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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

Creatine supplementation and neuromuscular fatigue above critical power

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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.

1 **Abstract**

2 The present study examined the effect of creatine supplementation on neuromuscular
3 fatigue and exercise tolerance when cycling above critical power (CP). Eleven males
4 performed an incremental cycling test, 4-5 constant-load trials to task failure (TTF) to
5 obtain asymptote (CP) and curvature constant (W') of the power-duration relationship,
6 followed by three constant-load supra-CP trials: 1) one TTF following placebo
7 supplementation (PLA); 2) one TTF following creatine supplementation (CRE); and 3)
8 one trial of equal duration to PLA following creatine supplementation (ISO).
9 Neuromuscular assessment of the right knee extensors was performed pre- and post-
10 exercise to measure maximal voluntary contraction (MVC), twitch forces evoked by
11 single (Q_{pot}) and paired high- (PS100) and low-frequency (PS10) stimulations and
12 voluntary activation. Creatine supplementation increased TTF in CRE vs. PLA by
13 ~11% ($P = 0.017$) and work done above CP by ~10% ($P = 0.015$), with no difference
14 ($P > 0.05$) in reductions in MVC (-24 ± 8 vs. $-20 \pm 9\%$), Q_{pot} (-39 ± 13 vs. $-32 \pm 14\%$),
15 PS10 (-42 ± 14 vs. $-36 \pm 13\%$), PS100 (-25 ± 10 vs. $-18 \pm 12\%$) and voluntary activation
16 (-7 ± 8 vs. $-5 \pm 7\%$) in CRE vs. PLA. No significant difference were found between ISO
17 and both PLA and CRE ($P > 0.05$). These findings suggest similar levels of
18 neuromuscular fatigue can be found following supra-CP cycling despite increases in
19 performance time and amount of work done above CP, supporting the notion of a
20 critical level of neuromuscular fatigue and challenging a direct causative link between
21 W' depletion and neuromuscular fatigue.

22

23

24 **Introduction**

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

34 nature has been questioned due to its sensitivity to interventions altering O₂ delivery
35 (Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012). The depletion of W' has been associated
36 with the accumulation of fatigue-related metabolites (i.e. P_i, H⁺, ADP, La⁻) to a critical
37 level (Burnley *et al.*, 2010; Ferguson *et al.*, 2010; 2007; Poole *et al.*, 1988) and it is
38 further suggested that these metabolic perturbations may also contribute to the
39 continued reduction in muscle efficiency, proposed as the 'fatigue cascade' by
40 Murgatroyd *et al.* (2011).

41

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

60

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

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

73

74 Indeed, creatine supplementation has the potential to test the relationship between W'
75 and neuromuscular fatigue reported by Schäfer *et al.* (2019) through manipulation of
76 an individual's anaerobic work capacity. Such interventions aiming to increase total
77 creatine stores ([TCr]; i.e. sum of phosphocreatine [PCr] and free creatine [Cr]) have
78 also been shown to enhance the muscular capacity for PCr hydrolysis leading to
79 longer time to task failure (Smith *et al.*, 2004; 1998b). Greater fatigue-induced
80 metabolic disturbances (i.e. higher [Pi], [Cr], [PCr/Cr]) have also been reported
81 following high-intensity knee-extension exercise under creatine loading compared to
82 placebo (Smith *et al.*, 2004; 1998b). An increase in muscle [TCr] by up to 20% ($\frac{1}{3}$ in
83 form of PCr) following creatine supplementation has previously been demonstrated
84 (Finn *et al.*, 2001; Casey *et al.*, 1996; Greenhaff *et al.* 1994; Harris *et al.*, 1992). The
85 effect of creatine supplementation on high-intensity performance has been intensively
86 studied since the 1990s (Rossiter *et al.*, 1996; Jacobs *et al.*, 1997; Smith *et al.*, 1998a;
87 McNaughton *et al.*, 1998; Miura *et al.*, 1999). Improvements in time to task failure of
88 up to 24% have been observed, with greater changes observed following shorter,
89 more intense exercise during which the contribution of the anaerobic energy turnover
90 becomes more predominant (Jacobs *et al.*, 1997; Prevost *et al.*, 1997; Smith *et al.*,
91 1998a; Branch, 2003). In addition, creatine supplementation increased W' by 10-25%,
92 without affecting CP (Smith *et al.*, 1998a; Miura *et al.*, 1999; Eckerson *et al.*, 2005).
93 These findings provide support for a significant role of muscle Cr/PCr content in high-
94 intensity performance and evidence the primarily anaerobic nature of W' .

95

96 Whereas the effect of creatine on performance is well-established, very little is known
97 about its effect on neuromuscular fatigue. Creatine supplementation has been
98 reported to influence neuromuscular measures (Stout *et al.* 2000; Smith *et al.*, 2007).
99 Stout *et al.* (2000) reported a greater physical working capacity at the fatigue threshold
100 (+ 20%), measured as the highest power output that does not result in an increase in
101 EMG activity over time, following five days of creatine loading, which was thought to

102 indicate a delay in the onset of neuromuscular fatigue. Similarly, Smith *et al.* (2007)
103 found an increase in the electromyographic fatigue threshold during cycle ergometry
104 (+ ~15%). However, whether creatine supplementation alters neuromuscular fatigue
105 at task failure following exercise above CP remains unclear. The integration of the CP
106 concept with electromyographic and mechanical measures of neuromuscular fatigue
107 may offer further insights into the limits of exercise tolerance within the severe-intensity
108 domain.

109

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

119

120

121 **Methods**

122 **Ethical Approval**

123 Written informed consent was obtained from each participant. The study was approved
124 by the University of Brighton Research Ethics & Governance Committee (ethics
125 approval reference number 11718) and conformed to the standards set by the latest
126 Declaration of Helsinki, except for registration in a database.

127

128 **Participants**

129 Eleven recreationally active, non-vegetarian male participants (mean \pm SD: age, 22.6
130 \pm 2.8 years; body mass, 75.8 \pm 11.5 kg; peak O₂ consumption ($\dot{V}O_{2peak}$), 51.7 \pm 8.3
131 ml.min⁻¹.kg⁻¹; peak power output (P_{peak}), 311 \pm 37 W) volunteered for this study. All
132 participants were familiar with cycle ergometry and the exercise procedures used in
133 our laboratory.

