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Creatine supplementation improves performance above critical power but does not influence the magnitude of neuromuscular fatigue at task failure.

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Running Title

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New Findings

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

  Does the magnitude of neuromuscular fatigue depend on the amount of work done (*W'*) at task failure when cycling above critical power (CP)?

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

  Creatine supplementation increases *W'* and enhances supra-CP performance, but induces similar magnitudes of neuromuscular fatigue at task failure compared to placebo. Increased *W'* does not lead to higher levels of neuromuscular fatigue. This supports the notion of a critical level of neuromuscular fatigue at task failure and challenges a direct causative link between *W'* depletion and neuromuscular fatigue.

Abstract

The present study examined the effect of creatine supplementation on neuromuscular fatigue and exercise tolerance when cycling above critical power (CP). Eleven males performed an incremental cycling test, 4-5 constant-load trials to task failure (TTF) to obtain asymptote (CP) and curvature constant (*W'*) of the power-duration relationship, followed by three constant-load supra-CP trials: 1) one TTF following placebo supplementation (PLA); 2) one TTF following creatine supplementation (CRE); and 3) one trial of equal duration to PLA following creatine supplementation (ISO). Neuromuscular assessment of the right knee extensors was performed pre- and post-exercise to measure maximal voluntary contraction (MVC), twitch forces evoked by single (Q_{pot}) and paired high- (PS100) and low-frequency (PS10) stimulations and voluntary activation. Creatine supplementation increased TTF in CRE *vs.* PLA by ~11% (P = 0.017) and work done above CP by ~10% (P = 0.015), with no difference (P > 0.05) in reductions in MVC (-24 ± 8 *vs.* -20 ± 9%), Q_{pot} (-39 ± 13 *vs.* -32 ± 14%), PS10 (-42 ± 14 *vs.* -36 ± 13%), PS100 (-25 ± 10 *vs.* -18 ± 12%) and voluntary activation (-7 ± 8 *vs.* -5 ± 7%) in CRE *vs.* PLA. No significant difference were found between ISO and both PLA and CRE (P > 0.05). These findings suggest similar levels of neuromuscular fatigue can be found following supra-CP cycling despite increases in performance time and amount of work done above CP, supporting the notion of a critical level of neuromuscular fatigue and challenging a direct causative link between *W'* depletion and neuromuscular fatigue.

Introduction

An individual's tolerance to high-intensity exercise can be mathematically calculated from modelling the power-duration relationship. The well-established critical power concept defines critical power (CP) as the asymptote and *W'* as the curvature constant of this hyperbolic relationship (Monod & Scherrer, 1965). When using this two-parameter model, one assumes that exercise above CP depletes *W'*, with task failure occurring when this mathematically finite amount of work is fully depleted (Monod & Scherrer, 1965; Moritani *et al.*, 1981; Poole *et al.*, 1988). Interestingly, *W'* has long been associated with the use of an anaerobic energy store (Jenkins & Quigley, 1993; Smith *et al.*, 1998a; Miura *et al.*, 1999; Miura *et al.*, 2000) although its solely anaerobic

nature has been questioned due to its sensitivity to interventions altering O_2 delivery (Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012). The depletion of *W'* has been associated with the accumulation of fatigue-related metabolites (i.e. P_i, H^+, ADP, La^-) to a critical level (Burnley et al., 2010; Ferguson et al., 2010; 2007; Poole et al., 1988) and it is further suggested that these metabolic perturbations may also contribute to the continued reduction in muscle efficiency, proposed as the 'fatigue cascade' by Murgatroyd *et al.* (2011).

More recently, a continuous decline in muscle [PCr] has been demonstrated during exercise above CP (Jones *et al.*, 2008). It has been suggested that task failure within the severe intensity domain, i.e. when exercising above CP, occurs when a critical level of intramuscular [PCr], [P_i] and/or pH is reached (Jones *et al.*, 2008; Vanhatalo *et al.*, 2010). These intramuscular metabolic disturbances have been associated with the development of substantial levels of peripheral fatigue, i.e. a reduction in the force-generating capacity of the muscle induced by alterations at or distal to the neuromuscular junction (Allen *et al.*, 2008; Burnley *et al.*, 2010). Interestingly, similar magnitudes of peripheral fatigue, i.e. reductions in evoked twitch forces (~35%), have been observed following exercise across a wide range of supra-CP intensities performed until task failure (Amann *et al.*, 2006; Romer *et al.,* 2007; Amann & Dempsey, 2008; Amann *et al.,* 2009; 2011; Johnson *et al.,* 2015; Thomas *et al.,* 2015; Hureau *et al.,* 2016) and led Amann *et al.* (2006) to introduce the concept of a "critical threshold of peripheral fatigue". The theory behind this concept proposes that group III and IV muscle afferents detect fatigue-related metabolites within the exercising muscles and regulate the central motor drive accordingly in order to limit the magnitude of peripheral fatigue and maintain muscle and overall homeostasis of the organism.

Only recently have studies combined the CP concept with neuro-stimulation techniques to further understand the neurophysiological limits of high-intensity exercise (Burnley *et al.*, 2012; Schäfer *et al.*, 2019). Schäfer *et al.* (2019) reported a positive correlation between an individual's anaerobic work capacity (*W'*) and changes in neuromuscular function (i.e. maximal voluntary contraction, MVC; potentiated twitch force, Q_{pot}; twitch forces evoked by low-frequency stimulations at 10 Hz, PS10) following cycling exercise above CP. This suggests a greater level of peripheral fatigue

at task failure in individuals able to accumulate a larger amount of work above CP. However, this is yet to be explored within individuals. In line with the above, the manipulation of an individual *W'* via creatine supplementation should increase the *W'* of a severe intensity exercise and induce greater levels of peripheral fatigue at task failure.

