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Acetaminophen ingestion improves muscle activation and performance during a 3-min all-out cycling test

Original investigation

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1 **ABSTRACT**

2 **Purpose:** Acute acetaminophen (ACT) ingestion has been shown to enhance cycling time-
3 trial performance. The purpose of this study was to assess whether ACT ingestion enhances
4 muscle activation and critical power (CP) during maximal cycling exercise. **Methods:**
5 Sixteen active male participants completed two 3-min all-out tests against a fixed resistance
6 on an electronically-braked cycle ergometer 60 minutes following ingestion of 1 g ACT or
7 placebo (maltodextrin, PL). CP was estimated as the mean power output over the final 30 s of
8 the test and W' (the curvature constant of the power-duration relationship) was estimated as
9 the work done above CP. The femoral nerve was stimulated every 30 s to measure membrane
10 excitability (M-wave) and surface electromyography (EMG_{RMS}) was recorded continuously
11 to infer muscle activation. **Results:** Compared to PL, ACT ingestion increased CP (ACT: 297
12 ± 32 vs PL: 288 ± 31 W, $P < 0.001$) and total work done (ACT: 66.4 ± 6.5 vs PL: 65.4 ± 6.4
13 kJ, $P = 0.03$) without impacting W' (ACT: 13.1 ± 2.9 vs PL: 13.6 ± 2.4 kJ, $P = 0.19$) or the M-
14 wave amplitude ($P = 0.66$) during the 3-min all-out cycling test. Normalized EMG_{RMS}
15 amplitude declined throughout the 3-min protocol in both PL and ACT conditions; however,
16 the decline in EMG_{RMS} was attenuated in the ACT condition, with the EMG_{RMS} amplitude
17 being greater compared to PL over the last 60 s of the test ($P = 0.04$). **Conclusion:** These
18 findings indicate that acute ACT ingestion might increase performance and CP during
19 maximal cycling exercise by enhancing muscle activation.

20

21 **Key words:** Analgesic; critical power; electromyography; muscle activation; neuromuscular
22 fatigue; exercise performance

23 INTRODUCTION

24 Fatigue is a complex, multi-factorial process that is linked to perturbations within the central
25 nervous system and the contracting skeletal muscles (Enoka & Duchateau, 2008; Hureau et
26 al. 2016). Recent studies suggest that fatigue development may be related, at least in part, to
27 pain sensation (Astokorki & Mauger 2017a; Astokorki & Mauger 2017b; O’Leary et al.
28 2017). Acetaminophen (ACT) is a commonly used medicine for general pain relief.
29 Ingestion of ACT lowers pain sensation through inhibiting the cyclooxygenase enzymes,
30 which stimulate nociceptor discharge through the synthesis of prostaglandins (Graham et al.
31 2013; Józwiak-Bębenista & Nowak, 2014), and modulating serotonergic, opioid and
32 cannabinoid pathways (Graham et al. 2013; Pickering et al. 2006, 2008). Acute ACT
33 ingestion has been shown to enhance endurance exercise performance consistent with the
34 notion that interventions which can modulate pain sensation have the potential to influence
35 exercise performance (Foster et al. 2014; Mauger et al. 2010, Morgan et al. 2018). Indeed,
36 similar to the effects of caffeine (O’Connor et al. 2004), Mauger et al. (2010) and Foster et al.
37 (2014) have both previously reported enhanced exercise performance and/or work output at a
38 given level of muscle pain following acute ACT ingestion. These results suggest that ACT
39 reduces pain at a given absolute work rate and/or permits a higher work rate for an equivalent
40 pain sensation.

41

42 In a recent study, Morgan et al. (2018) reported an attenuated decline in skeletal muscle
43 electromyography (EMG) amplitude, reflective of an increase in muscle activation, and an
44 increased critical torque during a maximal intermittent single-leg knee extensor test following
45 ACT ingestion. During cycling exercise, the power equivalent of the critical torque, the
46 critical power (CP), represents an important threshold for oxidative metabolic control and
47 exercise tolerance (Jones et al. 2010; Vanhatalo et al. 2011). Indeed, CP, which is the

48 asymptote of the hyperbolic relationship between power output and time to exhaustion,
49 reflects the highest work rate that can be sustained without a progressive loss of
50 intramuscular and systemic homeostasis (Black et al. 2016; Poole et al. 1988; Poole et al.
51 1990; Vanhatalo et al. 2016), and interacts with the curvature constant of this relationship,
52 W' , to define exercise tolerance within the severe exercise intensity domain (Jones et al.
53 2010; Vanhatalo et al. 2011). Since CP is linked to muscle activation and neuromuscular
54 fatigue development during exercise, as inferred from EMG responses (Burnley et al. 2012),
55 and since ACT ingestion can concomitantly influence EMG responses and the critical torque
56 (Morgan et al., 2018), ACT might also enhance CP by modulating aspects of central fatigue
57 development during large muscle mass exercise. This potential blunting in central fatigue
58 development could be mediated by inhibition of nociceptor sensitising prostaglandins
59 (Graham et al. 2013; Józwiak-Bębenista & Nowak, 2014) and/or enhanced corticospinal
60 excitability (Mauger & Hopker, 2013) permitting an increased CP and thus improved
61 endurance exercise performance.