134

135 **Study Design**

136 Participants reported to the laboratory on nine to ten different occasions over a 5 to 6
137 week period, with each test separated by a minimum of 24 h and performed at the
138 same time of day (± 2 h) to control for the effect of diurnal variation (Atkinson & Reilly,
139 1996) . The tests included a ramp incremental test for the determination of $\dot{V}O_{2peak}$, a
140 familiarisation to the experimental protocol, four to five constant-load trials performed
141 to task failure for the determination of CP and W' and three constant-load trials to
142 investigate the effect of creatine supplementation on neuromuscular function in the
143 fresh state and following constant-load cycling above CP. These last three main
144 experimental trials were separated by 5 to 7 days.

145

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

160

161 **Incremental Test and Familiarisation**

162 Power for the maximal ramp incremental test was initially set to 50-125 W depending
163 on individual fitness level and increased by 5 W every 12 s until task failure. P_{peak} and
164 $\dot{V}O_{2peak}$ were defined as the highest 15 s moving average.

165 Participants were familiarised with constant-load trials performed to task failure,
166 neuromuscular function assessment (NMFA) and a quick transition from the cycle
167 ergometer to the isometric rig during a separate visit.

168

169 **Determination of CP and W'**

170 Participants completed a series of four to five constant-load tests in a semi-
171 randomized order to elicit task failure within ~3 and 15 min (Poole *et al.*, 1988; Hill,
172 1993). Participants were not informed of the elapsed time or any other performance
173 measure throughout testing except cadence.

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

176

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

$$178 \quad t_{lim} = W' / (P - CP) \quad (1)$$

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

$$180 \quad W = CP \times t_{lim} + W' \quad (2)$$

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

$$182 \quad P = (1/t_{lim}) \times W' + CP \quad (3)$$

183

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

189

190 **Experimental Trials**

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

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

210 Single and paired square-wave electrical stimulation (200 μ s pulse width) were
211 delivered via adhesive surface electrodes to the femoral nerve (ValuTrode; Axelgaard,
212 Fallbrook, CA, USA) using a constant-current stimulator (DS7AH; Digitimer Ltd,
213 Welwyn Garden City, UK). Therefore, the cathode was positioned in the femoral
214 triangle and the anode midway between the iliac crest and the greater trochanter. The
215 stimulation threshold was determined by delivering two single stimuli separated by 5
216 s to the femoral nerve, and current was increased progressively (+20 mA) starting at
217 10 mA until no further increase in M-wave peak-to-peak amplitude and resting twitch
218 force was evoked. The stimulation intensity was set at 130% to ensure full spatial
219 motor unit recruitment. Determination of the stimulation threshold was conducted
220 before each first NMFA of every subsequent trial.

221 The first NMFA of each visit was preceded by a standardized isometric warm-up with
222 the right knee extensors, involving ten 3 s isometric contractions with progressively
223 increasing contraction intensity and maximal efforts during the last three contractions
224 (3 s on – 7 s off; adapted from Girard *et al.*, 2013). Additional MVCs were performed
225 if the coefficient of variation (CV) over three MVCs was $\geq 5\%$. Each NMFA involved
226 five isometric 3 s MVCs separated by 20 s rest. Paired stimuli at 100 Hz (PS100) were
227 delivered during and 2 s after the last three contractions, followed by paired stimuli at
228 10 Hz (PS10) and a single stimulus (Q_{pot}). Real-time visual feedback was displayed
229 throughout as recommended by Gandevia (2001) and the time window between
230 exercise termination and the first MVC for NMFA was standardised to 60 s for every
231 participant and every session.

232 Peak MVC was measured as the greatest 0.5 s mean force produced before electrical
233 stimulation and reported as the mean of five MVCs. Potentiated twitch force was
234 defined as the greatest peak twitch force in response to supramaximal stimulation.
235 The ratio between twitch forces evoked by low- and high-frequency paired stimuli
236 (PS10:PS100) was calculated to determine low-frequency fatigue. Within-twitch
237 measures [i.e. contraction time (CT), maximal rate of force development (MRFD),
238 maximal rate of relaxation (MRR) and half-relaxation time (HRT)] were derived from
239 each resting twitch. Voluntary activation was calculated using the interpolated paired
240 stimulation technique (Merton, 1954). One participant was excluded from the data
241 analysis for VA after values were identified as outliers using the interquartile range
242 (Tukey, 1977). M-wave peak-to-peak amplitude (PPA) was measured as the absolute
243 difference of the greatest and smallest value of the biphasic M-wave, and M-wave area
244 was determined as the integral of the absolute value of the M-wave. For twitch forces,
245 within-twitch parameters, VA and M-wave properties, the mean of three was reported.

246

247 **Supplementation, Urinary Creatinine and Body Mass**

248 All participants ingested 4x 5 g.d⁻¹ of dextrose (PLA) during the first 5-day
249 supplementation period. Prior to the second main trial, participants ingested 4 x 5 g.d⁻¹
250 ¹ of creatine monohydrate for five successive days and during the third
251 supplementation period, participants ingested a maintenance dose of 2 g.d⁻¹ of
252 creatine for each day between the second and the third main trial (Hultman *et al.*,
253 1996). Each dose was dissolved in 200 ml of warm water and flavoured with no added
254 sugar orange squash. Supplements were taken at regular intervals equally spread
255 throughout the day. Participants were blinded to the supplementation condition and
256 were asked to log the times supplements were taken for each supplementation period.
257 The self-reported compliance across participants was 100%.

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

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

265

266 **Blood Lactate Concentration**

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

275

276 **Statistical Analysis**

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

289

290

291 **Results**

292 **Incremental Test and Determination of CP and W'**

293 P_{peak} was 311 ± 37 W and $\dot{V}O_{2peak}$ achieved during the fast ramp test 51.7 ± 8.3 ml.min⁻¹.
294 $1.kg^{-1}$. Critical power and W' were 191 ± 37 W ($61.3 \pm 5.9\%$ P_{peak}) and 19.9 ± 6.2 kJ,
295 with associated standard errors of 2 ± 1 W and 1.1 ± 0.7 kJ. Mean power output for
296 the main trials was 302 ± 38 W ($97 \pm 7\%$ P_{peak}).