Indeed, creatine supplementation has the potential to test the relationship between *W'* and neuromuscular fatigue reported by Schäfer *et al.* (2019) through manipulation of an individual's anaerobic work capacity. Such interventions aiming to increase total creatine stores ([TCr]; i.e. sum of phosphocreatine [PCr] and free creatine [Cr]) have also been shown to enhance the muscular capacity for PCr hydrolysis leading to longer time to task failure (Smith et al., 2004; 1998b). Greater fatigue-induced metabolic disturbances (i.e. higher [P_i], [Cr], [PCr/Cr]) have also been reported following high-intensity knee-extension exercise under creatine loading compared to placebo (Smith et al., 2004; 1998b). An increase in muscle [TCr] by up to 20% ($^1/_3$ in form of PCr) following creatine supplementation has previously been demonstrated (Finn *et al.*, 2001; Casey *et al.*, 1996; Greenhaff *et al.* 1994; Harris *et al.*, 1992). The effect of creatine supplementation on high-intensity performance has been intensively studied since the 1990s (Rossiter *et al.*, 1996; Jacobs *et al.*, 1997; Smith *et al.*, 1998a; McNaughton *et al.*, 1998; Miura *et al.*, 1999). Improvements in time to task failure of up to 24% have been observed, with greater changes observed following shorter, more intense exercise during which the contribution of the anaerobic energy turnover becomes more predominant (Jacobs et al., 1997; Prevost *et al.*, 1997; Smith *et al.*, 1998a; Branch, 2003). In addition, creatine supplementation increased *W'* by 10-25%, without affecting CP (Smith *et al.*, 1998a; Miura *et al.*, 1999; Eckerson *et al.*, 2005). These findings provide support for a significant role of muscle Cr/PCr content in high-intensity performance and evidence the primarily anaerobic nature of *W'*.

Whereas the effect of creatine on performance is well-established, very little is known about its effect on neuromuscular fatigue. Creatine supplementation has been reported to influence neuromuscular measures (Stout *et al.* 2000; Smith *et al.*, 2007). Stout *et al.* (2000) reported a greater physical working capacity at the fatigue threshold (+ 20%), measured as the highest power output that does not result in an increase in EMG activity over time, following five days of creatine loading, which was thought to

indicate a delay in the onset of neuromuscular fatigue. Similarly, Smith *et al.* (2007) found an increase in the electromyographic fatigue threshold during cycle ergometry (+ ~15%). However, whether creatine supplementation alters neuromuscular fatigue at task failure following exercise above CP remains unclear. The integration of the CP concept with electromyographic and mechanical measures of neuromuscular fatigue may offer further insights into the limits of exercise tolerance within the severe-intensity domain.

Therefore, the aim of the present study was to provide experimental evidence for an association between the use of *W'* and the development of neuromuscular fatigue using creatine supplementation. We hypothesised that: (1) creatine supplementation would improve performance (i.e. time to task failure) by increasing the amount of work done above CP; (2) a greater amount of work done above CP would increase the magnitude of neuromuscular fatigue observed at task failure; (3) the same absolute amount of work completed above CP (i.e. exercise time in control *vs.* "isotime") would lead to the same magnitude of neuromuscular fatigue regardless of creatine supplementation.

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 11718) and conformed to the standards set by the latest Declaration of Helsinki, except for registration in a database.

Participants

Eleven recreationally active, non-vegetarian male participants (mean ± SD: age, 22.6 ± 2.8 years; body mass, 75.8 ± 11.5 kg; peak O_2 consumption ($\dot{V}O_{2peak}$), 51.7 ± 8.3 $ml.min^{-1}.kg^{-1}$; peak power output (P_{peak}), 311 ± 37 W) volunteered for this study. All participants were familiar with cycle ergometry and the exercise procedures used in our laboratory.

Study Design

Participants reported to the laboratory on nine to ten different occasions over a 5 to 6 week period, with each test separated by a minimum of 24 h and performed at the same time of day (± 2 h) to control for the effect of diurnal variation (Atkinson & Reilly, 1996) . 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 three constant-load trials to investigate the effect of creatine supplementation on neuromuscular function in the fresh state and following constant-load cycling above CP. These last three main experimental trials were separated by 5 to 7 days.

All tests were performed on an electromagnetically braked, computer-controlled cycle ergometer (SRM High Performance Ergometer with eight strain gauges; Schoberer Rad Meßtechnik, Jülich, Germany). Seat height, handlebar height and distance from the seat to the handlebar were adjusted and replicated for each participant for the duration of the study. Ventilatory and pulmonary gas exchange were measured using a breath-by-breath system (Metalyzer Sport; Cortex Biophysik, Leipzig, Germany). Each session was preceded by 3 min rest, 5 min at 50 W, 3 min rest and 4 min at 20 W. Participants were instructed to maintain a cadence of 80 rpm throughout all sessions and exercise was terminated when cadence dropped twice <75 rpm for >5 s despite strong verbal encouragement. 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, to refrain from alcohol (24 h) and caffeine consumption (12 h) before testing and to avoid its consumption throughout the supplementation period prior to each main trial.

