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

Original investigation

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ABSTRACT

Purpose: Acute acetaminophen (ACT) ingestion has been shown to enhance cycling time-trial performance. The purpose of this study was to assess whether ACT ingestion enhances muscle activation and critical power (CP) during maximal cycling exercise. **Methods**: Sixteen active male participants completed two 3-min all-out tests against a fixed resistance on an electronically-braked cycle ergometer 60 minutes following ingestion of 1 g ACT or placebo (maltodextrin, PL). CP was estimated as the mean power output over the final 30 s of the test and W′ (the curvature constant of the power-duration relationship) was estimated as the work done above CP. The femoral nerve was stimulated every 30 s to measure membrane excitability (M-wave) and surface electromyography (EMG_{RMS}) was recorded continuously to infer muscle activation. **Results:** Compared to PL, ACT ingestion increased CP (ACT: 297 ± 32 vs PL: 288 ± 31 W, $P<0.001$) and total work done (ACT: 66.4 ± 6.5 vs PL: 65.4 ± 6.4 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-wave amplitude ($P=0.66$) during the 3-min all-out cycling test. Normalized EMG_{RMS} amplitude declined throughout the 3-min protocol in both PL and ACT conditions; however, the decline in EMG_{RMS} was attenuated in the ACT condition, with the EMG_{RMS} amplitude being greater compared to PL over the last 60 s of the test ($P=0.04$). **Conclusion**: These findings indicate that acute ACT ingestion might increase performance and CP during maximal cycling exercise by enhancing muscle activation.

Key words: Analgesic; critical power; electromyography; muscle activation; neuromuscular 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.

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

MATERIALS AND METHODS

Participants

Sixteen trained male cyclists (mean ± SD: age 29 ± 9 y, height 1.79 ± 0.07 m, body mass 77 ± 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 informed consent to participate in the present study, which was approved by the local Ethics Committee (Sport and Health Sciences, University of Exeter). All subjects participated in local cycling competitions. Trained individuals were selected as it has been shown that endurance training influences pain tolerance (O'Leary et al. 2017). After being informed of the experimental procedures and associated risks, all participants completed a medical health questionnaire, which was checked by a medical doctor, to ensure it was safe to consume ACT prior to performing exhaustive exercise. The questionnaire incorporated questions pertaining to: known allergies to medications, current intake of medication and prior use of ACT as well as any history of illnesses, cigarette use, alcohol consumption, illegal drug use and chronic illnesses (personal and family history). None of the participants had a history of motor and/or neurological disorders or frequent chronic ingestion of pain relief medication (i.e. ACT, non-steroidal anti-inflammatory medication etc.). Participants were also advised to avoid ingestion of pain relief medication over the duration of the study and were provided with a list of prohibited medication(s). Participants were instructed to arrive at the laboratory in a

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

Experimental Design

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

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 $\dot{V}O_{2peak}$ (linear factor =
144 50%Δ power output/preferred cadence^2).

145

146 *Measurements*

147 *Breath-by-breath pulmonary gas exchange*

Throughout all laboratory tests, participants wore a mask connected to an impeller turbine transducer assembly (Cortex Metalyzer, Cortex, Leipzig, Germany). Inspired and expired gas volume and concentration signals were continuously sampled at 100 Hz. The analyser was calibrated before each test with gases of known concentration (O_2 15%, CO_2 4.5%), and a calibration syringe of known volume (3-L; Hans Rudolph, KS).

Electromyography

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

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

Peripheral Nerve Stimulation

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

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

Data Analysis

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

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

Statistics

Paired-samples *t*-tests were used to compare the CP, W′, total work done and cardiorespiratory responses between ACT and PL conditions. In addition, paired samples *t*-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 × 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 ± 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 ($P=0.77$).

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, η^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, η^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, η^2=0.56) and an interaction effect (P<0.0001, η^2=0.86, table 2). Post-hoc analysis revealed that the performance times were lower in the ACT condition compared

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

Neuromuscular Function

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

DISCUSSION

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

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

Power-duration relationship

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

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

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

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

Neuromuscular function

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

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

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

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

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

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

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

Conflict of interest

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

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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.

References

Anderson, B.J. 2008. Paracetamol (Acetaminophen): mechanisms of action. *Paediatr Anaesth.* **18**: 915-921.

Astokorki, A.H.Y., and Mauger, A.R. 2017a. Tolerance of exercise-induced pain at a fixed rating of perceived exertion predicts time trial cycling performance. *Scand. J. Med. Sci. Sports.* **27**(3): 309–317.