297

298 **Experimental Trials**

299 **Time to Task Failure**

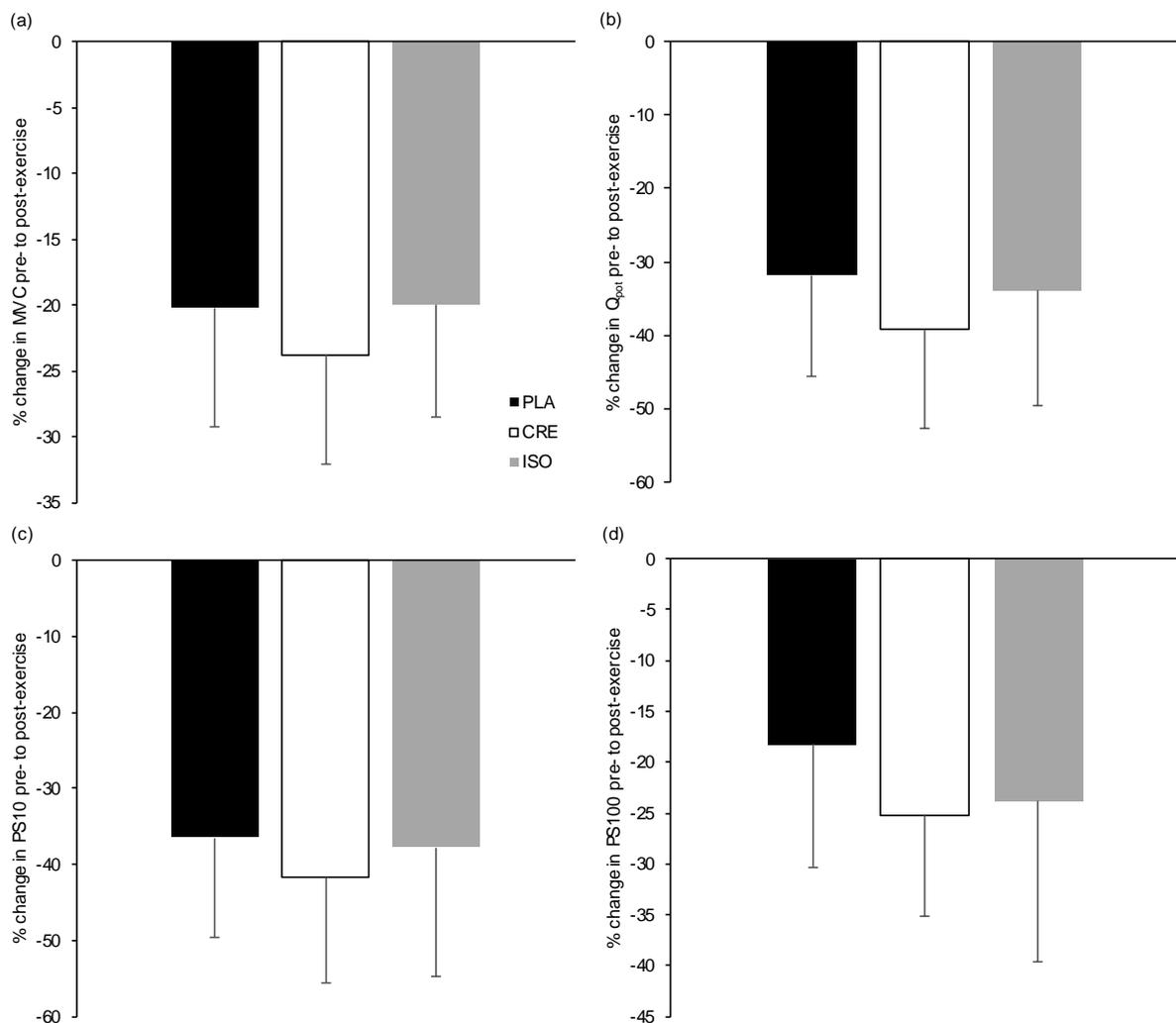
300 Time to task failure improved significantly with creatine supplementation (PLA: $184 \pm$
301 46 s vs. CRE: 205 ± 65 s; $t_{10} = -2.85$; $P = 0.017$, $d = 0.373$). Work done above CP
302 increased significantly from 19.3 ± 4.0 kJ for PLA to 21.2 ± 4.2 kJ for CRE ($t_{10} = -2.945$;
303 $P = 0.015$, $d = 0.463$). $\dot{V}O_{2peak}$ was not significantly different between experimental
304 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
305 ml.min⁻¹.kg⁻¹; $F_{1,299,12.989} = 1.692$, $P = 0.221$, $\eta_p^2 = 0.145$)

306

307 **Maximal Voluntary Force**

308 MVC decreased significantly from pre- to post-exercise by $20 \pm 9\%$ for PLA, $24 \pm 8\%$
309 for CRE and $20 \pm 9\%$ for ISO ($F_{1,10} = 102.301$, $P < 0.001$, $\eta_p^2 = 0.911$), with no
310 significant main effect for condition ($F_{2,20} = 1.818$, $P = 0.188$, $\eta_p^2 = 0.154$) and no
311 significant exercise x condition interaction ($F_{2,20} = 1.752$, $P = 0.199$, $\eta_p^2 = 0.149$; Figure
312 1; Table 1).

313



314

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

318

319

320 **Potentiated Twitch Force and Doublet Twitch Forces**

321 Potentiated twitch force, PS10 and PS100 were all significantly reduced after PLA (-
 322 32 ± 14, -36 ± 13 and -18 ± 12%, respectively), CRE (-39 ± 13, -42 ± 14 and -25 ±
 323 10%, respectively) and ISO (-34 ± 16, -38 ± 17 and -24 ± 16%) (Q_{pot}, F_{1,10} = 78.707, P
 324 < 0.001, η_p² = 0.887; PS10, F_{1,10} = 95.505, P < 0.001, η_p² = 0.905; PS100, F_{1,10} =
 325 70.312, P < 0.001, η_p² = 0.875; Figure 1; Table1). There was no significant main effect
 326 for condition for these variables (Q_{pot}, F_{2,20} = 0.332, P = 0.721, η_p² = 0.032; PS10, F_{2,20}
 327 = 0.833, P = 0.449, η_p² = 0.077; PS100, F_{2,20} = 0.708, P = 0.505, η_p² = 0.066) and no
 328 significant interaction effect for PS10 (F_{2,20} = 3.338, P = 0.056, η_p² = 0.250) and PS100

329 ($F_{2,20} = 2.122$, $P = 0.146$, $\eta_p^2 = 0.175$). However, a significant interaction effect was
 330 found for Q_{pot} ($F_{2,20} = 6.106$, $P = 0.009$, $\eta_p^2 = 0.379$). At baseline, Q_{pot} was significantly
 331 greater in CRE compared to PLA ($t_{10} = -4.265$; $P = 0.002$, $d = 0.448$) and ISO ($t_{10} =$
 332 2.888 ; $P = 0.016$, $d = 0.326$).