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. P_{peak} and $\dot{V}O_{2peak}$ were defined as the highest 15 s moving average.

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 during a separate visit.

Determination of CP and *W'*

Participants completed a series of four to five constant-load tests in a semi-randomized order to elicit task failure within ~3 and 15 min (Poole *et al.*, 1988; Hill, 1993). Participants were not informed of the elapsed time or any other performance measure throughout testing except cadence.

For each participant, three different models were used to obtain estimates for CP and *W'* (least-squares regression model), as follows:

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

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

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

$$W = CP \times t_{lim} + W' \quad (2)$$

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

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

The regression model that best fitted the data for each participant (lowest standard error (SE) for CP and *W'*) was selected and an additional fifth trial was performed if these SEs were >2% and >10% of CP and *W'*, respectively (Murgatroyd *et al.*, 2011; Dekerle *et al.*, 2015). The 95% confidence interval for the CP estimate was calculated to ensure that power outputs for the main trials were confidently above CP.

Experimental Trials

Power output for the subsequent three experimental trials was predicted to fully deplete *W'* within 3 min and was calculated for each participant from interpolation of the power-time relationship. Trials were performed at 97 ± 7%P_{peak} 1) until task failure following placebo supplementation (PLA); 2) until task failure following creatine supplementation (CRE); and 3) for an equal duration to PLA following creatine supplementation (ISO). CRE and ISO were performed in a randomised order. Ventilation and pulmonary gas exchange were recorded continuously throughout

cycling exercise. Neuromuscular function assessment was performed before and 60 s post-exercise. Therefore, participants were seated on a custom-built isometric chair adjusted to enable hip and knee joint angles of 90 deg (Becker & Awiszus, 2001) and two cross-shoulder straps were used to minimize upper body movement. The EMG activity of the vastus lateralis was recorded using surface electrodes (Kendall H59P; Covidien, Mansfield, MA, USA) positioned based on the SENIAM recommendations (Hermens *et al.*, 2000). The reference electrode was fixed to the right patella. Consistent electrode placement between sessions was ensured by marking each electrode position with indelible ink. EMG data were amplified (gain x1000), digitized at 4 kHz and band-pass filtered (2-20 kHz). All data were recorded and processed offline using a data acquisition system (PowerLab 26T with LabChart 7; ADInstrument Ltd, Oxford, UK).

Single and paired square-wave electrical stimulation (200 µs pulse width) were delivered via adhesive surface electrodes to the femoral nerve (ValuTrode; Axelgaard, Fallbrook, CA, 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. The 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. The stimulation intensity was set at 130% to ensure full spatial motor unit recruitment. Determination of the stimulation threshold was conducted before each first NMFA of every subsequent trial.

The first NMFA of each visit was preceded by a standardized isometric warm-up with the right knee extensors, involving ten 3 s isometric contractions with progressively increasing contraction intensity and maximal efforts during the last three contractions (3 s on – 7 s off; adapted from Girard *et al.*, 2013). Additional MVCs were performed if the coefficient of variation (CV) over three MVCs was ≥5%. Each NMFA involved 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}). Real-time visual feedback was displayed throughout as recommended by Gandevia (2001) and the time window between exercise termination and the first MVC for NMFA was standardised to 60 s for every participant and every session.

Peak MVC was measured as the greatest 0.5 s mean force produced before electrical stimulation and reported as the mean of five MVCs. Potentiated twitch force was defined as the greatest peak twitch force in response to supramaximal stimulation. The ratio between twitch forces evoked by low- and high-frequency paired stimuli (PS10:PS100) was calculated to determine low-frequency fatigue. 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. Voluntary activation was calculated using the interpolated paired stimulation technique (Merton, 1954). One participant was excluded from the data analysis for VA after values were identified as outliers using the interquartile range (Tukey, 1977). M-wave peak-to-peak amplitude (PPA) was measured as the absolute difference of the greatest and smallest value of the biphasic M-wave, and M-wave area was determined as the integral of the absolute value of the M-wave. For twitch forces, within-twitch parameters, VA and M-wave properties, the mean of three was reported.

Supplementation, Urinary Creatinine and Body Mass

All participants ingested 4x 5 $g.d^{-1}$ of dextrose (PLA) during the first 5-day supplementation period. Prior to the second main trial, participants ingested 4 x 5 $g.d^{-1}$ of creatine monohydrate for five successive days and during the third supplementation period, participants ingested a maintenance dose of 2 $g.d^{-1}$ of creatine for each day between the second and the third main trial (Hultman *et al*., 1996). Each dose was dissolved in 200 ml of warm water and flavoured with no added sugar orange squash. Supplements were taken at regular intervals equally spread throughout the day. Participants were blinded to the supplementation condition and were asked to log the times supplements were taken for each supplementation period. The self-reported compliance across participants was 100%.

Participants collected a 24 h urine sample on day 5 of the first (PLA) and second (CRE or ISO) supplementation period. Urinary volume was determined and a 1.5 ml aliquot was transferred to a labelled sample and stored frozen at -20°C until analysis (within a maximum of 4 months). Urinary creatinine concentration was determined calorimetrically using the Jaffe reaction (Jaffe, 1886).

Body mass was measured during the first visit and prior to each of the three main trials.