Astokorki, A.H.Y., and Mauger, A.R. 2017b. Transcutaneous electrical nerve stimulation reduces exercise-induced perceived pain and improves endurance exercise performance. *Eur. J. Appl. Physiol.* **117**(3): 483–492.

Black, M.I., Durant, J., Jones, A.M., and Vanhatalo, A. 2014. Critical power derived from a 3 min all-out test predicts 16.1-km road time-trial performance. *Eur. J. Sport Sci.* **14**: 217-223.

Black, M.I., Jones, A.M., Blackwell, J.R., Bailey, S.J., Wylie, L.J., McDonagh, S.T.J., Thompson, C., Kelly, J., Sumners, P., Mileva, K.N., et al. 2017. Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *J. Appl. Physiol.* **122**(3): 446–459.

Blain, G.M., Mangum, T.S., Sidhu, S.K., Weavil, J.C., Hureau, T.J., Jessop, J.E., Bledsoe, A.D., Richardson, R.S., and Amann, M. 2016. Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *J. Physiol.* **594**(18): 5303-5315.

459 Burke, D. 2002. Effects of activity on axonal excitability: implications for motor control
460 studies. *Adv. Exp. Med. Biol.* **508**: 33-37.
461
462 Burnley, M., Vanhatalo, A., and Jones, A.M. 2012. Distinct profiles of neuromuscular fatigue
463 during muscle contractions below and above the critical torque in humans. *J. Appl. Physiol.*
464 **113**: 215-223.
465
466 Chidnok, W., Fulford, J., Bailey, S.J., DiMenna, F.J., Skiba, P.F., Vanhatalo, A., and Jones,
467 A.M. 2013. Muscle metabolic determinants of exercise tolerance following exhaustion:
468 relationship to the "critical power." *J. Appl. Physiol.* **115**(2): 243–250.
469
470 Cleak, M.J., and Eston, R.G. 1992. Muscle soreness, swelling, stiffness and strength loss after
471 intense eccentric exercise. *Br. J. Sports Med.* **26**: 267–272.
472
473 Da Silva, E., Pinto, R.S., Cadore, E.L., and Kruel, L.F. 2015. Nonsteroidal anti-inflammatory
474 drug use and endurance during running in male long-distance runners. *J. Athl. Train.* **50**:
475 295–302.
476
477 Enoka, R.M., and Duchateau, J. 2008. Muscle fatigue: what, why and how it influences
478 muscle function. *J. Physiol.* **586**: 11-23.
479
480 Felippe, L.C., Ferreira, G.A., Learsi, S.K., Boari, D., Bertuzzi, R., and Lima-Silva, A.E.
481 2018. Caffeine increases both total work performed above critical power and peripheral
482 fatigue during a 4-km cycling time trial. *J. Appl. Physiol.* **24**(6): 1491-1501.
483

484 Florence, S., and Weir, J.P. 1997. Relationship of critical velocity to marathon running
485 performance. *Eur. J. Appl. Physiol. Occ. Physiol.* **75**(3): 274–8.
486
487 Foster, J., Taylor, L., Chrismas, B.C., Watkins, S.L., and Mauger, A.R. 2014. The influence
488 of acetaminophen on repeated sprint cycling performance. *Eur. J. Appl. Physiol.* **114**: 41-48.
489
490 Goodall, S., Ross, E.Z., and Romer, L.M. 2010. Effect of graded hypoxia on supraspinal
491 contributions to fatigue with unilateral knee-extensor contractions. *J. Appl. Physiol.* **109**(6):
492 1842-51.
493
494 Graham, G.G., Davies, M.J., Day, R.O., Mohamudally, A., and Scott, K.F. 2013. The modern
495 pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity
496 and recent pharmacological findings. *Inflammopharmacology,* **21**: 201-232.
497
498 Hureau, T.J., Romer, L.M., and Amann, M. 2016. The 'sensory tolerance limit': A
499 hypothetical construct determining exercise performance? *Eur. J. Sport Sci.* **7**: 1-12.
500
501 Janal, M.N., Glusman, M., Kuhl, J.P., and Clark, W.C. 1994. Are runners stoical? An
502 examination of pain sensitivity in habitual runners and normally active controls. *Pain*, **58**(1):
503 109–116.
504
505 Jones, A.M., Vanhatalo, A., Burnley, M., Morton, R.H., and Poole, D.C. 2010. Critical
506 power: Implications for determination of VO2max and exercise tolerance. *Med. Sci. Sports*
507 *Exerc.* **42**(10): 1876–1890.
508