333
 334
 335

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

339
 340

341 **M-wave properties**

342 M-wave PPA showed no significant main effect for time ($F_{1,10} = 2.469$, $P = 0.147$, η_p^2
 343 $= 0.198$), condition ($F_{2,20} = 0.226$, $P = 0.799$, $\eta_p^2 = 0.022$) or time x condition interaction
 344 ($F_{2,20} = 0.841$, $P = 0.446$, $\eta_p^2 = 0.078$; Table 1). M-wave area was significantly greater
 345 following exercise ($F_{1,10} = 9.483$, $P = 0.012$, $\eta_p^2 = 0.487$) with no significant difference
 346 between conditions ($F_{2,20} = 0.258$, $P = 0.775$, $\eta_p^2 = 0.025$) and no time x condition
 347 interaction ($F_{2,20} = 1.853$, $P = 0.183$, $\eta_p^2 = 0.156$; Table 1).

348

349 **Voluntary Activation**

350 Voluntary activation decreased significantly pre- to post-exercise by 5 ± 7 , 7 ± 8 and 7
 351 $\pm 9\%$ for PLA, CRE and ISO ($F_{1,9} = 7.529$, $P = 0.023$, $\eta_p^2 = 0.456$), with no main effect
 352 for condition ($F_{2,18} = 1.822$, $P = 0.190$, $\eta_p^2 = 0.168$) and no interaction effect ($F_{2,18} =$
 353 1.308 , $P = 0.295$, $\eta_p^2 = 0.127$; Table 1).

354
 355

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

| Parameter | PLA | | CRE | | ISO | |
|------------------------------|-----------|------------|-----------|------------|-----------|------------|
| | PRE | POST | PRE | POST | PRE | POST |
| <i>Neuromuscular fatigue</i> | | | | | | |
| 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·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·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 (μV·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

358

359

360 Urinary Creatinine and Body Mass

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

363

364 **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 |

365

366

367 Blood Lactate Concentration

368 Blood lactate concentrations increased significantly pre- to post-exercise from 1.57 ±
369 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

370 $\pm 2.10 \text{ mmol.l}^{-1}$ for PLA, CRE and ISO ($F_{1,10} = 200.642$, $P < 0.001$, $\eta_p^2 = 0.953$), with
371 no main effect for condition ($F_{2,20} = 0.077$, $P = 0.846$, $\eta_p^2 = 0.008$) and no interaction
372 effect ($F_{2,20} = 0.052$, $P = 0.949$, $\eta_p^2 = 0.005$).

373

374

375 **Discussion**

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

381

382

383 *Creatine and high-intensity cycling performance above CP*

384

385 Numerous studies have investigated the performance enhancing effect of creatine, in
386 particular on high-intensity and sprint performance (Prevost *et al.*, 1997; Jacobs *et al.*,
387 1997; Aaserud *et al.*, 1998; Mujika *et al.*, 2000; Skare *et al.*, 2001). In the present
388 study, time to task failure ($\sim 97\% P_{\text{peak}}$) improved by $\sim 11\%$ (184 vs. 205 s, $P = 0.017$,
389 $d = 0.373$) following 5 days of creatine supplementation. Jacobs *et al.* (1997) reported
390 an improvement of 8% following short-term creatine supplementation when cycling to
391 exhaustion at $125\% \dot{V}O_{2\text{max}}$ (130 vs. 141 s, $P < 0.001$, $d = 1.571$). Prevost *et al.* (1997)
392 reported a larger mean improvement in TTF of 24% at a higher exercise intensity
393 ($150\% \dot{V}O_{2\text{max}}$). A similar observation of greater improvements at higher exercise
394 intensities was described by Smith *et al.* (1998a) with an increase by $\sim 11\%$ (93 vs.
395 103 s) and $\sim 7\%$ (236 vs. 253 s) in TTF for work rates eliciting task failure in ~ 90 -250
396 s. The efficacy of creatine seems greater for shorter efforts, i.e. when the relative
397 contribution of the anaerobic pathways to the total energy turnover becomes more
398 predominant (Branch, 2003). Performance improvements might be attributed to an
399 increase in muscular [PCr] concentration and therefore, a greater accessibility of
400 immediate energy storage (ATP) (Greenhaff *et al.*, 1994). Greater PCr availability
401 within the muscle cell has been associated with slower [PCr] kinetics in single-leg
402 exercise (Jones *et al.*, 2009). The role of [PCr] kinetics in the regulation of

403 mitochondrial respiration may suggest slower $\dot{V}O_2$ kinetics following creatine
404 supplementation (Jones *et al.*, 2002). However, alterations in the $\dot{V}O_2$ response due
405 to creatine supplementation remain equivocal (Rico-Sanz & Marco, 2000; Jones *et al.*,
406 2002). Some studies failed to support performance enhancing effects of creatine
407 supplementation during all-out cycling bouts of 15 s to 3 min (Cooke *et al.*, 1995;
408 Schneider *et al.*, 1997; Finn *et al.*, 2001; Vanhatalo & Jones, 2009). Febbraio *et al.*
409 (1995) found no differences in TTF when cycling at 115 or 125% $\dot{V}O_{2max}$ following
410 creatine loading. Possible explanations for no performance enhancing effects in these
411 studies may include differences in the exercise design (i.e. all-out vs. time to task
412 failure), duration, sample size, and the sensitivity of the protocol to detect changes in
413 performance and/or anaerobic capacity.

414

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

424 Interestingly, large variations between participants in performance improvements and
425 therefore changes in work done above CP (-8 to +27%) were found. The major reason
426 put forward to explain the discrepancy in creatine's efficacy between participants often
427 refers to individual differences in initial muscle [TCr] (responders vs. non-responders),
428 so that individuals with low initial [TCr] show greater responses to creatine
429 supplementation compared to individuals with high initial [TCr]. Greenhaff *et al.* (1994)
430 classified individuals with [TCr] of close to or <120 mmol.kg⁻¹ dry weight (dw) prior to
431 creatine ingestion as "responders", showing substantial increases in muscle [TCr] of
432 ~25% (+ 29 ± 3 mmol.kg⁻¹ dw) compared to "non-responders" (5-7%; + 8-9 mmol.kg⁻¹
433 dw). Syrotuik & Bell (2004) have identified three responder's types: true responders
434 (>20 mmol.kg⁻¹ dw from preload levels), quasi responders (>10 and <20 mmol.kg⁻¹ dw
435 from preload levels) and non-responders (<10 mmol.kg⁻¹ dw from preload levels). In
436 the present study, [TCr] was not measured. However, the significant mean change in

