

Effect of Acetaminophen on Endurance Cycling Performance in Trained Triathletes in Hot and Humid Conditions

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1 **TITLE PAGE**

2 **Effect of acetaminophen on endurance cycling performance in trained triathletes in hot**
3 **and humid conditions**

4 Original Investigation

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21

22 **ABSTRACT**

23 **Purpose:** The ergogenic effect of various pre- and per-cooling strategies during endurance
24 exercise in hot and humid environmental conditions has been extensively investigated but the
25 effect of acetaminophen (ACT, also known as paracetamol) on endurance performance in
26 trained individuals in these conditions is unknown. The aim of this study was to determine the
27 effect of ACT on physiological and perceptual variables during steady state and time trial
28 cycling performance of trained triathletes in hot and humid conditions.

29 **Methods:** In a randomized, double-blind crossover design, eleven triathletes completed ~60
30 min steady state cycling at 63% peak power output followed by a time trial ($7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{body}$
31 mass^{-1}) in hot and humid conditions ($\sim 30^\circ\text{C}$, $\sim 69\%$ relative humidity) 60 min after consuming
32 either $20 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{body mass}^{-1}$ ACT or a color matched placebo (PLA). Time trial completion
33 time, gastrointestinal temperature, skin temperature, thermal sensation, thermal comfort, rating
34 of perceived exertion and fluid balance were recorded throughout each session.

35 **Results:** Time trial completion time was greater in the ACT condition, however this difference
36 was not statistically significant despite a moderate effect for poorer performance compared to
37 PLA (64.6 s, CI = -11.08 to 140.3 s, $d=0.57$, $p=0.086$). There were no differences in
38 gastrointestinal and skin temperature, thermal sensation and comfort, or fluid balance between
39 trials.

40 **Conclusion:** In conclusion, the antipyretic and analgesic effects previously associated with
41 ACT ingestion were not apparent in trained triathletes and existing pre- and per-cooling
42 strategies appear to be more appropriate for endurance cycling performance in the heat.

43 **KEYWORDS**

44 cooling, heat, thermoregulation, ergogenic aid, Olympic distance triathlon

45 INTRODUCTION

46 It is well known that hot and humid environmental conditions reduce endurance exercise
47 performance in trained individuals^{1,2}. The impaired performance is typically related to a
48 combination of factors including increased cardiovascular strain, decreased neural drive, as
49 well as dehydration during prolonged exercise bouts³. Accordingly, numerous studies have
50 investigated the efficacy of various cooling strategies to mitigate the detrimental effects of hot
51 environmental conditions on endurance exercise performance. The methods of cooling can be
52 categorized as interventions before (pre-cooling) or during (per-cooling) exercise and have
53 been shown to improve prolonged aerobic exercise in the heat^{4,5}.

54 Effective pre- and per-cooling techniques generally aim to reduce core and/or skin temperature,
55 decreasing the net heat storage in the body and increasing the magnitude of heat production
56 required to upregulate autonomic thermoregulatory mechanisms⁶. The most effective methods
57 include cold water immersion, wearing iced garments and ice-slurry ingestion which have been
58 shown to reduce pre-exercise core and skin temperatures and increase exercise performance in
59 the heat⁷. In spite of this, implementing pre-cooling methods can present logistical challenges
60 in the field and effects can be transient especially during endurance sports^{5,8,9}. Therefore
61 pharmaceutical compounds may represent alternate, less onerous, long-lasting ergogenic aids
62 for endurance athletes participating in major events in hot conditions. Acetaminophen (ACT),
63 also known as paracetamol, is an ergogenic aid that has been associated with increased cycling
64 performance in trained and recreationally active humans but its potential to improve endurance
65 performance in hot conditions is unknown^{10,11}.

66 Acetaminophen is a widely used medication with antipyretic and analgesic properties and is
67 commonly used to reduce fever and pain during illness¹². An acetaminophen dose of 20 mg·kg⁻¹
68 ·body mass⁻¹ has been shown to maximise plasma acetaminophen concentration ~2 h post
69 ingestion¹³, reduce core temperature when resting¹³ and exercising^{10,11} and increase moderate

70 intensity cycling performance by 17% in hot conditions (~30°C, ~50% RH) for recreationally
71 active individuals compared to placebo¹¹. Conversely, studies have also shown only modest
72 physiological or ergogenic effects of acetaminophen during 30-60 min steady state endurance
73 exercise bouts in similar populations^{14,15}. Therefore, while there may be an apparent ergogenic
74 effect of acetaminophen, it is unclear whether its ingestion improves performance of trained
75 endurance athletes undertaking a cycling bout in hot and humid conditions. The aim of the
76 present study was to determine the effect of acetaminophen ingestion on cycling time trial
77 performance in endurance trained athletes in hot and humid environmental conditions. We
78 hypothesized that core (gastrointestinal) temperature, skin temperature and thermal sensation
79 would be reduced by acetaminophen and enhance endurance cycling performance in a
80 thermally challenging environment.