509 Jones, A.M., Wilkerson, D.P., DiMenna, F., Fulford, J., and Poole, D.C. 2008. Muscle
510 metabolic responses to exercise above and below the "critical power" assessed using ^{31}P-
511 MRS. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**: 585-593.
512
513 Kalmar, J.M., and Cafarelli, E. 2004. “Central Fatigue and Transcranial Magnetic
514 Stimulation: Effect of Caffeine and the Confound of Peripheral Transmission Failure.” *J.*
515 *Neuros Methods*, **138**(1–2): 15–26.
516
517 Kelly, J., Vanhatalo, A., Wilkerson, D.P., Wylie, L.J., and Jones, A.M. 2013. Effects of
518 nitrate on the power-duration relationship for severe-intensity exercise. *Med. Sci. Sports*
519 *Exerc.* **45**(9): 1798–1806.
520
521 Mauger, A.R., and Hopker, J.G. 2013. The effect of acetaminophen ingestion on cortico-
522 spinal excitability. *Can. J. Physiol. Pharmacol*. **91:** 187–189.
523
524 Mauger, A.R., Jones, A.M., and Williams, C.A. 2010. Influence of acetaminophen on
525 performance during time trial cycling. *J. Appl. Physiol.* **108**: 98-104.
526
527 Mauger, A.R., Taylor, L., Harding, C., Wright, B., Foster, J., and Castle, P.C. 2014. Acute
528 acetaminophen (paracetamol) ingestion improves time to exhaustion during exercise in the
529 heat. *Exp. Physiol.* **99**: 164–171.
530
531 Morgan, P.T., Bowtell, J.L., Vanhatalo, A., Jones, A.M., and Bailey, S.J. 2018. Acute
532 acetaminophen ingestion improves performance and muscle activation during maximal
533 intermittent knee extensor exercise. *Eur. J. Appl. Physiol.* **118**(3): 595–605.

534

535 Neyroud, D., Vallotton, A., Millet, G.Y., Kayser, B., and Place, N. 2014. The effect of
536 muscle fatigue on stimulus intensity requirements for central and peripheral fatigue
537 quantification. *Eur. J. Appl. Physiol.* **114**(1): 205-15.

538

539 Nosaka, K., and Clarkson, P.M. 1996. Changes in indicators of inflammation after eccentric
540 exercise of the elbow flexors. *Med. Sci. Sports Exerc.* **28**: 953–961.

541

542 O'Connor, P.J., Motl, R.W., Broglio, S.P., and Ely, M.R. 2004. "Dose-Dependent Effect of
543 Caffeine on Reducing Leg Muscle Pain during Cycling Exercise Is Unrelated to Systolic
544 Blood Pressure." *Pain*, **109**(3): 291–98.

545

546 O'Leary, T.J., Collett, J., Howells, K., and Morris, M.G. 2017. High but not moderate-
547 intensity endurance training increases pain tolerance: a randomised trial. *Eur. J. Appl.*
548 *Physiol.* **117**(11): 2201–2210.

549

550 Paton, C.D., and Hopkins, W.G. 2006. Variation in performance of elite cyclists from race to
551 race. *Eur. J. Sport Sci.* **6**(1): 25–31.

552

553 Pickering, G., Estève, V., Loriot, M.A., Eschalier, A., and Dubray, C. 2008. Acetaminophen
554 reinforces descending inhibitory pain pathways. *Clin. Pharmacol. Ther.* **84**: 47-51.

555

556 Pickering, G., Loriot, M.A., Libert, F., Eschalier, A., Beaune, P., and Dubray, C. 2006.
557 Analgesic effect of acetaminophen in humans: first evidence of a central serotonergic
558 mechanism. *Clin. Pharmacol. Ther.* **79**: 371-378.

559

560 Poole, D.C., Burnley, M., Vanhatalo, A., Rossiter, H.B., and Jones, A.M. 2016. Critical
561 power: An important fatigue threshold in exercise physiology. *Med. Sci. Sports Exerc.*
562 **48**(11): 2320–2334.

563

564 Poole, D.C., Ward, S.A., Gardner, G.W., and Whipp, B.J. 1988. Metabolic and respiratory
565 profile of the upper limit for prolonged exercise in man. *Erg.* **31**: 1265-79.