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

439

440 Inferences regarding individual creatine retention in the body pool might be drawn from
441 creatinine excretion. During the first few days of creatine supplementation, the majority
442 of the ingested creatine remains within the body until the muscle's capacity to extract
443 creatine from the blood is exhausted. Despite continuous supplementation, ~90% of
444 ingested creatine is excreted into urine (Chanutin & Guy, 1926; Terjung *et al.*, 2000).
445 The rate of creatine degradation to creatinine, the end product of the creatine
446 metabolism, approximates 2 g.d⁻¹ (Walker, 1979). The amount of creatinine excreted
447 in the urine is directly proportional to the muscle creatine concentration (Hultman *et al.*,
448 1996). An increase in urinary creatinine excretion has previously been
449 demonstrated following 5 to 6 days of creatine supplementation (60% Hultman *et al.*,
450 1996; ~22% Mujika *et al.*, 2000). In the present study, we observed no increase in
451 urinary creatinine excretion following 5 days of creatine supplementation. This may be
452 due to large variations in urinary creatinine excretion between participants, with 6 out
453 of 11 showing an increase of up to +142% and 5 showing a decrease of up to -60%.
454 Similarly, large inter-individual variations in urinary creatinine following creatine
455 loading were also reported by Hultman *et al.* (1996). Syrotuik & Bell (2004) reported
456 that individuals classified as responders showed the lowest urinary creatine
457 concentrations at baseline and the greatest absolute increase after 5 days of
458 supplementation compared to non-responders. However, in line with the present
459 study, data for urinary creatinine did not show a clear trend between responders and
460 non-responders (Syrotuik & Bell, 2004).

461

462

463 *Creatine and neuromuscular fatigue after high-intensity cycling above CP*

464

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

470 fatigue at task failure (Figure 1) despite greater amount of work performed above CP
471 (~ +10%), which contradicts our second hypothesis.

472 The effect of creatine on neuromuscular fatigue is not well documented, with only a
473 few studies investigating changes in surface EMG during submaximal and
474 supramaximal cycling exercise following short-term creatine loading (Stout *et al.*,
475 2000; Smith *et al.*, 2007). Creatine supplementation has been shown to delay the
476 onset of neuromuscular fatigue during an incremental exercise, i.e. measured as the
477 highest power output leading to no increase in EMG activity during a constant-load
478 exercise bout (Stout *et al.*, 2000; Smith *et al.*, 2007). During exercise above this onset
479 of neuromuscular fatigue, the predominant reliance on anaerobic glycolysis and the
480 subsequent changes in intramuscular metabolites have been suggested to impair
481 excitation-contraction coupling and ultimately, alter motor unit recruitment, measured
482 as an increase in EMG amplitude, so that either additional motor units are recruited or
483 the firing rate of already active motor units is increased (Moritani *et al.*, 1993; Stout *et al.*
484 *et al.*, 2000; Smith *et al.*, 2007). Creatine supplementation might reduce the reliance on
485 anaerobic glycolysis and thus, reduce the associated metabolic perturbations by
486 increasing the amount of ATP provided through the creatine-kinase reaction (Volek &
487 Kraemer, 1996; Prevost *et al.*, 1997); this would then delay alterations in motor unit
488 recruitment patterns. Both Stout *et al.* (2000) and Smith *et al.* (2007) did not use
489 neurostimulation techniques to investigate neuromuscular fatigue. To the best of the
490 authors' knowledge, this is the first study investigating both peripheral and central
491 components of neuromuscular fatigue following creatine supplementation, and
492 focusing on the severe intensity domain (i.e. > CP). Thus, based on the findings
493 mentioned above, creatine supplementation may delay the development of
494 neuromuscular fatigue (Stout *et al.*, 2000; Smith *et al.*, 2007), but to attain similar levels
495 of neuromuscular fatigue at task failure. The metabolic perturbations associated with
496 the anaerobic glycolysis energy turnover may be lesser, but those associated with PCr
497 breakdown would be greater during CRE, leading to similar impairment of excitation-
498 contraction coupling in both PLA and CRE. Collectively, similar levels of
499 neuromuscular fatigue observed across all conditions in the present study provide
500 support for a critical level of peripheral fatigue in the population tested.

501 The present findings do not seem to align with the positive correlation reported
502 between the size of W' and the magnitude of neuromuscular fatigue (Schäfer *et al.*,
503 2019). It is worth noting this relationship was also found in the present study (Q_{pot} vs.

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

514

515

516 *Limitations*

517

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

526 Full depletion of W' was not controlled and therefore, an earlier termination of the
527 voluntary task before 'true' exhaustion during PLA and CRE could have confounded
528 the results (i.e. behavioural effect). However, it must be noted that similar
529 neuromuscular changes were reported in Schäfer *et al.* (2019), who controlled for the
530 full depletion of W' (MVC, 20 ± 10 vs. $20 \pm 9\%$; Q_{pot} , 35 ± 13 vs. $32 \pm 14\%$; PS10, 38
531 ± 13 vs. $36 \pm 13\%$; PS100, 18 ± 9 vs. $18 \pm 12\%$).

532 The delayed assessment of neuromuscular measures will have likely caused an
533 underestimation of the magnitude of neuromuscular fatigue due to substantial
534 recovery of neuromuscular function within the first 1-3 min post-exercise (Froyd *et al.*,
535 2013). To control and limit a potential recovery effect, the present study standardised

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

538 Although the muscle [TCr] was not measured in the present study, previous
539 investigations using similar supplementation protocols reported an increase in muscle
540 [TCr] by up to 20% and therefore, similar changes would be expected for the present
541 study (Harris *et al.*, 1992; Greenhaff *et al.*, 1994; Casey *et al.*, 1996; Hultman *et al.*,
542 1996; Finn *et al.*, 2001).

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

546

547

548 **Conclusion**

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

554

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558 **References**

559 Aaserud, R., Gramvik, P., Olsen, S. R. and Jensen, J. (1998). Creatine
560 supplementation delays onset of fatigue during repeated bouts of sprint running.
561 *Scandinavian Journal of Medicine & Science in Sports*, 8: 247–251.

562 <http://doi.org/10.1111/j.1600-0838.1998.tb00478.x>

563 Allen, D. G., Lamb, G. D. and Westerblad, H. (2008). Impaired calcium release during
564 fatigue. *Journal of Applied Physiology*, 104: 296–305.