62

63 Although the improvement in cycling performance that has been reported following ACT
64 ingestion (Foster et al. 2014; Mauger et al. 2010) may also be linked to enhanced
65 neuromuscular function and a higher CP, as observed during single leg exercise (Morgan et
66 al. 2018), the exercise modality and the volume of skeletal muscle mass engaged are known
67 to influence the degree of neuromuscular and peripheral fatigue development. Specifically,
68 greater peripheral fatigue development has been observed at the same relative intensity
69 during knee-extensor exercise compared to cycling exercise (Rossman et al. 2012, 2014).
70 Therefore, the mechanisms underpinning the potential ergogenic effect of ACT on larger
71 muscle mass exercise such as cycling, which is more relevant for sports performance,
72 requires further research.

73

74 The purpose of the present study was, therefore, to assess the effect of acute ACT ingestion
75 on neuromuscular fatigue development and its potential underlying mechanisms during large
76 muscle mass exercise. We tested the hypotheses that, compared to placebo, acute
77 consumption of 1 g ACT would increase total work done, CP and muscle activation during a
78 3-min all-out cycling test.

79

80 **MATERIALS AND METHODS**

81 *Participants*

82 Sixteen trained male cyclists (mean \pm SD: age 29 ± 9 y, height 1.79 ± 0.07 m, body mass 77
83 ± 8 kg, $\dot{V}O_{2peak}$ 60.8 ± 7.0 ml \cdot kg $^{-1}\cdot$ min $^{-1}$, range: 52-77 ml \cdot kg $^{-1}\cdot$ min $^{-1}$) provided written
84 informed consent to participate in the present study, which was approved by the local Ethics
85 Committee (Sport and Health Sciences, University of Exeter). All subjects participated in
86 local cycling competitions. Trained individuals were selected as it has been shown that
87 endurance training influences pain tolerance (O'Leary et al. 2017). After being informed of
88 the experimental procedures and associated risks, all participants completed a medical health
89 questionnaire, which was checked by a medical doctor, to ensure it was safe to consume ACT
90 prior to performing exhaustive exercise. The questionnaire incorporated questions pertaining
91 to: known allergies to medications, current intake of medication and prior use of ACT as well
92 as any history of illnesses, cigarette use, alcohol consumption, illegal drug use and chronic
93 illnesses (personal and family history). None of the participants had a history of motor and/or
94 neurological disorders or frequent chronic ingestion of pain relief medication (i.e. ACT, non-
95 steroidal anti-inflammatory medication etc.). Participants were also advised to avoid
96 ingestion of pain relief medication over the duration of the study and were provided with a
97 list of prohibited medication(s). Participants were instructed to arrive at the laboratory in a

98 rested and fully hydrated state, at least 3 h post-prandial, and to avoid strenuous exercise, and
99 consumption of caffeine and alcohol in the 24 h prior to each testing session.

100

101 *Experimental Design*

102 Participants visited the laboratory on 5 occasions over a 5- to 6-week period with all tests
103 conducted at a similar time of day (± 90 min). All tests were conducted on an electronically
104 braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). On the first
105 laboratory visit, participants performed a ramp-incremental cycling test for the determination
106 of the linear factor (as described below), gas exchange threshold (GET), peak aerobic power
107 output and the peak oxygen uptake ($\dot{V}O_{2\text{peak}}$). During this initial laboratory visit, the seat and
108 handlebar positions were adjusted for comfort and replicated for all tests. The second and
109 third laboratory visits were used to familiarise participants to the measurements and
110 experimental protocol as described below. During these visits (i.e. visits 2-3), participants
111 completed a 3-min all-out cycling test to ensure the coefficient of variation for work done and
112 CP between visits was $<1\%$ and that the criteria to ensure a valid test were fulfilled (Jones et
113 al. 2010). For each 3-min test, achievement of $\dot{V}O_{2\text{peak}}$ ($>95\%$), as verified by the $\dot{V}O_{2\text{peak}}$
114 achieved during the ramp incremental ramp tests, was an obligatory criterion for a valid test.
115 In the one instance where these criteria were not fulfilled, the participant completed a further
116 familiarisation trial prior to commencing the experimental trials. During these sessions, the
117 settings and placement of EMG and peripheral nerve stimulation electrodes were recorded for
118 each subject as a reference for electrode placement in subsequent experimental trials (see
119 below for further details). These trials were not included in the subsequent data analysis.
120 Participants then performed the fatiguing protocol under two experimental conditions:
121 placebo (PL) and ACT. Experimental sessions were separated by 3-7 days.