Blood Lactate Concentration

Blood lactate concentration ([La^-]) was determined from an arterialized fingertip capillary blood sample using lithium-heparin coated microvette tubes (CB3000, Sarsedt, Germany). Blood samples were collected at rest and immediately following the post-exercise NMFA. Prior to collection, the fingertip was cleaned with an alcohol wipe, left to air dry and punctured using a single use lancet (Accu-Chek Safe T-Pro, Roche Diagnostics, West Sussex, UK). Blood samples were analysed for [La^-] using an automated, electrochemical lactate and glucose analyser (YSI 2300, Yellow Springs Instruments, Ohio, USA).

Statistical Analysis

All data were analysed using a standardized package (SPSS v.25 for Windows; IBM Corporation, Armonk, NY, USA) and reported as means ± SD, unless stated otherwise. Data was checked for normal distribution using the Shapiro-Wilk test and sphericity was assessed using Mauchly's test. Two-way repeated measures ANOVA on the factors 'condition' (CRE, PLA, ISO) and 'time' (pre, post) were used to test for differences in neuromuscular and physiological measures. Post-hoc analysis was performed following a significant main or interaction effect using Bonferroni post hoc adjusted pairwise comparisons. Student's paired-sample *t*-tests were used to compare performance times and work done above CP between PLA and CRE. Effect sizes are presented as partial eta squared (η_p^2) for main and interaction effects and Cohen's *d* was calculated to estimate effect sizes for pairwise comparisons. The level of significance was set at $P < 0.05$.

Results

Incremental Test and Determination of CP and *W'*

P_{peak} was 311 ± 37 W and $\dot{V}O_{2peak}$ achieved during the fast ramp test 51.7 ± 8.3 $ml.min^{-1}.kg^{-1}$. Critical power and *W'* were 191 ± 37 W (61.3 ± 5.9% P_{peak}) and 19.9 ± 6.2 kJ, with associated standard errors of 2 ± 1 W and 1.1 ± 0.7 kJ. Mean power output for the main trials was 302 ± 38 W (97 ± 7% P_{peak}).

Experimental Trials

Time to Task Failure

Time to task failure improved significantly with creatine supplementation (PLA: 184 ± 46 s *vs.* CRE: 205 ± 65 s; t_{10} = -2.85; P = 0.017, *d* = 0.373). Work done above CP increased significantly from 19.3 ± 4.0 kJ for PLA to 21.2 ± 4.2 kJ for CRE (t_{10} = -2.945; P = 0.015, *d* = 0.463). $\dot{V}O_{2peak}$ was not significantly different between experimental trials (PLA: 49.5 ± 6.7 $ml.min^{-1}.kg^{-1}$ *vs*. CRE: 48.5 ± 6.8 $ml.min^{-1}.kg^{-1}$ *vs*. ISO 48.1 ± 6.5 $ml.min^{-1}.kg^{-1}$; $F_{1.299,12.989}$ = 1.692, P = 0.221, η_p^2 = 0.145)

Maximal Voluntary Force

MVC decreased significantly from pre- to post-exercise by 20 ± 9% for PLA, 24 ± 8% for CRE and 20 ± 9% for ISO ($F_{1,10}$ = 102.301, P < 0.001, η_p^2 = 0.911), with no significant main effect for condition ($F_{2,20}$ = 1.818, P = 0.188, η_p^2 = 0.154) and no significant exercise *x* condition interaction ($F_{2,20}$ = 1.752, P = 0.199, η_p^2 = 0.149; Figure 1; Table 1).