565 <https://doi.org/10.1152/jappphysiol.00908.2007>

566 Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F. and Dempsey,
567 J. A. (2011). Implications of group III and IV muscle afferents for high-intensity

568 endurance exercise performance in humans. *The Journal of Physiology*, 589:
569 5299–5309. <https://doi.org/10.1113/jphysiol.2011.213769>

570 Amann, M. and Dempsey, J. A. (2008). Locomotor muscle fatigue modifies central
571 motor drive in healthy humans and imposes a limitation to exercise performance.
572 *The Journal of Physiology*, 586: 161–173.
573 <https://doi.org/10.1113/jphysiol.2007.141838>

574 Amann, M., Eldridge, M. W., Lovering, A. T., Stickland, M. K., Pegelow, D. F. and
575 Dempsey, J. A. (2006). Arterial oxygenation influences central motor output and
576 exercise performance via effects on peripheral locomotor muscle fatigue in
577 humans. *The Journal of Physiology*, 575: 937–952.
578 <https://doi.org/10.1113/jphysiol.2006.113936>

579 Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F. and Dempsey, J. A. (2009).
580 Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral
581 muscle fatigue development in humans. *The Journal of Physiology*, 587: 271–
582 283. <https://doi.org/10.1113/jphysiol.2008.163303>

583 Atkinson, G. and Reilly, T. (1996). Circadian variation in sports performance. *Sports*
584 *Medicine*, 21,: 292-312. <https://doi.org/10.2165/00007256-199621040-00005>

585 Becker, R. and Awiszus, F. (2001). Physiological alterations of maximal voluntary
586 quadriceps activation by changes of knee joint angle. *Muscle & Nerve*, 24: 667–
587 672. <https://doi.org/10.1002/mus.1053>

588 Branch, J. D. (2003). Effect of creatine supplementation on body composition and
589 performance: a meta-analysis. *International Journal of Sport Nutrition and*
590 *Exercise Metabolism*, 13: 198–226. <https://doi.org/10.1123/ijsnem.13.2.198>

591 Burnley, M., Vanhatalo, A., Fulford, J. and Jones, A. M. (2010). Similar metabolic
592 perturbations during all-out and constant force exhaustive exercise in humans: a
593 ³¹P magnetic resonance spectroscopy study. *Experimental Physiology*, 95:
594 798–807. <https://doi.org/10.1113/expphysiol.2010.052688>

595 Burnley, M., Vanhatalo, A. and Jones, A. M. (2012). Distinct profiles of neuromuscular
596 fatigue during muscle contractions below and above the critical torque in humans.
597 *Journal of Applied Physiology*, 113: 215–223.
598 <https://doi.org/10.1152/jappphysiol.00022.2012>

599 Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E. and Greenhaff, P. L.
600 (1996). Creatine ingestion favorably affects performance and muscle metabolism
601 during maximal exercise in humans. *American Journal of Physiology*, 271: 31–

602 37. <https://doi.org/10.1152/ajpendo.1996.271.1.E31>

603 Chanutin, A. and Guy, L. P. (1926). The fate of creatine when administered to man. *J*

604 *Biol Chem*, 67: 29–41.

605 Cooke, W. H., Grandjean, P. W. and Barnes, W. S. (1995). Effect of oral creatine

606 supplementation on power output and fatigue during bicycle ergometry. *Journal*

607 *of Applied Physiology*, 78: 670-673. <https://doi.org/10.1152/jappl.1995.78.2.670>

608 Dekerle, J., Mucci, P. and Carter, H. (2012). Influence of moderate hypoxia on

609 tolerance to high-intensity exercise. *European Journal of Applied Physiology*, 112:

610 327–335. <https://doi.org/10.1007/s00421-011-1979-z>

611 Dekerle, J., De Souza, K. M., De Lucas, R. D., Guglielmo, L. G. A., Greco, C. C. and

612 Denadai, B. S. (2015). Exercise tolerance can be enhanced through a change in

613 work rate within the severe intensity domain: Work above critical power is not

614 constant. *PLoS ONE*, 10: 1–15. <https://doi.org/10.1371/journal.pone.0138428>

615 Eckerson, J. M., Stout, J. R., Moore, G. A., Stone, N. J., Iwan, K. A., Gebauer, A. N.

616 and Ginsberg, R. (2005). Effect of creatine phosphate supplementation on

617 anaerobic working capacity and body weight after two and six days of loading in

618 men and women. *Journal of Strength and Conditioning Research*, 19: 756–763.

619 <https://doi.org/10.1519/R-16924.1>

620 Febbraio, M. A., Carey, M. F. and Unit, E. M. (1995). Effect of creatine

621 supplementation on intramuscular. *Acta Physiologica Scandinavica*, 155: 387–

622 395. <https://doi.org/10.1111/j.1748-1716.1995.tb09988.x>

623 Finn, J., Ebert, T., Withers, R., Carey, M., Mackay, M., Phillips, J. and Febbraio, M.

624 (2001). Effect of Creatine Supplementation on metabolism and performance in

625 humans during Intermittent Sprint cycling. *Eur J Appl Physiol*, 17: 238–243.

626 <https://doi.org/10.1007/s004210170011>

627 Froyd, C., Millet, G. Y. and Noakes, T. D. (2013). The development of peripheral

628 fatigue and short-term recovery during self-paced high-intensity exercise. *Journal*

629 *of Physiology*, 591: 1339–1346. <https://doi.org/10.1113/jphysiol.2012.245316>

630 Gandevia, S. (2001). Spinal and supraspinal factors in human muscle fatigue.

631 *Physiological Reviews*, 81: 1725–89.

632 <https://doi.org/10.1152/physrev.2001.81.4.1725>

633 Girard, O., Bishop, D. J. and Racinais, S. (2013). Neuromuscular Adjustments of the

634 Quadriceps Muscle after Repeated Cycling Sprints. *PLoS ONE*, 8: 1–10.

635 <https://doi.org/10.1371/journal.pone.0061793>

636 Greenhaff, P. L., Bodin, K., Soderlund, K. and Hultman, E. (1994). Effect of oral
637 creatine supplementation on skeletal muscle phosphocreatine resynthesis.
638 *American Journal of Physiology-Endocrinology and Metabolism*, 266: E725–
639 E730. <https://doi.org/10.1152/ajpendo.1994.266.5.E725>

640 Harris, R. C., Söderlund, K. and Hultman, E. (1992). Elevation of creatine in resting
641 and exercised muscle of normal subjects by creatine supplementation. *Clinical*
642 *Science*, 83: 367–374.

