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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  $\pm 32$  vs PL:  $288 \pm 31$  W,  $P < 0.001$ ) and total work done (ACT:  $66.4 \pm 6.5$  vs PL:  $65.4 \pm 6.4$   
13 kJ,  $P = 0.03$ ) without impacting  $W'$  (ACT:  $13.1 \pm 2.9$  vs PL:  $13.6 \pm 2.4$  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  $29 \pm 9$  y, height  $1.79 \pm 0.07$  m, body mass  $77$   
83  $\pm 8$  kg,  $\dot{V}O_{2peak}$   $60.8 \pm 7.0$  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 ( $\pm 90$  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 ( $\dot{V}O_{2peak}$ ). 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  $\dot{V}O_{2peak}$  ( $>95\%$ ), as verified by the  $\dot{V}O_{2peak}$   
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 (O<sub>2</sub> 15%, CO<sub>2</sub> 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 ( $\pm 5$  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,  $180^\circ$ ) 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  $\mu$ 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  $EMG_{\text{RMS}}$ , 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  $6 \times 30$ -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  $\dot{V}O_{2\text{peak}}$  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 ( $\eta^2$ ) 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  $\dot{V}O_{2\text{peak}}$  measured in the ramp incremental test was  $4.50 \pm 0.41 \text{ L}\cdot\text{min}^{-1}$  ( $61 \pm 6$   
243  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and the peak aerobic power output was  $393 \pm 29 \text{ W}$ . The GET occurred at  
244  $1.98 \pm 0.26 \text{ L}\cdot\text{min}^{-1}$  and  $152 \pm 22 \text{ W}$ . The  $\dot{V}O_{2\text{peak}}$  achieved during the 3-min test following  
245 PL ( $4.51 \pm 0.59 \text{ L}\cdot\text{min}^{-1}$ ,  $60 \pm 7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) and ACT ingestion ( $4.53 \pm 0.57 \text{ L}\cdot\text{min}^{-1}$ ,  $61$   
246  $\pm 8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) 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  $820 \pm 139$  W during the first 5 s of the test to  $288 \pm 31$  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  $838 \pm 127$  W during the first 5 s of the test to  $297 \pm 32$  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 ( $368 \pm 36$  W)  
260 compared to PL ( $363 \pm 36$  W,  $P = 0.007$ ,  $d = 0.13$ ). CP (ACT:  $297 \pm 32$  W vs. PL:  $288 \pm 31$  W,  
261  $P < 0.0001$ ,  $d = 0.28$ ) and total work done (ACT:  $66.4 \pm 6.5$  kJ vs.  $65.4 \pm 6.4$  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:  $838 \pm 127$  W vs. PL:  $820 \pm 139$  W,  $P = 0.10$ ,  $d = 0.16$ )  
264 or  $W'$  (ACT:  $13.1 \pm 2.9$  vs. PL:  $13.6 \pm 2.4$  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:  $65.8 \pm 6.5$  kJ vs. Visit 5:  $66.0 \pm 6.4$  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<sub>RMS</sub> 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<sub>RMS</sub> was attenuated following  
282 ACT ingestion (from  $92 \pm 5$  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<sub>RMS</sub> 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<sub>RMS</sub> 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<sub>RMS</sub> 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<sub>RMS</sub> 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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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**); <sup>a</sup>significantly different from  
625 30 s; <sup>b</sup>significantly different from 60 s; <sup>c</sup>significantly different from 90 s; <sup>d</sup>significantly  
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. <sup>a</sup>significantly different from baseline;  
642 <sup>b</sup>significantly 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; <sup>a</sup>significantly different from 30 s; <sup>b</sup>significantly different from 60 s;  
651 <sup>c</sup>significantly different from 90 s; <sup>d</sup>significantly different from 120 s; <sup>e</sup>significantly 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.