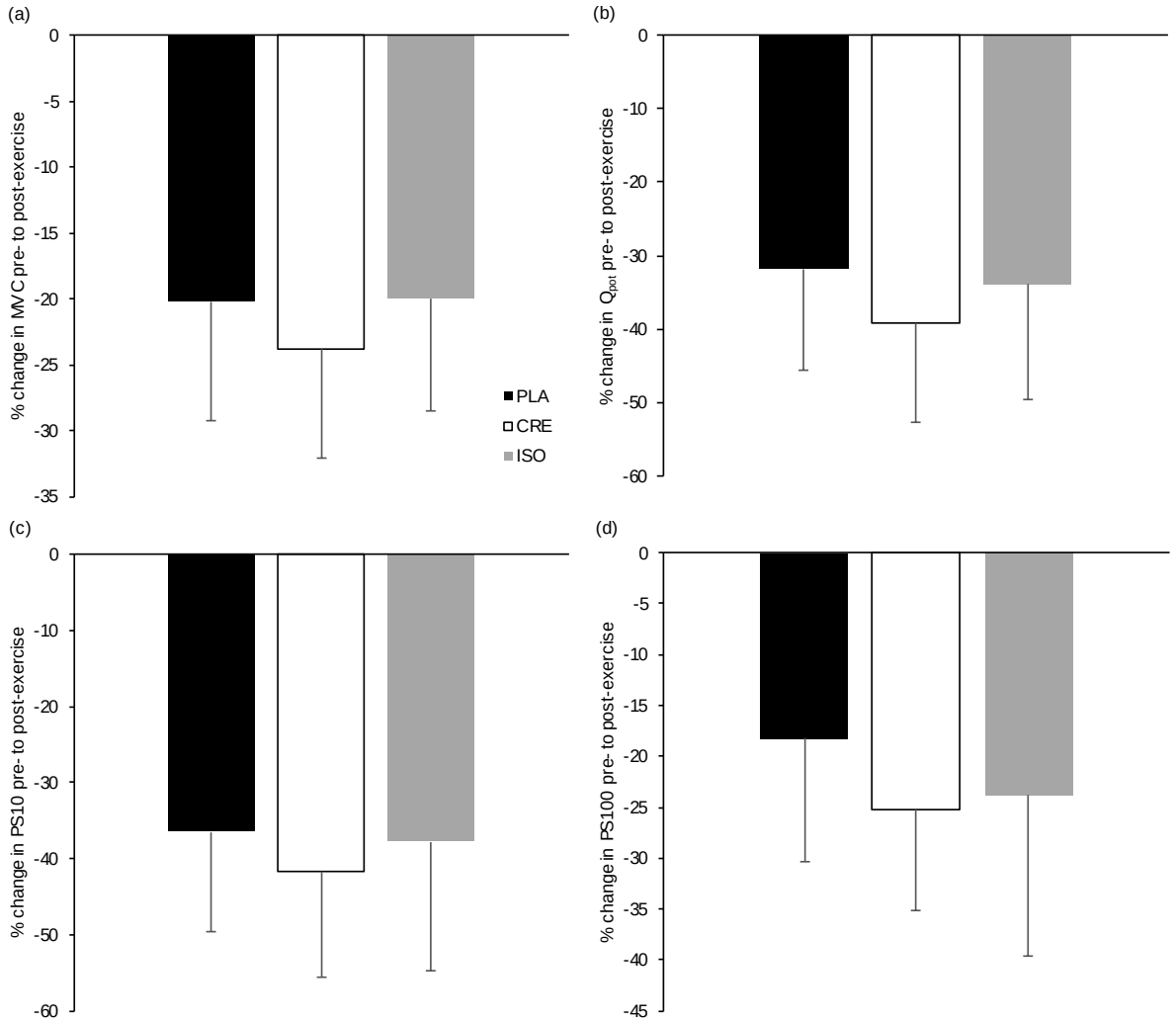

Figure 1. Pre- to post-trial percentage change in maximal voluntary contraction (MVC; A), potentiated twitch force (Qpot; B), low-frequency (10 Hz) doublet force (PS10; C) and high-frequency (100 Hz) doublet force (PS100; D) for Placebo (PLA), Creatine (CRE) and Iso-time (ISO).

Potentiated Twitch Force and Doublet Twitch Forces

Potentiated twitch force, PS10 and PS100 were all significantly reduced after PLA (-32 ± 14, -36 ± 13 and -18 ± 12%, respectively), CRE (-39 ± 13, -42 ± 14 and -25 ± 10%, respectively) and ISO (-34 ± 16, -38 ± 17 and -24 ± 16%) (Q_{pot}, $F_{1,10} = 78.707$, $P < 0.001$, $\eta_p^2 = 0.887$; PS10, $F_{1,10} = 95.505$, $P < 0.001$, $\eta_p^2 = 0.905$; PS100, $F_{1,10} = 70.312$, $P < 0.001$, $\eta_p^2 = 0.875$; Figure 1; Table1). There was no significant main effect for condition for these variables (Q_{pot}, $F_{2,20} = 0.332$, $P = 0.721$, $\eta_p^2 = 0.032$; PS10, $F_{2,20} = 0.833$, $P = 0.449$, $\eta_p^2 = 0.077$; PS100, $F_{2,20} = 0.708$, $P = 0.505$, $\eta_p^2 = 0.066$) and no significant interaction effect for PS10 ($F_{2,20} = 3.338$, $P = 0.056$, $\eta_p^2 = 0.250$) and PS100

($F_{2,20}$ = 2.122, P = 0.146, η_p^2 = 0.175). However, a significant interaction effect was found for Q_{pot} ($F_{2,20}$ = 6.106, P =0.009, η_p^2 = 0.379). At baseline, Q_{pot} was significantly greater in CRE compared to PLA (t_{10} = -4.265; P = 0.002, *d* = 0.448) and ISO (t_{10} = 2.888; P = 0.016, *d* = 0.326).

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 Placebo (PLA), Creatine (CRE) and Iso-time (ISO).

M-wave properties

M-wave PPA showed no significant main effect for time ($F_{1,10}$ = 2.469, P = 0.147, η_p^2 = 0.198), condition ($F_{2,20}$ = 0.226, P = 0.799, η_p^2 = 0.022) or time *x* condition interaction ($F_{2,20}$ = 0.841, P = 0.446, η_p^2 = 0.078; Table 1). M-wave area was significantly greater following exercise ($F_{1,10}$ = 9.483, P = 0.012, η_p^2 = 0.487) with no significant difference between conditions ($F_{2,20}$ = 0.258, P = 0.775, η_p^2 = 0.025) and no time *x* condition interaction ($F_{2,20}$ = 1.853, P = 0.183, η_p^2 = 0.156; Table 1).

Voluntary Activation

Voluntary activation decreased significantly pre- to post-exercise by 5 ± 7, 7 ± 8 and 7 ± 9% for PLA, CRE and ISO ($F_{1,9}$ = 7.529, P = 0.023, η_p^2 = 0.456), with no main effect for condition ($F_{2,18}$ = 1.822, P = 0.190, η_p^2 = 0.168) and no interaction effect ($F_{2,18}$ = 1.308, P = 0.295, η_p^2 = 0.127; Table 1).