643 Hermens, H. J., Freriks, B., Disselhorst-Klug, C. and Rau, G. (2000). Development of
644 recommendations for SEMG sensors and sensor placement procedures. *Journal*
645 *of Electromyography and Kinesiology*, 10: 361–374.
646 [https://doi.org/10.1016/S1050-6411\(00\)00027-4](https://doi.org/10.1016/S1050-6411(00)00027-4)

647 Hill, D. W. (1993). The critical power concept. A Review. *Sports Medicine*, 16: 237–
648 254.

649 Hultman, E., Söderlund, K., Timmons, J. A., Cederblad, G. and Greenhaff, P. L.
650 (1996). Muscle creatine loading in men. *Journal of Applied Physiology*, 81: 232–
651 237. <https://doi.org/10.1152/jappl.1996.81.1.232>

652 Hureau, T. J., Romer, L. M. and Amann, M. (2016). The 'sensory tolerance limit': A
653 hypothetical construct determining exercise performance? *European Journal of*
654 *Sport Science*, 18: 13-24. <https://doi.org/10.1080/17461391.2016.1252428>

655 Jacobs, I., Bleue, S. and Goodman, J. (1997). Creatine ingestion increases anaerobic
656 capacity and maximum accumulated oxygen deficit. *Canadian Journal of Applied*
657 *Physiology*, 22: 231–243. <https://doi.org/10.1139/h97-015>

658 Jaffe, M. (1886). Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn
659 erzeugt und über eine neue Reaction des Kreatinins. *Journal of Physical*
660 *Chemistry*, 10: 391–400. <https://doi.org/10.1515/bchm1.1886.10.5.391>

661 Jenkins, D. G. and Quigley, B. M. (1993). The influence of high-intensity exercise
662 training on the Wlim-Tlim relationship. *Medicine and Science in Sports and*
663 *Exercise*, 25: 275–282. <https://doi.org/10.1249/00005768-199302000-00019>

664 Johnson, M. A., Sharpe, G. R., Williams, N. C. and Hannah, R. (2015). Locomotor
665 muscle fatigue is not critically regulated after prior upper body exercise. *Journal*
666 *of Applied Physiology*, 119: 840–850.
667 <https://doi.org/10.1152/jappphysiol.00072.2015>

668 Jones, A.M., Wilkerson, D.P. and Fulford, J. (2009). Influence of dietary creatine
669 supplementation on muscle phosphocreatine kinetics during knee-extensor

670 exercise in humans. *American Journal of Physiology. Regulatory, Integrative and*
671 *Comparative Physiology*, 296: R1078-R1087.
672 <https://doi.org/10.1152/ajpregu.90896.2008>

673 Jones, A., Wilkerson, D., DiMenna, F., Fulford, J. and Poole, D. (2008). Muscle
674 metabolic responses to exercise above and below the 'critical power' assessed
675 using ³¹P-MRS. *American journal of physiology. Regulatory, Integrative and*
676 *Comparative Physiology*, 294: R585–R593.
677 <https://doi.org/10.1152/ajpregu.00731.2007>

678 Jones, A.M., Carter, H., Pringle, J.S. and Campbell, I.T. (2002) Effect of creatine
679 supplementation on oxygen uptake kinetics during submaximal cycle exercise.
680 *Journal of Applied Physiology*, 92: 2571-2577.
681 <https://doi.org/10.1152/jappphysiol.01065.2001>

682 McNaughton, L. R., Dalton, B. and Tarr, J. (1998). The effects of creatine
683 supplementation on high-intensity exercise performance in elite performers.
684 *European Journal of Applied Physiology*, 78: 236–240.
685 <https://doi.org/10.1007/s004210050413>

686 Merton, P. (1954). Voluntary strength and fatigue. *Journal of Physiology*, 123: 553–
687 564. <https://doi.org/10.1113/jphysiol.1954.sp005070>

688 Miura, A., Kino, F., Kajitani, S., Sato, H., Sato, H. and Fukuba, Y. (1999). The effect
689 of oral creatine supplementation on the curvature constant parameter of the
690 power-duration curve for cycle ergometry in humans. *Japanese Journal of*
691 *Physiology*, 49: 169–174. <https://doi.org/10.2170/jjphysiol.49.169>

692 Miura, A., Sato, H., Sato, H., Whipp, B. J. W. and Fukuba, Y. (2000). The effect of
693 glycogen depletion on the curvature constant parameter of the power-duration
694 curve for cycle ergometry. *Ergonomics*, 43: 133–141.
695 <https://doi.org/10.1080/001401300184693>

696 Monod, H. and Scherrer, J. (1965). The Work Capacity of a Synergic Muscular Group.
697 *Ergonomics*, 8: 329–338. <https://doi.org/10.1080/00140136508930810>

698 Moritani, T., Nagata, A., Devries, H. A. and Muro, M. (1981). Critical power as a
699 measure of physical work capacity and anaerobic threshold. *Ergonomics*, 24:
700 339–350. <https://doi.org/10.1080/00140138108924856>

701 Moritani, T., Takaishi, T. and Matsumoto, T. (1993). Determination of maximal power
702 output at neuromuscular fatigue threshold. *Journal of Applied Physiology*, 74:
703 1729–1734. <https://doi.org/10.1152/jappl.1993.74.4.1729>