566

567 Rossman, M.J., Garten, R.S., Venturelli, M., Amann, M., and Richardson, R.S. 2014. The
568 role of active muscle mass in determining the magnitude of peripheral fatigue during
569 dynamic exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **306**(12): 934–940.

570

571 Rossman, M.J., Venturelli, M., Mcdaniel, J., Amann, M., and Richardson, R.S. 2012. Muscle
572 mass and peripheral fatigue: A potential role for afferent feedback? *Acta Physiologica*,
573 **206**(4): 242–250.

574

575 Sidhu, S.K., Cresswell, A.G., and Carroll, T.J. 2012. Motor cortex excitability does not
576 increase during sustained cycling exercise to volitional exhaustion. *J. Appl. Physiol.* **113**(3):
577 401–409.

578

579 Skiba, P.F., Chidnok, W., Vanhatalo, A., and Jones, A. 2012. Modeling the expenditure and
580 reconstitution of work capacity above critical power. *Med. Sci. Sports Exerc.* **44**(8): 1526–
581 1532.

582

583 Smith, H.S. 2009. Potential analgesic mechanisms of acetaminophen. *Pain Physician,* **12**:
584 269-80.
585
586 Smith, J.C., Dangelmaier, B.S., and Hill, D.W. 1999. Critical power is related to cycling time
587 trial performance. *Int. J. Sports Med.* **20**: 374-378.
588
589 Tesarz, J., Gerhardt, A., Schommer, K., Treede, R.D., and Eich, W. 2013. Alterations in
590 endogenous pain modulation in endurance athletes: An experimental study using quantitative
591 sensory testing and the cold-pressor task. *Pain,* **154**(7): 1022–1029.
592
593 Toussaint, K., Yang, X.C., Zielinski, M.A., Reigle, K.L., Sacavage, S.D., Nagar, S., and
594 Raffa, R.B. 2010. What do we (not) know about how paracetamol (acetaminophen) works? *J*
595 *Clin Pharm. Ther*. **35**: 617-638.
596
597 Vanhatalo, A., Black, M.I., DiMenna, F.J., Blackwell, J.R., Schmidt, J.F., Thompson, C.,
598 Wylie, L.J., Mohr, M., Bangsbo, J., Krustrup, P., et al. 2016. The mechanistic bases of the
599 power–time relationship: muscle metabolic responses and relationships to muscle fibre type.
600 *J. Physiol*. **594**(15): 4407–4423.
601
602 Vanhatalo, A., Doust, J.H., and Burnley, M. 2008. A 3 min all-out cycling test is sensitive to
603 a change in critical power. *Med. Sci. Sports Exerc.* **40**(9): 1693–1699.
604
605 Vanhatalo, A., Doust, J.H., and Burnley, M. 2007. Determination of critical power using a 3-
606 min all-out cycling test. *Med. Sci. Sports Exerc.* **39**(3): 548–555.
607

608 Vanhatalo, A., Jones, A.M., and Burnley, M. 2011. Application of critical power in sport. *Int.*
609 *J. Sports Physiol. Perform.* **6**(1): 128–136.
610
611 Walton, C., Kalmar, J., and Cafarelli, E. 2003. Caffeine increases spinal excitability in
612 humans. *Muscle Nerve*, **28:** 359–36

613 **Figure captions**

614 *Figure 1*

615 Group mean ± 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 ± 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); [a]significantly different from
625 30 s; [b]significantly different from 60 s; [c]significantly different from 90 s; [d]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

Figure 3

M-wave amplitude responses in the *m*.vastus lateralis during the 3-min cycling test for placebo (clear circles) and acetaminophen (filled circles) trials. Mean ± SE M-wave responses are presented in panel A with the M-wave response from a representative individual presented in panel B, for PL (grey line) and ACT (black line), for the first 30 and final 30 s, respectively of the 3-min protocol. [a]significantly different from baseline; [b]significantly different from 30 s ($P<0.05$).

Figure 4

Surface electromyography (EMG) responses (expressed relative to M-wave amplitude) in the *m*.vastus lateralis during the 3-min cycling test for placebo (clear circles) and acetaminophen (filled circles) trials. Mean ± SE EMG responses are presented in panel A with the EMG response from a representative individual presented in panel B, for PL (grey line) and ACT (black line), for the first 30 and final 30 s, respectively of the 3-min protocol. *Significantly different from placebo; [a]significantly different from 30 s; [b]significantly different from 60 s; [c]significantly different from 90 s; [d]significantly different from 120 s; [e]significantly different from 150 s ($P<0.05$).

Figure 5

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