81

82 **METHODS**

83 **Participants**

84 Thirteen endurance trained (level 3) triathletes¹⁶ volunteered to take part in the study (sex: 9
85 male/4 female, age: 29.2 ± 8.4 years, stature: 175.3 ± 7.6 cm, body mass 67.2 ± 9.0 kg, peak
86 oxygen uptake ($\dot{V}O_{2peak}$): 64.5 ± 8.5 mL·kg⁻¹·min⁻¹, peak power output: 338.3 ± 61.6 Watts,
87 body surface area: 1.8 ± 0.2 m²). Participants were excluded if they had a recent injury and
88 illness or were in recovery and if they were on medication which affected thermoregulatory
89 autonomic responses or analgesia. A within-participant randomized double-blind crossover
90 study design was employed with trials undertaken after either oral ingestion of acetaminophen
91 or a placebo. Due to injury and illness, n = 2 did not complete experimental trials. Written
92 informed consent was obtained from all participants prior to commencement of the study and

93 ethical approval was granted by the Bond University Human Research Ethics Committee
94 (GW02854).

95 **Preliminary Testing**

96 Participants were instructed to refrain from ingesting analgesic agents (e.g. non-steroidal anti-
97 inflammatory drugs) for 48 h before each laboratory visit and abstained from caffeine and
98 alcohol consumption for the 24 h prior to each experimental trial. Prior to commencing
99 experimental trials participants attended a preliminary testing session undertaken in temperate
100 conditions (23.4 ± 1.2 °C, $63.1 \pm 7.6\%$ relative humidity). Participants arrived at the laboratory
101 a minimum two hours prior to the initial trial commencing after an overnight fast and voided
102 their bladder. Body mass was determined using electronic scales (WM204, Wedderburn,
103 Australia) and height using a wall-mounted stadiometer (Harpenden stadiometer, Holtain
104 Limited, UK). Dual energy X-ray absorptiometry (DXA; Lunar Prodigy DXA machine, GE
105 Healthcare, USA) was performed by a licensed practitioner using methods described
106 previously¹⁷ to determine body composition.

107 Participants were provided with a pre-exercise snack before beginning a cycling familiarization
108 session. Subsequently, they completed a 10 min warm-up at self-selected intensity followed by
109 a maximal incremental test to volitional fatigue as described previously¹⁸. Breath-by-breath
110 respiratory gas data was averaged as 30 s epochs throughout the test via metabolic cart (Quark
111 C-PET; Cosmed, Italy). Finally, after a ~5 min self-selected cooldown and ~5 min passive rest,
112 participants completed a familiarization session which included a 10 min steady state ride at
113 63% peak power output (~70% $\dot{V}O_{2\text{peak}}$) followed by a short passive recovery (2 min), and the
114 same $7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ performance time trial used in the experimental trial (described
115 subsequently). Participants were familiarized with perceptual scales^{19,20} during the 10 min
116 steady state cycling bout. All participants were endurance trained athletes experienced in

117 undertaking high intensity self-paced cycling in competition, and the preliminary trial ensured
118 that participants were familiar with the laboratory procedures and requirements of the
119 performance test.

120 **Experimental Trial**

121 *Dietary Standardization*

122 Upon arrival for experimental trials (Figure 1), participants provided a mid-stream urine sample
123 collected upon waking which was analyzed using a refractometer (PAL-10S, Atago, Japan) to
124 determine hydration status via urine specific gravity (USG). Any participant who arrived
125 dehydrated (USG ≥ 1.020) were required to consume 600mL of sports drink (GatoradeTM – 36g
126 CHO). A standardized diet (220 kJ·kg⁻¹·BM⁻¹; 8g CHO·kg⁻¹·BM⁻¹) was provided for the 24 h
127 prior to trials. Participants arrived fasted and were provided with a pre-exercise snack (40 kJ·kg⁻¹·
128 BM⁻¹; 2g CHO·kg⁻¹·BM⁻¹) 90 min prior to commencing exercise. During steady state cycling
129 5mL water·kg⁻¹·BM⁻¹ was provided every 20 min to be consumed within 5 min of presentation.
130 During the period between steady state cycling and the time trial, 3mL water·kg⁻¹·BM⁻¹ and a
131 carbohydrate energy gel (Koda energy gelTM – 495kJ; 30g CHO) was ingested. Participants
132 were permitted to drink water ad-libitum during the initial time trial with the volume of water
133 matched during the subsequent trial.