Table 1. Neuromuscular measures at pre-exercise (PRE) and after exhaustive constant-load cycling (POST) for placebo (PLA), creatine (CRE) and iso-time (ISO)

	PLA		CRE		ISO	
Parameter	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
Neuromuscular fatigue						
MVC (N)	566 ± 128	451 ± 105*	584 ± 124	447 ± 113*	583 ± 127	472 ± 134*

Peripheral fatigue						
Q_{pot} (N)	171 ± 23†	117 ± 29*	182 ± 26	111 ± 30*	174 ± 23†	116 ± 32*
PS10 (N)	251 ± 50	160 ± 48*	267 ± 49	158 ± 56*	259 ± 48	161 ± 52*
PS100 (N)	242 ± 33	198 ± 41*	248 ± 37	187 ± 43*	242 ± 32	188 ± 55*
PS10:PS100	1.04 ± 0.14	0.80 ± 0.16*	1.08 ± 0.10	0.83 ± 0.16*	1.07 ± 0.13	0.87 ± 0.21*
CT (ms)	76 ± 8	70 ± 3*	78 ± 7	73 ± 5*	77 ± 7	73 ± 6*
MRFD ($N \cdot ms^{-1}$)	5.98 ± 1.05	3.87 ± 1.54*	5.95 ± 1.40	3.21 ± 1.05*	5.47 ± 0.65	3.41 ± 1.02*
MRR ($N \cdot ms^{-1}$)	-1.81 ± 0.35	-1.12 ± 0.27*	-1.69 ± 0.29	-0.94 ± 0.30*	-1.71 ± 0.23	-0.98 ± 0.23*
HRT (ms)§	82.5 ± 8.1	91.6 ± 11.5*	85.8 ± 12.4	96.8 ± 9.8*	88.5 ± 11.7	94.8 ± 17.8*
Surface EMG						
M-wave PPA (mV)	7.8 ± 1.9	8.1 ± 2.3	8.1 ± 2.1	8.7 ± 2.7	8.0 ± 2.2	8.2 ± 2.5
M-wave area ($\mu V \cdot s^{-1}$)	33.5 ± 11.1	36.8 ± 12.5*	33.2 ± 7.6	39.0 ± 11.4*	32.7 ± 10.8	35.5 ± 11.0*
Central fatigue						
VA (%)#	88 ± 6	84 ± 7*	93 ± 4	86 ± 9*	89 ± 5	84 ± 10*

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 *vs.* PRE, †P < 0.05 *vs.* CRE at PRE, §main effect for condition P = 0.031; #n=10

Urinary Creatinine and Body Mass

Urinary creatinine excretion, urinary volume and body mass were not significantly different between the two supplementation conditions (Table 2).

Table 2. Effect of creatine supplementation on urinary creatinine, urinary volume and body mass

	PLA	**CRE**	**Significant difference**	**Effect size**
Urinary creatinine excretion	115 ± 61 $mg.dL^{-1}$	140 ± 86 $mg.dL^{-1}$	t_{10} = -0.896 (P = 0.391)	*d* = 0.339
Urinary volume	108 ± 43 $mL.h^{-1}$	105 ± 48 $mL.h^{-1}$	t_{10} = -0.398 (P = 0.699)	*d* = 0.066
Body mass	75.7 ± 11.4 kg	76.1 ± 11.8 kg	t_{10} = -1.507 (P = 0.163)	*d* = 0.052

Blood Lactate Concentration

Blood lactate concentrations increased significantly pre- to post-exercise from 1.57 ± 0.34 to 9.05 ± 1.66 $mmol.l^{-1}$, 1.51 ± 0.32 to 9.02 ± 2.11 $mmol.l^{-1}$ and 1.53 ± 0.39 to 9.16

± 2.10 mmol.l^{-1} for PLA, CRE and ISO ($F_{1,10}$ = 200.642, P < 0.001, η_p^2 = 0.953), with no main effect for condition ($F_{2,20}$ = 0.077, P = 0.846, η_p^2 = 0.008) and no interaction effect ($F_{2,20}$ = 0.052, P = 0.949, η_p^2 = 0.005).

Discussion

The present study is the first to demonstrate that an improvement in high-intensity cycling performance above CP following creatine supplementation does not influence the magnitude of neuromuscular fatigue at task failure. The magnitude of neuromuscular fatigue does therefore not seem to depend on the amount of work done above CP.

Creatine and high-intensity cycling performance above CP

Numerous studies have investigated the performance enhancing effect of creatine, in particular on high-intensity and sprint performance (Prevost *et al.*, 1997; Jacobs *et al.*, 1997; Aaserud *et al.*, 1998; Mujika *et al.*, 2000; Skare *et al.*, 2001). In the present study, time to task failure (~97% P_{peak}) improved by ~11% (184 *vs.* 205 s, P = 0.017, *d* = 0.373) following 5 days of creatine supplementation. Jacobs *et al.* (1997) reported an improvement of 8% following short-term creatine supplementation when cycling to exhaustion at 125%$\dot{V}O_{2max}$ (130 *vs.* 141 s, P < 0.001, *d* = 1.571). Prevost *et al.* (1997) reported a larger mean improvement in TTF of 24% at a higher exercise intensity (150%$\dot{V}O_{2max}$). A similar observation of greater improvements at higher exercise intensities was described by Smith *et al.* (1998a) with an increase by ~11% (93 *vs.* 103 s) and ~7% (236 *vs.* 253 s) in TTF for work rates eliciting task failure in ~90-250 s. The efficacy of creatine seems greater for shorter efforts, i.e. when the relative contribution of the anaerobic pathways to the total energy turnover becomes more predominant (Branch, 2003). Performance improvements might be attributed to an increase in muscular [PCr] concentration and therefore, a greater accessibility of immediate energy storage (ATP) (Greenhaff *et al.*, 1994). Greater PCr availability within the muscle cell has been associated with slower [PCr] kinetics in single-leg exercise (Jones et al., 2009). The role of [PCr] kinetics in the regulation of

