscles not directly involved in exercise (Hureau *et al.*, 2016).

The present study confirms and expands the findings of Burnley *et al.* (2012) to whole-body exercise. The authors reported substantial reductions in the force generating capacity following single-leg contractions at different intensities above critical torque (~40-55%). These impairments were predominantly associated with alterations at or distal to the neuromuscular junction (PS100: ~32-35%). These changes are greater than those of the present study, which may be due to an underestimation of the magnitude of fatigue as a result of a delayed NMFA following cycling exercise in the present study or due to differences in exercise modalities per se (knee extensions vs. cycling exercise). Indeed, greater magnitudes of peripheral fatigue have been demonstrated following exercise involving smaller muscle mass by Rossman *et al.* (2014; 2012).

Interestingly, the reductions in potentiated twitch force observed following P-12 (-31%) are substantially greater than those following a ~11 min constant-load trial performed until task failure reported by Thomas *et al.* (2016) (-16%). The decision-making behaviour involved in the performance of a time to task failure may lead to a premature end of the task, i.e. before 100% of *W'* is depleted, and participants may therefore stop before reaching their 'true' physiological limits. This would further lead to an underestimation of the magnitude of neuromuscular fatigue.

In the present study, PS10 and PS100 were significantly reduced following exercise, but with no significant difference between P-3 and P-12. A proportionally greater reduction in twitch force was observed for PS10 (~27%), described as low-frequency fatigue (LFF) (Verges *et* *al.*, 2009). These findings are in accordance with the reduction in PS10:100 (~30%) reported by Temesi *et al.* (2017) following 6-min cycling exercise within the severe intensity domain (80% P_{peak}). LFF has been associated with a reduction of Ca^{2+} release from the SR (Balog, 2010; Allen *et al.* 2008a; Keeton & Binder-Macleod, 2006; Rassier & MacIntosh, 2000).

Reductions in Q_{pot} may be mediated by alterations of the sarcolemmal excitability, measured by changes in M-wave PPA and M-wave area. Whereas M-wave PPA did not differ significantly pre- to post-exercise in both trials, changes in M-wave area were more pronounced following P-3 compared to P-12 (+14% vs. +2%). This would suggest for a greater disturbance in the propagation or transmission of action potentials following high-intensity cycling exercise in the upper part of the severe intensity domain. In contrast, Black *et al.* (2017) found changes in M-wave amplitude and area of greater extent following moderate and heavy exercise compared to severe intensity exercise, suggesting that sarcolemmal excitability is more affected after longer, low-intensity exercise. Previous studies described controversial results with some studies reporting increases, decreases or no changes in M-wave properties (for review see Rodriguez-Falces & Place, 2018). These discrepancies may be explained by differences in the muscle studied and/or methodological differences (i.e. stimulation technique, electrode placement). Furthermore, the modest reliability reported for surface EMG recordings of voluntary and evoked contractions (Ball & Scurr, 2010; Buckthorpe *et al.* 2012; Rota *et al.* 2013) may be considered when discussing meaningful change.

Central fatigue after high-intensity cycling above CP

Changes in VA of 5 to 10% have been found following exercise within the severe intensity domain (Temesi *et al.* 2017; Thomas *et al*. 2016; 2015; Johnson *et al.* 2015; Goodall *et al.* 2015; Sidhu *et al.* 2014), with greater reductions in the lower part of the domain (Thomas *et al.*, 2016; Burnley *et al.*, 2012). The present study is in line with these findings, observing moderate reductions in VA but with no difference between the trials (P-3: -11%; P-12: -12%). Exercise-induced reduction in VA implies that central fatigue develops due to a suboptimal neural drive from the motor cortex (supra-spinal fatigue) and/or changes in the intrinsic properties of the motor neurons (spinal fatigue) (Taylor *et al.* 2006; Gandevia, 2001; 1998).