122

123 *Experimental protocol*

124 All trials (visits 1-5) started with a standardised warm-up routine (10 min at 100-150 W,
125 corresponding to <90% GET, followed by 5 min of passive rest) and testing of the optimal
126 EMG electrode (for recording muscle activation), anode and cathode placement and
127 stimulation intensity for peripheral nerve stimulation. Single peripheral nerve stimulation
128 pulses were manually triggered at rest to determine the characteristics of the M-wave
129 response to supra-maximal nerve stimulation. Neuromuscular function was assessed pre-,
130 during- and post-trial (<10 s) as described below.

131

132 The experimental protocol comprised a 3-min period of unloaded pedalling at the
133 participant's preferred cadence, followed by a 3-min all-out sprint, 60 min following
134 ingestion of either PL (1 g maltodextrin) or 1 g ACT (visits 4-5). This timing was selected to
135 coincide with the attainment of the peak plasma [ACT] concentration (Anderson et al. 2008).
136 The placebo was made from dextrose powder inserted into gelatine capsules designed to have
137 an identical appearance and weight to ACT capsules but without the analgesic and antipyretic
138 effects. The order of trials for visits 4 and 5 were administered in a double-blind, randomised
139 fashion using a counter-balanced cross-over experimental design. The 3-min all-out cycling
140 protocol used in this study replicated the procedures described previously by Vanhatalo et al.
141 (2007, 2008). The fixed resistance for the all-out sprint was set using the linear mode of the
142 ergometer such that on reaching their preferred cadence, the participants would achieve a
143 power output equivalent to 50% of the difference between GET and $\dot{V}O_{2\text{peak}}$ (linear factor =
144 $50\% \Delta \text{ power output / preferred cadence}^2$).

145

146 *Measurements*

147 *Breath-by-breath pulmonary gas exchange*

148 Throughout all laboratory tests, participants wore a mask connected to an impeller turbine
149 transducer assembly (Cortex Metalyzer, Cortex, Leipzig, Germany). Inspired and expired gas
150 volume and concentration signals were continuously sampled at 100 Hz. The analyser was
151 calibrated before each test with gases of known concentration (O₂ 15%, CO₂ 4.5%), and a
152 calibration syringe of known volume (3-L; Hans Rudolph, KS).

153

154 *Electromyography*

155 Neuromuscular function was assessed pre-, during- and immediately post each of the trials.
156 Pre- and post-trial neuromuscular function was tested with the participant cycling at 80 RPM
157 with a low resistance (20 W) as described below. Surface EMG activity was measured from
158 *m. vastus lateralis*, *m. vastus medialis*, *m. rectus femoris* and *m. biceps femoris* muscles of the
159 right leg to continuously record muscle activity during exercise using active bipolar bar
160 electrodes with a single differential configuration (DE2.1, DelSys Inc, Boston, MA, USA).
161 Bipolar electrodes were positioned over the muscle belly parallel to the longitudinal axis of
162 each muscle (SENIAM guidelines). The placement of electrodes was considered optimal on
163 achieving the largest and most reproducible M-wave signal from the *m.vastus lateralis* and
164 *m.vastus medialis* whilst noting minimal activity in the *m.bicep femoris*. Placement of
165 electrodes was optimised during each laboratory visit. Double-sided adhesive tape and a
166 hypoallergenic medical tape were used to ensure the EMG sensor stability for recording
167 electrodes. The skin area underneath each EMG electrode was shaved, then exfoliated and
168 cleaned with alcohol to minimise the skin impedance. The EMG signal was pre-amplified
169 (1000 x), band-pass filtered (20–450 Hz, Bagnoli-8, DelSys Inc, Boston, MA, USA), and
170 then transferred to a computer with a sampling frequency of 2 kHz. EMG data were recorded
171 continuously and digitised synchronously with 16 bit resolution via an A/D converter (± 5 V
172 range, CED 1401 power, Cambridge, UK). EMG was average rectified using the root mean

173 square method (EMG_{RMS}). EMG_{RMS} throughout the trial was then normalised to the EMG
174 signal during the first 30 s of the 3-min test to provide a percentage of the maximal signal.
175 Finally, EMG_{RMS} was normalised to the local (closest) standardised M-wave amplitude and
176 presented as a percentage of the maximal signal. In addition, M-wave amplitude was
177 normalised by pre-exercise, resting values, and presented as a percentage. This method of
178 normalizing the EMG trace to the M-wave may enable a more accurate assessment of
179 changes in muscle activation that are likely occurring upstream of the neuromuscular junction
180 (i.e. spinal and/or supraspinal in origin). The ground electrode was placed over the patella of
181 the right leg.