134 *Environmental Conditions*

135 Each trial was undertaken in a room/structure where environmental conditions were maintained
136 at ~30 °C, ~70% RH (Acetaminophen trial: 29.9±0.7 °C, 68.7±2.7%; Placebo trial: 29.7±0.7
137 °C, 68.7±2.8%) and recorded by dual Kestrel 5400 monitors (Kestrel Instruments, USA).

138 *Steady State and Time Trial Cycling*

139 All experimental trials were undertaken in the morning (0530-1030) at the same time for each
140 participant and were separated by 4-7 days. Prior to the exercise bout (60 min) participants

141 ingested gelatin capsules containing either $20\text{mg}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ acetaminophen (ACT) or a color-
142 matched placebo (cornflour; PLA). Participants completed both steady state and time trial
143 cycling on an electromagnetically braked ergometer (Lode Instruments; Groningen, The
144 Netherlands) which was set up to replicate each participant's individual bicycle settings and
145 facing wind speed $\sim 1.5\text{m}\cdot\text{s}^{-1}$ was facilitated using a fan. Initially participants entered the tent
146 and rested passively in a seated position for 10 minutes before mounting the ergometer and
147 beginning the cycling bout. Subjects had 5 min to progress mechanical work to the required
148 63% peak power output which they then maintained for 55 min. The steady state cycling was
149 followed by a short passive recovery period (2 min). Participants then commenced the $7\text{kJ}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$
150 time trial using a mode on the ergometer where power output is dependent on each
151 participant's cadence and changes in a linear manner. This relationship was individualised
152 using a linear factor that permits each participant to cycle at $\sim 85\%$ PPO at their preferred
153 cadence similar to previous approaches²¹. Time to completion was recorded using a handheld
154 stopwatch. Participants received no feedback or verbal encouragement during the time trial
155 except notification of each 10% of work completed.

156 *Gastrointestinal and Skin Temperature*

157 Gastrointestinal temperature (T_{gi}) was measured as an indicator of core temperature.
158 Participants ingested a thermistor pill $\sim 9\text{-}10$ h prior to arrival for each experimental trial (e-
159 Celsius Performance; Bodycap, France). T_{gi} was recorded at 30s intervals which were
160 subsequently converted to 60s epochs for analysis. Skin temperature (T_{sk}) was determined
161 using a thermochron attached to the skin (iButton; Maxim Integrated, USA) with medical tape
162 (Fixomull, BSN Medical, Germany). Thermochrons were placed on the back (infraspinale),
163 mid-bicep, mid-thigh and medial mid-calf. Mean skin temperature was calculated using the
164 unweighted mean of sites on the back, bicep, thigh, and calf²².

165 *Heart Rate*

166 Heart rate (HR) was recorded at a frequency of 1Hz by telemetry (Polar H10; Kempele,
167 Finland) and was recorded using a proprietary app (Polar Beat; Polar Electro, Kempele,
168 Finland). The data was saved to a cloud-based software (Polar Flow; Polar Electro, Kempele,
169 Finland) before being exported for analysis.

170 *Perceptual Measures*

171 Perceived thermal comfort on a 4-point Likert scale (1=comfortable, 4=uncomfortable)¹⁹,
172 perceived thermal sensation on a 17-point Likert scale (0.0=unbearably cold, 4.0=neutral,
173 8.0=unbearably hot)²⁰ and rating of perceived exertion (RPE)²³ were assessed verbally after
174 reference to the visual scale. Data was obtained immediately before the steady state cycling,
175 every 10 min during steady state cycling and immediately following time trial completion.