mitochondrial respiration may suggest slower $\dot{V}O_2$ kinetics following creatine supplementation (Jones et al., 2002). However, alterations in the $\dot{V}O_2$ response due to creatine supplementation remain equivocal (Rico-Sanz & Marco, 2000; Jones *et al.*, 2002).Some studies failed to support performance enhancing effects of creatine supplementation during all-out cycling bouts of 15 s to 3 min (Cooke *et al.,* 1995; Schneider *et al.*, 1997; Finn *et al.*, 2001; Vanhatalo & Jones, 2009). Febbraio *et al.* (1995) found no differences in TTF when cycling at 115 or 125%$\dot{V}O_{2max}$ following creatine loading. Possible explanations for no performance enhancing effects in these studies may include differences in the exercise design (i.e. all-out *vs.* time to task failure), duration, sample size, and the sensitivity of the protocol to detect changes in performance and/or anaerobic capacity.

W' is mathematically equivalent to a given amount of work that can be performed above CP (Monod & Scherrer, 1965; Moritani *et al.*, 1981; Poole *et al.*, 1988) and is greater in CRE compared to PLA (~ +10%). One may therefore assume creatine supplementation successfully increased anaerobic work capacity in the present study. Accordingly, similar supplementation protocols have previously been shown to increase *W'* by 10-25% (Smith *et al.*, 1998a; Miura *et al.*, 1999; Eckerson *et al.*, 2005), with no changes in CP (Smith *et al.*, 1998a; Miura *et al.*, 1999). The reported improvements in performance and work done above CP support the efficacy of creatine supplementation in the present study.

Interestingly, large variations between participants in performance improvements and therefore changes in work done above CP (-8 to +27%) were found. The major reason put forward to explain the discrepancy in creatine's efficacy between participants often refers to individual differences in initial muscle [TCr] (responders *vs.* non-responders), so that individuals with low initial [TCr] show greater responses to creatine supplementation compared to individuals with high initial [TCr]. Greenhaff *et al.* (1994) classified individuals with [TCr] of close to or <120 mmol.kg^{-1} dry weight (dw) prior to creatine ingestion as "responders", showing substantial increases in muscle [TCr] of ~25% (+ 29 ± 3 mmol.kg^{-1} dw) compared to "non-responders" (5-7%; + 8-9 mmol.kg^{-1} dw). Syrotuik & Bell (2004) have identified three responder's types: true responders (>20 mmol.kg^{-1} dw from preload levels), quasi responders (>10 and <20 mmol.kg^{-1} dw from preload levels) and non-responders (<10 mmol.kg^{-1} dw from preload levels). In the present study, [TCr] was not measured. However, the significant mean change in

TTF indicate good responsiveness overall to the creatine supplementation for our participants.

Inferences regarding individual creatine retention in the body pool might be drawn from creatinine excretion. During the first few days of creatine supplementation, the majority of the ingested creatine remains within the body until the muscle's capacity to extract creatine from the blood is exhausted. Despite continuous supplementation, ~90% of ingested creatine is excreted into urine (Chanutin & Guy, 1926; Terjung *et al.*, 2000). The rate of creatine degradation to creatinine, the end product of the creatine metabolism, approximates 2 $g.d^{-1}$ (Walker, 1979). The amount of creatinine excreted in the urine is directly proportional to the muscle creatine concentration (Hultman *et al.*, 1996). An increase in urinary creatinine excretion has previously been demonstrated following 5 to 6 days of creatine supplementation (60% Hultman *et al.*, 1996; ~22% Mujika *et al.*, 2000). In the present study, we observed no increase in urinary creatinine excretion following 5 days of creatine supplementation. This may be due to large variations in urinary creatinine excretion between participants, with 6 out of 11 showing an increase of up to +142% and 5 showing a decrease of up to -60%. Similarly, large inter-individual variations in urinary creatinine following creatine loading were also reported by Hultman *et al.* (1996). Syrotuik & Bell (2004) reported that individuals classified as responders showed the lowest urinary creatine concentrations at baseline and the greatest absolute increase after 5 days of supplementation compared to non-responders. However, in line with the present study, data for urinary creatinine did not show a clear trend between responders and non-responders (Syrotuik & Bell, 2004).

Creatine and neuromuscular fatigue after high-intensity cycling above CP

In line with our third hypothesis, no difference in neuromuscular fatigue was found following ISO and CRE, i.e. when the same total work / the same duration of exercise was performed (Figure 1). This would support for a given amount of work done above CP (equal for ISO and CRE) to induce a given level of neuromuscular fatigue. However, creatine supplementation did not lead to greater levels of neuromuscular

fatigue at task failure (Figure 1) despite greater amount of work performed above CP (~ +10%), which contradicts our second hypothesis.