182

183 *Peripheral Nerve Stimulation*

184 Electrical stimulation was applied using a constant current stimulator (Digitimer Stimulator
185 DS7AH, Digitimer, UK). Initially, the crank angle at which peripheral nerve stimulation was
186 to be delivered during the trials was determined for each subject as described by Black et al.
187 (2017) and as performed by Sidhu et al. (2012). Stimulations were delivered at the identified
188 crank angle specific to each trial ($62 \pm 7^\circ$ relative to full knee extension, 180°) to align with
189 maximal EMG_{RMS} amplitude. A custom written sequencer script triggered 3 single
190 stimulations, independently, with at least 1 and up to 10 pedal revolutions between stimuli.
191 During the 3-min cycling test, these stimulations were delivered every 30 s. M-waves were
192 elicited in *m.vastus lateralis* and *m.vastus medialis* by supramaximal percutaneous electrical
193 stimulation of the femoral nerve (200 μ s duration), approximately 3–5 cm below the inguinal
194 ligament in the femoral triangle. The cathode was systematically moved vertically and
195 horizontally and the amplitude of the muscle action potential (i.e. M-wave) was monitored to
196 identify the optimal position of the cathode for attaining maximal peak-to-peak M-wave
197 (M_{max}) amplitude. To determine the stimulation intensity, single stimuli were delivered in 20

198 mA step-wise increments from 100 mA until a plateau (i.e. M_{\max}) in the M-wave was
199 observed. A supramaximal pulse of 130% M_{\max} current (Burke, 2002; Goodall et al. 2010;
200 Neyroud et al. 2014) was applied during the exercise tests (mean stimulation intensity: $251 \pm$
201 48 mA). The procedures for optimal electrode placement and stimulation intensity were
202 completed during each laboratory visit (visits 2-5).

203

204 *Data Analysis*

205 Data were analysed using a custom written script developed in Spike2 software (CED,
206 Cambridge, UK). CP was estimated as the mean power output over the final 30 s of the test,
207 and the W' was estimated as the work done above the CP (Vanhatalo et al. 2007, 2008). Peak
208 $\dot{V}O_2$ was determined as the highest 15-s interval (i.e. $\dot{V}O_{2\text{peak}}$). Total work was calculated as
209 the area under the power-time curve. Peak power output attained in the 3-min test was
210 defined as the maximal 1-s interval. The changes in power output, M_{Max} and EMG_{RMS} , were
211 used to quantify neuromuscular fatigue development and changes in muscle activation. All
212 neuromuscular parameters and power output were averaged across the protocol into 6×30 -s
213 bin averages. Estimates of CP and W' were also used to predict the time taken to complete a
214 range of total work done (W) targets (50, 75, 100, 125, 150, 175, 200, 225, 250, 500, 750,
215 1000 kJ) as previously described (Kelly et al. 2013).

216

$$217 \quad T_{\text{lim}} = (W - W') / CP \quad (\text{equation 1})$$

218

219 *Statistics*

220 Paired-samples t -tests were used to compare the CP, W' , total work done and
221 cardiorespiratory responses between ACT and PL conditions. In addition, paired samples t -
222 tests were used to assess parameters of neuromuscular function at task end between trials (i.e.

223 M_{\max} and EMG_{RMS}). The profiles of power output, M-wave amplitude and EMG_{RMS} before,
224 during and after the 3-min test were analysed using two-way ANOVAs (time \times condition)
225 with repeated measures (using 30 s averages; i.e. 6 time points) between PL and ACT. A two-
226 way repeated-measures ANOVA was also used to assess differences in predicted
227 performance times. Where the ANOVA revealed a significant interaction effect, post-hoc
228 comparisons were completed using a Bonferroni correction. A Pearson's product moment
229 correlation coefficient was used to determine the relationship between the change in EMG
230 amplitude and the change in power production between conditions. A one-way ANOVA was
231 used to assess differences in $\dot{V}O_{2peak}$ obtained during the incremental ramp test and both 3-
232 min trials (PL and ACT). To assess the possibility of an order effect of trials, a paired
233 samples *t-test* was conducted on total work done for visits 4 and 5. For calculation of effect
234 size, partial eta squared (η^2) was used for omnibus tests. Cohen's *d* was used to calculate the
235 effect size for paired *t-tests* and post-hoc comparisons. All statistical tests were performed
236 both on % change and raw data. Where sphericity was violated, a Greenhouse Geisser
237 correction factor was used. For all tests, results were considered statistically significant when
238 $P < 0.05$. Data are presented as mean \pm SD, unless otherwise indicated. All statistical analyses
239 were conducted using IBM SPSS Statistics version 24.