Exploratory research using bivariate correlation between changes in neuromuscular function and indices of aerobic and anaerobic capacities

The magnitude of the changes in neuromuscular fatigue showed large variability between participants (reduction in MVC: CV ~50% for P-3; CV ~46% for P-12). De Souza *et al.* (2016) reported similar results with great between-participant variability (>50% CV) in peak torque reduction after a fatiguing cycling exercise (70% *W'* depletion in 3 and 10 min) and suggested that this may be due to an inverse relationship between CP and the change in peak torque. Further, Coelho *et al.* (2015) reported an inverse relationship between the reduction in isokinetic power and $\dot{V}O_{2peak}$ following a maximal incremental cycling test. In agreement, in the present study, changes in MVC following P-12 were inversely related to CP (r = -0.74; P = 0.006) and $\dot{V}O_{2peak}$ (r = -0.66; P = 0.019; Fig. 3). Participants of high $\dot{V}O_{2peak}$ also displayed smaller changes in Q_{pot} (r = -0.65; P = 0.023) and PS10 (r = -0.58; P = 0.047; Fig. 3). Aerobically fitter participants seem to be coping better with the development of peripheral fatigue during severe intensity exercise of longer duration. This may be because of a faster and greater O_2 delivery alongside structural adaptations within the exercising muscles (Murgatroyd *et al.* 2011; Rossiter, 2011) which may reduce or delay the accumulation of fatigue-related metabolites.

In contrast, for P-3, the changes in MVC were not significantly correlated with $\dot{V}O_{2peak}$ or CP (P > 0.05). A larger *W'* was associated with larger reductions in Q_{pot} (r = 0.65; P = 0.022) and PS10 (r = 0.62; P = 0.033; Fig. 2). Although bivariate correlations do not prove a causal relationship exists, these significant relationships support the links between utilisation of *W'* and development of peripheral fatigue as suggested by Murgatroyd *et al.* (2011). It would be worth noting here that individuals with greater MVC pre-exercise also showed greater reductions following P-12 (r = 0.85; P < 0.001) and P-3 (r = 0.79; P = 0.002) but did not have greater *W'* (P-12: r = 0.35; P = 0.27; P-3: r = 0.37; P = 0.24). It may be suggested that aerobic capacity is of greater relevance during P-12 as the aerobic contribution relative to the total energy turnover increases with increasing exercise duration, whereas during P-3 the anaerobic work capacity contributes to a relatively larger extent to the total energy turnover.

Delimitations and limitations

In the protocol of the present study, muscular activation must have differed between P-3 and P-12 (intensity x time effect) for all flexors and extensors contributing to external power production, and most likely so for the knee extensors, i.e. the muscle group tested during the NMA protocol. An intensity x time effect on VL activation during the two cycling trials would have affected the physiological processes underpinning muscle force generation of both MVC and twitch forces recorded following cycling. This would likely interfere with the association proposed in the present framework between the use of *W’* and the key measures of NMF.

A major methodological limitation in studies investigating neuromuscular fatigue following locomotor exercise lies in the time delay between exercise termination and neuromuscular assessment due to the transition from the cycle ergometer or treadmill to the dynamometer. However, the present study standardised the time window for the transition to 60 s for each trial and all neuromuscular assessments were completed within 100 s. The delayed assessment of neuromuscular measures likely caused an underestimation of the magnitude of neuromuscular fatigue due to significant recovery of neuromuscular function within the first 1-2 min after exercise termination (Froyd *et al.* 2013). Furthermore, the isometric contraction used to assess muscular force generating capacity does not represent the dynamic contraction pattern during cycling exercise. Therefore, results should be interpreted with caution.

In conclusion, the present study demonstrates for the first time that the magnitude of neuromuscular fatigue observed following full depletion of *W’* is similar when supra-CP exercise is performed close to the lower or upper boundary of the severe intensity domain. Further exploratory analysis showed that smaller changes in the force-generating capacity are seen in individuals with greater aerobic capacities for the longer severe-intensity exercise, but in individuals with greater anaerobic capacities for the shorter severe-intensity cycling exercise. Thus, despite no difference in the magnitude of neuromuscular fatigue following a short vs. long bout of severe intensity exercise, the present results suggest for different physiological mechanisms to underlie exercise tolerance within the lower vs. upper boundary of this intensity domain. Future research should aim to provide experimental evidence for a causal relationship between *W’*, CP and neuromuscular fatigue in order to further understand exercise tolerance within the severe intensity domain.

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Additional information

Competing interests

None declared.

Author contributions

All experiments were performed in the Welkin Human Performance Laboratories, University of Brighton, UK.

Conception and design of the work: L.U.S., J.D., M.H. Acquisition, analysis and interpretation of data for the work: L.U.S., J.D., M.H. Drafting of the work or revising it critically for important intellectual content: L.U.S., J.D., M.H. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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