The effect of creatine on neuromuscular fatigue is not well documented, with only a few studies investigating changes in surface EMG during submaximal and supramaximal cycling exercise following short-term creatine loading (Stout *et al.*, 2000; Smith *et al.*, 2007). Creatine supplementation has been shown to delay the onset of neuromuscular fatigue during an incremental exercise, i.e. measured as the highest power output leading to no increase in EMG activity during a constant-load exercise bout (Stout *et al.*, 2000; Smith *et al.*, 2007). During exercise above this onset of neuromuscular fatigue, the predominant reliance on anaerobic glycolysis and the subsequent changes in intramuscular metabolites have been suggested to impair excitation-contraction coupling and ultimately, alter motor unit recruitment, measured as an increase in EMG amplitude, so that either additional motor units are recruited or the firing rate of already active motor units is increased (Moritani *et al.*, 1993; Stout *et al.*, 2000; Smith *et al.*, 2007). Creatine supplementation might reduce the reliance on anaerobic glycolysis and thus, reduce the associated metabolic perturbations by increasing the amount of ATP provided through the creatine-kinase reaction (Volek & Kraemer, 1996; Prevost *et al.*, 1997); this would then delay alterations in motor unit recruitment patterns. Both Stout *et al.* (2000) and Smith *et al.* (2007) did not use neurostimulation techniques to investigate neuromuscular fatigue. To the best of the authors' knowledge, this is the first study investigating both peripheral and central components of neuromuscular fatigue following creatine supplementation, and focusing on the severe intensity domain (i.e. > CP). Thus, based on the findings mentioned above, creatine supplementation may delay the development of neuromuscular fatigue (Stout *et al.*, 2000; Smith *et al.*, 2007), but to attain similar levels of neuromuscular fatigue at task failure. The metabolic perturbations associated with the anaerobic glycolysis energy turnover may be lesser, but those associated with PCr breakdown would be greater during CRE, leading to similar impairment of excitation-contraction coupling in both PLA and CRE. Collectively, similar levels of neuromuscular fatigue observed across all conditions in the present study provide support for a critical level of peripheral fatigue in the population tested.

The present findings do not seem to align with the positive correlation reported between the size of *W'* and the magnitude of neuromuscular fatigue (Schäfer *et al.*, 2019). It is worth noting this relationship was also found in the present study (Q_{pot} *vs.*

W': r = 0.76 and r = 0.56 for CRE and PLA, respectively; P < 0.05). However, these correlations were found for fairly heterogeneous samples of participants, based on their *W'* (Schäfer *et al.*, 2019: 19.9 ± 6.0 kJ; CV of 30%; present study: 19.9 ± 6.2 kJ; CV of 31%), whereas the present study tested within-subject changes. It may be that the intra-individual changes in *W'* induced by creatine supplementation in the present study were too small to reveal differences in neuromuscular fatigue at task failure. Other experimental interventions such as an anaerobic training programme, long enough to affect *W'* more greatly, may lead to larger changes in markers of neuromuscular fatigue at task failure. This would challenge the notion of a critical level of peripheral fatigue.

Limitations

The authors decided against a double-blinded, fully-randomised, cross-over design due to the approximately 6-week wash-out period required following creatine supplementation (Hultman *et al.*, 1996). The duration of the study and the variations in individual fitness levels over time could have affected the performance trials. Therefore, all participants started with the placebo trial and only the second and third main trials (CRE and ISO) were randomised. However, adequate familiarisation prior to the main trials was ensured and participants were blind to the order of the supplements until all experimentation had been completed.

Full depletion of *W'* was not controlled and therefore, an earlier termination of the voluntary task before 'true' exhaustion during PLA and CRE could have confounded the results (i.e. behavioural effect). However, it must be noted that similar neuromuscular changes were reported in Schäfer *et al.* (2019), who controlled for the full depletion of *W'* (MVC, 20 ± 10 *vs.* 20 ± 9%; Q_{pot}, 35 ± 13 *vs.* 32 ± 14%; PS10, 38 ± 13 *vs.* 36 ± 13%; PS100, 18 ± 9 *vs.* 18 ± 12%).

The delayed assessment of neuromuscular measures will have likely caused an underestimation of the magnitude of neuromuscular fatigue due to substantial recovery of neuromuscular function within the first 1-3 min post-exercise (Froyd *et al.*, 2013). To control and limit a potential recovery effect, the present study standardised

timings within the neuromuscular assessment protocol, and the transition time between exercise termination and start of the neuromuscular assessment (60 s).

Although the muscle [TCr] was not measured in the present study, previous investigations using similar supplementation protocols reported an increase in muscle [TCr] by up to 20% and therefore, similar changes would be expected for the present study (Harris *et al.*, 1992; Greenhaff *et al.*, 1994; Casey *et al.*, 1996; Hultman *et al.*, 1996; Finn *et al.*, 2001).

Because of the duration of the study's implementation and the requirement for a high number of times to task failure to model the P-t relationship, the present study did not include the addition of 4-5 visits following creatine supplementation to re-assess *W'*.

Conclusion

In conclusion, the present study confirmed a performance enhancing effect of creatine supplementation and indicates that the level of neuromuscular fatigue is not dependent on the amount of work done above CP. These findings challenge a direct causative link between utilisation of *W'* and neuromuscular fatigue and support the notion that a critical level of peripheral fatigue may exist.

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Additional Information

Competing interests

This work was completed at the University of Brighton. All authors contributed to the conception and design of the study. Schäfer collected and analysed the data. Schäfer, Dekerle and Hayes wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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