240

241 **RESULTS**

242 Mean $\dot{V}O_{2peak}$ measured in the ramp incremental test was 4.50 ± 0.41 L \cdot min $^{-1}$ (61 ± 6
243 ml \cdot kg $^{-1}\cdot$ min $^{-1}$) and the peak aerobic power output was 393 ± 29 W. The GET occurred at
244 1.98 ± 0.26 L \cdot min $^{-1}$ and 152 ± 22 W. The $\dot{V}O_{2peak}$ achieved during the 3-min test following
245 PL (4.51 ± 0.59 L \cdot min $^{-1}$, 60 ± 7 ml \cdot kg $^{-1}\cdot$ min $^{-1}$) and ACT ingestion (4.53 ± 0.57 L \cdot min $^{-1}$, 61
246 ± 8 ml \cdot kg $^{-1}\cdot$ min $^{-1}$) were not significantly different to the values achieved during the ramp
247 incremental test ($P=0.77$).

248

249 *3-min all-out cycling test*

250 The $\dot{V}O_2$ profile during the 3-min test for PL and ACT conditions is shown in figure 1 (panel
251 A). In addition, the mean power output profile for all participants (and differences in CP)
252 during the 3-min all-out cycling test is shown in figure 1 (panel B) for the PL and ACT
253 conditions. Panel C represents changes to power output throughout the duration of the 3-min
254 test in all trials and is provided in 30-s averages. During the PL trial, power output declined
255 from 820 ± 139 W during the first 5 s of the test to 288 ± 31 W during the last 30 s of the 3-
256 min test ($P < 0.0001$, $\eta^2 = 0.99$; table 1). However, during the ACT trial, power output declined
257 from 838 ± 127 W during the first 5 s of the test to 297 ± 32 W during the final 30 s of the
258 test (table 1). There was a significant interaction effect (time \times condition; $P = 0.04$, $\eta^2 = 0.26$)
259 with the mean power output in the 3-min cycling test being greater in ACT (368 ± 36 W)
260 compared to PL (363 ± 36 W, $P = 0.007$, $d = 0.13$). CP (ACT: 297 ± 32 W vs. PL: 288 ± 31 W,
261 $P < 0.0001$, $d = 0.28$) and total work done (ACT: 66.4 ± 6.5 kJ vs. 65.4 ± 6.4 kJ; $P = 0.03$,
262 $d = 0.15$) was higher with ACT compared to PL (table 1; figure 2). However, there was no
263 difference in peak power output (ACT: 838 ± 127 W vs. PL: 820 ± 139 W, $P = 0.10$, $d = 0.16$)
264 or W' (ACT: 13.1 ± 2.9 vs. PL: 13.6 ± 2.4 kJ; $P = 0.19$, $d = 0.20$) during the 3-min cycling test
265 between conditions. No order effect was observed between visit 4 and visit 5 for total work
266 done (Visit 4: 65.8 ± 6.5 kJ vs. Visit 5: 66.0 ± 6.4 kJ; $P = 0.75$, $d = 0.03$).

267

268 When the CP and W' were combined to predict the time required to complete fixed work
269 targets between 50 and 1000 kJ, using equation 1, the ANOVA revealed a main effect by
270 condition ($P < 0.0001$, $\eta^2 = 0.56$) and an interaction effect ($P < 0.0001$, $\eta^2 = 0.86$, table 2). Post-
271 hoc analysis revealed that the performance times were lower in the ACT condition compared

272 with the PL condition for all time-trials with the exception of the two shortest (i.e. 50 and 75
273 kJ), with the improvement ranging from 1.1% (100 kJ) to 3.0% (1000 kJ).

274

275 *Neuromuscular Function*

276 From pre to post exercise, there was a main effect for time on M-wave amplitude in the
277 *m.vastus lateralis* ($P=0.003$, $\eta^2=0.29$, figure 3), which declined as the protocol progressed.
278 However, there was no main effect by condition ($P=0.66$, $\eta^2=0.01$) or time \times condition
279 interaction effect ($P=0.70$, $\eta^2=0.03$). EMG_{RMS} in the *m.vastus lateralis* decreased from $94 \pm$
280 4% over the first 30 s to $54 \pm 17\%$ over the final 30 s of the 3-min all-out test in the PL trial
281 ($P<0.0001$, $\eta^2=0.50$; figure 4). However, this decline in EMG_{RMS} was attenuated following
282 ACT ingestion (from 92 ± 5 over the first 30 s to $72 \pm 18\%$ over the final 30 s of the 3-min
283 all-out test), with there being a time \times condition interaction effect ($P=0.04$, $\eta^2=0.23$). Post-
284 hoc analysis revealed EMG_{RMS} was elevated at 150 s ($P=0.02$, $d=0.84$) and 180 s ($P=0.001$,
285 $d=1.31$) in ACT compared to PL (figure 4). There was a significant positive correlation
286 between the change in EMG amplitude and the change in power production over the last 30 s
287 of exercise between conditions ($r=0.88$, $P=0.04$, figure 5).

