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

INTRODUCTION

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

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

asymptote of the hyperbolic relationship between power output and time to exhaustion,

reflects the highest work rate that can be sustained without a progressive loss of

intramuscular and systemic homeostasis (Black et al. 2016; Poole et al. 1988; Poole et al.

1990; Vanhatalo et al. 2016), and interacts with the curvature constant of this relationship,

W', to define exercise tolerance within the severe exercise intensity domain (Jones et al.

2010; Vanhatalo et al. 2011). Since CP is linked to muscle activation and neuromuscular

fatigue development during exercise, as inferred from EMG responses (Burnley et al. 2012),

and since ACT ingestion can concomitantly influence EMG responses and the critical torque

(Morgan et al., 2018), ACT might also enhance CP by modulating aspects of central fatigue

development during large muscle mass exercise. This potential blunting in central fatigue

development could be mediated by inhibition of nociceptor sensitising prostaglandins

(Graham et al. 2013; Jóźwiak-Bębenista & Nowak, 2014) and/or enhanced corticospinal

excitability (Mauger & Hopker, 2013) permitting an increased CP and thus improved

endurance exercise performance.

Although the improvement in cycling performance that has been reported following ACT

ingestion (Foster et al. 2014; Mauger et al. 2010) may also be linked to enhanced

neuromuscular function and a higher CP, as observed during single leg exercise (Morgan et

al. 2018), the exercise modality and the volume of skeletal muscle mass engaged are known

to influence the degree of neuromuscular and peripheral fatigue development. Specifically,

greater peripheral fatigue development has been observed at the same relative intensity

during knee-extensor exercise compared to cycling exercise (Rossman et al. 2012, 2014).

Therefore, the mechanisms underpinning the potential ergogenic effect of ACT on larger

muscle mass exercise such as cycling, which is more relevant for sports performance,

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 ± 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·kg^{-1}·min^{-1}, range: 52-77 ml·kg^{-1}·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

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 $\dot{V}O_{2peak}$ (linear factor =

144 50%Δ 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_2 15%, CO_2 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 ($62 \pm 7°$ 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 µs 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. $\dot{V}O_{2peak}$). 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                           $T_{lim} = (W-W') / CP$ (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·min^{-1} (61 ± 6

243   ml·kg^{-1}·min^{-1}) and the peak aerobic power output was 393 ± 29 W. The GET occurred at

244   1.98 ± 0.26 L·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·min^{-1}, 60 ± 7 ml·kg^{-1}·min^{-1}) and ACT ingestion (4.53 ± 0.57 L·min^{-1}, 61

246   ± 8 ml·kg^{-1}·min^{-1}) were not significantly different to the values achieved during the ramp

247   incremental test ($P=0.77$).

248

3-min all-out cycling test

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

When the CP and W′ were combined to predict the time required to complete fixed work targets between 50 and 1000 kJ, using equation 1, the ANOVA revealed a main effect by condition ($P<0.0001$, $\eta^2=0.56$) and an interaction effect ($P<0.0001$, $\eta^2=0.86$, table 2). Post-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, η^2=0.29, figure 3), which declined as the protocol progressed.

278   However, there was no main effect by condition (P=0.66, η^2=0.01) or time $\times$ condition

279   interaction effect (P=0.70, η^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, η^2=0.50; figure 4). However, this decline in EMG_{RMS} 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, η^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 Felippe 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.

Conflict of interest

Acknowledgements

Author contribution

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

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Figure captions

Figure 1

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

Figure 2

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

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 ± 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. [a]significantly different from baseline;

642   [b]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 ± 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; [a]significantly different from 30 s; [b]significantly different from 60 s;

651   [c]significantly different from 90 s; [d]significantly different from 120 s; [e]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.