- 704 Mujika, I., Padilla, S., Ibanez, J., Izquierdo, M. and Gorostiaga, E. (2000). Creatine
705 supplementation and sprint performance in soccer players. *Medicine & Science*
706 *in Sports & Exercise*, 32: 518–525. [https://doi.org/10.1097/00005768-](https://doi.org/10.1097/00005768-200002000-00039)
707 [200002000-00039](https://doi.org/10.1097/00005768-200002000-00039)
- 708 Murgatroyd, S. R., Ferguson, C., Ward, S. A., Whipp, B. J. and Rossiter, H. B. (2011).
709 Pulmonary O₂ uptake kinetics as a determinant of high-intensity exercise
710 tolerance in humans. *Journal of Applied Physiology*, 110: 1598–1606.
711 <https://doi.org/10.1152/jappphysiol.01092.2010>
- 712 Poole, D., Ward, S. A., Gardner, G. W. and Whipp, B. J. (1988). Metabolic and
713 respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics*,
714 31: 1265–1279. <https://doi.org/10.1080/00140138808966766>
- 715 Prevost, M. C., Nelson, A. G. and Morris, S. G. (1997). Creatine supplementation
716 enhances intermittent work performance. *Research Quarterly for Exercise and*
717 *Sport*, 68: 233–240. <https://doi.org/10.1080/02701367.1997.10608002>
- 718 Rico-Sanz, J. and Marco, M.T. (2000). Creatine enhances oxygen uptake and
719 performance during alternating intensity exercise. *Medicine and Science in Sports*
720 *and Exercise*, 32(2): 379-385.
- 721 Romer, L. M., Haverkamp, H. C., Amann, M., Lovering, A. T., Pegelow, D. F. and
722 Dempsey, J. A. (2007). Effect of acute severe hypoxia on peripheral fatigue and
723 endurance capacity in healthy humans. *American Journal of Physiology.*
724 *Regulatory, Integrative and Comparative Physiology*, 292: R598–R606.
725 <https://doi.org/10.1152/ajpregu.00269.2006>
- 726 Rossiter, H. B., Cannell, E. R. and Jakeman, P. M. (1996). The effect of oral creatine
727 supplementation on the 1000-m performance of competitive rowers. *Journal of*
728 *Sports Sciences*, 14: 175–179. <https://doi.org/10.1080/02640419608727699>
- 729 Schäfer, L. U., Hayes, M. and Dekerle, J. (2019). The magnitude of neuromuscular
730 fatigue is not intensity dependent when cycling above critical power but relates to
731 aerobic and anaerobic capacities. *Experimental Physiology*, 104: 209–219.
732 <https://doi.org/10.1113/EP087273>
- 733 Skare, O., Skadberg, Ø. and Wisnes, A. R. (2001). Creatine supplementation
734 improves sprint performance in male sprinters. *Scandinavian Journal of Medicine*
735 *Science and Sports*, 11: 96–102. [https://doi.org/10.1034/j.1600-](https://doi.org/10.1034/j.1600-0838.2001.011002096.x)
736 [0838.2001.011002096.x](https://doi.org/10.1034/j.1600-0838.2001.011002096.x)
- 737 Smith, A., Walter, A., Herda, T., Ryan, E., Moon, J., Cramer, J. and Stout, J. (2007).

738 Effects of creatine loading on electromyographic fatigue threshold during cycle
739 egrometry in college-aged women. *Journal of International Society of Sports*
740 *Nutrition*, 4. <https://doi.org/10.1186/1550-2783-4-20>

741 Smith, S.A., Montain, S.J., Zientara, G.P. and Fielding, R.A. (2004). Use of
742 phosphocreatine kinetics to determine the influence of creatine on muscle
743 mitochondrial respiration: an in vivo ³¹P-MRS study of oral creatine ingestion.
744 *Journal of Applied Physiology*, 96: 2288-2292.
745 <https://doi.org/10.1152/japplphysiol.01021.2003>

746 Smith, J. C., Stephens, D. P., Hall, E. L., Jackson, A. W. and Earnest, C. P. (1998a).
747 Effect of oral creatine ingestion on parameters of the work rate-time relationship
748 and time to exhaustion in high-intensity cycling. *European Journal of Applied*
749 *Physiology and Occupational Physiology*, 77: 360–365.
750 <https://doi.org/10.1007/s004210050345>

751 Smith, S.A., Montain, S.J., Matott, R.P., Zientara, G.P., Jolesz, F.A. and Fielding, R.A.
752 (1998b) Creatine supplementation and age influence muscle metabolism during
753 exercise. *Journal of Applied Physiology*, 85(4): 1349-1356.
754 <https://doi.org/10.1152/jappl.1998.85.4.1349>

755 Stout, J., Eckerson, J., Ebersole, K., Moore, G., Perry, S., Housh, T., ... Batheja, A.
756 (2000). Effect of creatine loading on neuromuscular fatigue threshold. *Journal of*
757 *Applied Physiology*, 88: 109–112. <https://doi.org/10.1152/jappl.2000.88.1.109>

758 Syrotuik, D. G. and Bell, G. J. (2004). Acute creatine monohydrate supplementation:
759 A descriptive physiological profile of responders vs. nonresponders. *Journal of*
760 *Strength and Conditioning Research*, 18: 610–617.
761 <https://doi.org/10.1519/12392.1>

762 Terjung, R., Clarkson, P., Eichner, E. R., Greenhaff, P. L., Hespel, P. J., Israel, R. G.,
763 ... Williams, M. (2000). American College of Sports Medicine roundtable. The
764 physiological and health effects of oral creatine supplementation. *Medicine &*
765 *Science in Sports & Exercise*, 32: 706–717. [https://doi.org/10.1097/00005768-](https://doi.org/10.1097/00005768-200003000-00024)
766 [200003000-00024](https://doi.org/10.1097/00005768-200003000-00024)

767 Thomas, K., Goodall, S., Stone, M., Howatson, G., Clair Gibson, A. S. and Ansley, L.
768 (2015). Central and Peripheral Fatigue in Male Cyclists after 4-, 20-, and 40-km
769 Time Trials. *Medicine & Science in Sports & Exercise*, 47: 537–546.
770 <https://doi.org/10.1249/MSS.0000000000000448>

771 Tukey, J.W. Exploratory data analysis. Reading, Mass: Addison-Wesley Publishing

772 Company.

773 Vanhatalo, A., Fulford, J., Dimenna, F. J. and Jones, A. M. (2010). Influence of
774 hyperoxia on muscle metabolic responses and the power – duration relationship
775 during severe-intensity exercise in humans: a ³¹P magnetic resonance
776 spectroscopy study. *Experimental Physiology*, 95: 528–540.
777 <https://doi.org/10.1113/expphysiol.2009.050500>

778 Vanhatalo, A. and Jones, A. M. (2009). Influence of creatine supplementation on the
779 parameters of the ‘All-out critical power test’. *Journal of Exercise Science and*
780 *Fitness*, 7: 9–17. [https://doi.org/10.1016/S1728-869X\(09\)60002-2](https://doi.org/10.1016/S1728-869X(09)60002-2)

781 Volek, J. and Kraemer, W. (1996). Creatine supplementation: Its effect on human
782 muscular performance and body composition. *Journal of Strength and*
783 *Conditioning Research*, 10: 200–210.

784 Walker, J.B. (1979). Creatine: Biosynthesis, Regulation, and Function. *Advances in*
785 *Enzymology and Related Areas of Molecular Biology*, 50: 177-242.
786 <https://doi.org/10.1002/9780470122952.ch4>

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790 **Additional Information**

791

792 *Competing interests*

793 This work was completed at the University of Brighton. All authors contributed to the
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