288

289 **DISCUSSION**

290 Consistent with our hypotheses, the principal original findings of this study were that acute
291 ACT ingestion enhanced total work done and CP, and attenuated the decline in EMG
292 amplitude, in trained individuals during a 3-min all-out cycling test. The ACT-induced
293 increase in CP was predicted to translate into a 1-3% reduction in the time required to
294 complete a range of target work cycling trials (100-1000 kJ). The results of this study provide
295 some insight into the mechanisms by which ACT ingestion is ergogenic during large muscle

296 mass exercise and suggest that enhanced performance following ACT ingestion is
297 attributable, at least in part, to increases in CP and muscle activation.

298

299 *Power-duration relationship*

300 Our finding of an increase in total work done following acute ACT ingestion in the 3-min all-
301 out cycling test is consistent with previous observations of enhanced exercise performance
302 following acute ACT ingestion of similar doses (1-1.5 g; Foster et al. 2014; Mauger et al.
303 2010, Morgan et al. 2018). In the present study, neuromuscular fatigue development was
304 assessed during the completion of a 3-min all-out cycling test to offer insight into the
305 potential underlying mechanisms for the ergogenic effects of ACT ingestion. Consistent with
306 our previous finding of a 4% increase in critical torque when utilising a single-limb knee-
307 extension model (Morgan et al. 2018), CP achieved during a 3-min all-out cycling test was
308 improved by ~3% following the acute ingestion of ACT in the present study. Moreover, and
309 consistent with our previous findings (Morgan et al. 2018), W' was not altered following
310 ACT ingestion in the current study.

311

312 The potential practical significance of the 3% improvement in CP becomes clear when
313 applied to an exercise performance scenario. An important practical application of the CP is
314 that this parameter, in conjunction with W' , can be used to robustly predict cycling TT
315 performance (Black et al. 2014, 2017; Burnley et al. 2012; Chidnok et al. 2013; Florence &
316 Weir, 1997; Skiba et al. 2012; Smith et al. 1999). Accordingly, the influence of a given
317 intervention on CP and W' can be used to predict the effect that that intervention might have
318 on endurance exercise performance. For example, although Kelly et al. (2013) reported no
319 statistically significant increase in either CP (+1.4%) or W' (+8.4%) following dietary nitrate
320 supplementation, when the combined effect on these parameters was integrated, an

321 improvement of 2-3% in cycling time-trial performance was predicted. Similarly, in the
322 current study, endurance performance was predicted to be improved by ~1-3% following
323 acute ACT ingestion in the work trial simulations (~5-60 min). Since this magnitude of
324 performance enhancement following acute ACT ingestion exceeds 0.6%, which is suggested
325 to be the smallest 'worthwhile' improvement in road TT cycling (Paton & Hopkins, 2006),
326 our results suggest that acute ACT ingestion may enable a practically meaningful
327 improvement in endurance exercise performance. It should also be noted that, although we
328 did not directly assess the effect of acute ACT ingestion on cycling TT performance in the
329 current study, the predicted 1-3% is similar to the empirically demonstrated 1.8%
330 improvement in 10-mile cycling TT performance reported previously (Mauger et al. 2010).

331

332 Interestingly, improvements in exercise performance with acute ACT ingestion have been
333 reported in trained participants in both the current study and in previous studies (Mauger et
334 al. 2010) despite evidence that endurance training increases pain tolerance (Jones et al. 2014;
335 O'Leary et al. 2017) such that trained individuals are more likely to have a greater tolerance
336 to pain (Janal et al. 1994; Tesarz et al. 2013). However, it should be stressed that, although
337 the current and previous studies support an ergogenic effect of acute ACT consumption
338 (Foster et al. 2014; Mauger et al. 2010, 2014; Morgan et al. 2018), regular ACT use, or
339 exceeding a single dose of 1 g, is not recommended given the hepatotoxicity of ACT
340 (Graham et al. 2013).

341

342 *Neuromuscular function*

343 In addition to influencing the degree of muscle metabolic perturbation and the trajectory of
344 the $\dot{V}O_2$ slow component during exercise (Jones et al. 2008, 2010; Poole et al. 1988;
345 Vanhatalo et al. 2011), CP is linked to muscle activation characteristics during exercise, as

346 inferred from EMG responses, and is a critical threshold for neuromuscular fatigue
347 development (Burnley et al. 2012). Indeed, concomitant with our observation of an increased
348 CP in the current study, the decline in EMG amplitude during the 3-min all-out test was
349 attenuated in ACT compared to PL. These findings are strikingly similar to our recent study,
350 which reported a blunted decline in the EMG amplitude and an increased critical torque
351 during a 5-min maximal intermittent single-legged knee extension exercise task (Morgan et
352 al. 2018). Together, these results suggest that improved maintenance of muscle activation
353 contributes to the elevated CP and total work done following ACT ingestion. However, the
354 blunting of neuromuscular fatigue development following ACT ingestion was not
355 accompanied by improvements in peripheral muscle excitability, as inferred from
356 measurements of M-wave amplitude between the ACT and PL trials, suggesting that this
357 alteration occurred due to mechanisms upstream of the neuromuscular junction.

358

359 Our results support the notion that the ergogenic effect of ACT is principally mediated
360 centrally (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010).
361 However, while we are not aware of any evidence to suggest that ACT might influence
362 peripheral muscle excitability (Mauger & Hopker, 2013), or that interventions aimed at
363 reducing inflammation improve performance during whole body exercise (i.e. Cleak, &
364 Eston. 1992; Da Silva et al. 2015; Nosaka & Clarkson, 1996; Tokmakdis et al. 2003), we
365 cannot exclude that peripheral factors that were not assessed in the current study, such as
366 inflammation and/or alterations to muscle metabolism, may have contributed to the ergogenic
367 effect of ACT. Moreover, due to the nature of cycling exercise, it is technically challenging to
368 directly test cortical alterations via changes to voluntary activation using the interpolated
369 twitch technique (Doyle-Baker et al. 2017).

370

371 Whilst we have previously investigated the contribution of central and peripheral factors to
372 the improved performance following ACT ingestion in a small muscle mass model (Morgan
373 et al. 2018), the mechanisms underpinning fatigue development, and therefore ACT's
374 potential ergogenic effect, could differ for large muscle mass exercise (Rossman et al. 2012,
375 2014). We observed a strong correlation between the change in end-exercise EMG_{RMS} and
376 the change in power output (i.e. CP) within the last 30 s of the 3-min cycling test ($r=0.88$)
377 following ACT ingestion compared to placebo. However, the change in EMG_{RMS} was much
378 larger than the change in CP. Although the mechanisms for this effect remain to be defined,
379 this observation is in agreement, with Felipe et al. (2018). Specifically, these authors
380 reported that, compared to placebo, caffeine ingestion increased mean power output by ~4%
381 during a 4-km cycling test, resulting in a 2% reduction in time to complete the 4-km distance,
382 alongside a ~17% increase in muscle recruitment (as inferred by EMG).

383

384 It is possible that, through lowering pain sensation (Foster et al. 2014; Mauger et al. 2010),
385 ACT might have permitted the development of, and/or tolerance to, a greater degree of
386 intramuscular metabolic perturbation beyond that required to evoke a 'critical' threshold of
387 peripheral fatigue, thereby permitting improved exercise performance (Blain et al. 2016).
388 Alternatively, since the effects of ACT are believed to be largely centrally mediated
389 (Anderson, 2008; Graham et al. 2013; Smith, 2009; Toussaint et al. 2010), it is possible that
390 ACT ingestion attenuated the development of central fatigue. A blunting in central fatigue
391 development following ACT ingestion would be expected to permit enhanced central motor
392 output, possibly through a reduction in inhibitory feedback via cyclooxygenase inhibition and
393 a resultant decline in the synthesis of prostaglandins.

394

395 The higher EMG_{RMS} during the latter stages of the 3-min all-out cycling test observed
396 following ACT ingestion may have been a consequence of enhanced corticospinal
397 excitability (Mauger & Hopker, 2013). Greater corticospinal excitability following ACT
398 ingestion, as inferred from a greater motor-evoked potential in the study of Mauger & Hopker
399 (2013), may be linked to enhanced firing of motor units, and increased spinal excitability, as
400 has been reported with caffeine consumption (i.e. Kalmar & Cafarelli, 2004; Walton et al.
401 2003). Together, these effects on motor cortical and/or spinal excitability may explain the
402 enhanced muscle activation and the subsequent greater amount of work performed with ACT
403 ingestion in the current study. However, since cortical and peripheral contributions to fatigue
404 development were not directly tested in this study, further research is required to resolve the
405 underlying mechanisms for the ACT-mediated enhancement in muscle activation and
406 performance during maximal exercise.

407

408 In conclusion, acute ACT ingestion increased total work done during a 3-min all-out cycling
409 test in agreement with earlier reports of an ergogenic effect of ACT ingestion on cycling
410 performance. The improved performance in the 3-min all-out test was accompanied by an
411 increase in CP and better preservation of the EMG amplitude during the latter stages of the
412 protocol. When the ACT-induced increase in CP was used to predict the effects of acute ACT
413 ingestion on cycling performance, the estimated 1-3% improvement was in line with previous
414 experimental observations. Therefore, our results extend previous reports by revealing that
415 ACT ingestion improves performance concomitant with enhanced CP and muscle activation
416 during a 3-min all-out cycling test. These observations provide insight into the ergogenic
417 effect of ACT ingestion during large muscle mass exercise.

418 **Conflict of interest**

419 The authors report no conflict of interest in the publication of this research

420

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425 manipulation.

426

427 **Author contribution**

428 P.T. Morgan, A. Vanhatalo, A.M. Jones and S.J. Bailey conceived and designed the research.
429 P.T. Morgan conducted all experiments. J.L. Bowtell provided assistance with pilot testing
430 prior to experimental data collection as well supporting data analysis. P.T. Morgan wrote the
431 manuscript. J.L. Bowtell, A. Vanhatalo, A.M. Jones and S.J. Bailey helped supervise the
432 project throughout. All authors contributed to the interpretation of results and read, edited and
433 approved the manuscript.

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613 **Figure captions**

614 *Figure 1*

615 Group mean \pm SE $\dot{V}O_2$ during acetaminophen (ACT, filled circles) and placebo (PL, clear
616 circles) is presented in panel A. The dashed line represents the $\dot{V}O_{2peak}$ attained in the
617 incremental ramp test. Panel B illustrates the mean \pm SE power output profile during the 3-
618 min maximal cycling protocol for placebo (clear circles) and acetaminophen (filled circles)
619 trials **derived from 15 s averages**. Note that after attainment of peak power output a few
620 seconds into the test, power output falls over the first **~90-120 s** before reaching stable values
621 (the end-test power output; i.e. CP). **CP is significantly elevated in the last 30 s of the ACT**
622 **condition. Significant changes to power output over time (derived from 30 s averages)**
623 **throughout the 3-min cycling test for both ACT and PL conditions are shown in panel C.**
624 *Significantly different from PL (**i.e. main effect of condition**); ^asignificantly different from
625 30 s; ^bsignificantly different from 60 s; ^csignificantly different from 90 s; ^dsignificantly
626 different from 120 s (**main effect of time, $P < 0.05$**).

627

628 *Figure 2*

629 Group mean total work done in the placebo (PL) and acetaminophen (ACT) conditions are
630 shown in the open and closed bars, respectively (**Panel A**). Individual responses in the PL and
631 ACT conditions are shown by the open circles and linked with dashed lines. *Significantly
632 different from PL ($P < 0.05$). **Panel B represents the group mean critical power (CP) in the PL**
633 **and ACT conditions in the open and closed bars, respectively. Individual responses in the PL**
634 **and ACT conditions are shown by the open circles and linked with dashed lines.**

635

636 *Figure 3*

637 M-wave amplitude responses in the *m.vastus lateralis* during the 3-min cycling test for
638 placebo (clear circles) and acetaminophen (filled circles) trials. Mean \pm SE M-wave
639 responses are presented in panel A with the M-wave response from a representative
640 individual presented in panel B, for PL (grey line) and ACT (black line), for the first 30 and
641 final 30 s, respectively of the 3-min protocol. ^asignificantly different from baseline;
642 ^bsignificantly different from 30 s ($P<0.05$).

643

644 *Figure 4*

645 Surface electromyography (EMG) responses (expressed relative to M-wave amplitude) in the
646 *m.vastus lateralis* during the 3-min cycling test for placebo (clear circles) and acetaminophen
647 (filled circles) trials. Mean \pm SE EMG responses are presented in panel A with the EMG
648 response from a representative individual presented in panel B, for PL (grey line) and ACT
649 (black line), for the first 30 and final 30 s, respectively of the 3-min protocol. *Significantly
650 different from placebo; ^asignificantly different from 30 s; ^bsignificantly different from 60 s;
651 ^csignificantly different from 90 s; ^dsignificantly different from 120 s; ^esignificantly different
652 from 150 s ($P<0.05$).

653

654 *Figure 5*

655 Correlation between the change in electromyography amplitude (EMG, %) and the change in
656 critical power (CP) between conditions (acetaminophen and placebo). The solid line
657 represents the line of best fit.