176 *Fluid Balance*

177 Participant's towel-dried and were weighed nude to determine body mass immediately before
178 and immediately after exposure to the hot environment (WM204; Wedderburn, Australia). If
179 participants voided their bladder during the 2 min between steady state riding and the time trial,
180 they were weighed before and after to record excreted fluid loss. Participant's drink bottles
181 were weighed using portable scales (KD-192, Tanita, Japan) before and after the trial to

182 determine total fluid consumption. Whole body sweat loss (WBSL) was calculated using the
183 following formula:

184 Equation 1

$$185 \quad \text{WBSL(L)} = (\text{Body mass PRE(kg)} - \text{Body Mass POST(kg)}) + \\ 186 \quad \text{Total Fluid Consumption(L)} - \text{Urine Loss(L)}$$

187 **Statistical Analyses**

188 Physiological and perceptual measures were analyzed between conditions using a repeated-
189 measures (condition \times time) analysis of variance (ANOVA). Sphericity was tested using
190 Mauchly's test and when sphericity was violated a Greenhouse-Geisser correction was used. If
191 significant interactions were observed, post-hoc pairwise comparisons were conducted using a
192 Bonferroni correction. Time to completion and mean power output of the time trial, fluid
193 balance and USG was analyzed using a paired t-test. Effect sizes were calculated using Cohen's
194 d with thresholds for small (0.2), moderate (0.5) and large (0.8) interpreted according to Cohen
195 (2013). Statistical analyses were conducted using GraphPad Prism (version 8.4.2, GraphPad
196 Software Inc, USA) and effect sizes were calculated using Microsoft Excel (Microsoft
197 Cooperation, Redmond, USA). Data are mean \pm standard deviation, 95% confidence intervals
198 are reported and statistical significance was set at $P < 0.05$.

199 **RESULTS**

200 **Performance**

201 There was a moderate effect for slower time to completion (ACT: 1987 \pm 326s, CON:
202 1923 \pm 257s, CI = -11.1 to 140.3s, $d=0.57$, $p=0.086$, Figure 2) and lower mean power output
203 (ACT: 246 \pm 44W, CON: 252 \pm 46W, CI = -14.2 to 1.2W, $d=0.57$, $p=0.089$) of the cycling time
204 trial in the ACT compared to the PLA condition, but these mean differences did not reach
205 statistical significance. No order effect between trials was evident ($p=0.382$).

206 **Gastrointestinal Temperature, Skin Temperature and Heart Rate**

207 ACT had no effect on T_{gi} during both steady state cycling and the cycling time trial compared
208 with PLA. There was a main effect of time ($p<0.001$; Figure 3A) on gastrointestinal
209 temperature with a large increase observed during both steady state cycling (ACT: $\Delta 1.5\pm 0.4^{\circ}\text{C}$,
210 $p<0.001$; PLA: $\Delta 1.6\pm 0.4^{\circ}\text{C}$, $p<0.001$) and the time trial (ACT: $\Delta 0.7\pm 0.3^{\circ}\text{C}$, $p=0.002$; PLA:
211 $\Delta 0.7\pm 0.3^{\circ}\text{C}$, $p=0.005$). Similar gastrointestinal temperatures at the completion of steady state
212 cycling (ACT: $38.2\pm 0.5^{\circ}\text{C}$, PLA: $38.3\pm 0.4^{\circ}\text{C}$) and following the time trial (ACT: $38.9\pm 0.5^{\circ}\text{C}$,
213 PLA: $39.0\pm 0.4^{\circ}\text{C}$) were also observed between conditions.

214 Mean T_{sk} was not different in the ACT trial compared to the PLA trial at any timepoint ($p =$
215 0.595 ; Figure 3B). Mean T_{sk} fluctuated throughout the trials, increasing during the early period
216 (20 min) of steady state cycling (ACT: 1.0°C , CI = -0.3 to 2.3°C , $d=1.0$, $p = 0.216$; PLA: 0.7°C ,
217 CI = -0.3 to 1.6°C , $d=2.1$, $p = 0.523$) before decreasing until the completion of the steady state
218 bout (ACT: $33.6\pm 0.8^{\circ}\text{C}$; PLA: $33.7\pm 0.6^{\circ}\text{C}$). There was a moderate effect for the decrease in
219 T_{sk} during the ACT trial (-0.3°C , CI = -0.7 to 0.1°C , $d=-0.58$) whilst in the PLA trial it was a
220 small effect (-0.2°C , CI = -0.7 to 0.2°C , $d=-0.29$).

221 There was a main effect of time (main effect: $p<0.001$) for HR which increased and was highly
222 comparable between trials during steady state cycling (ACT: 157 ± 12 bpm, PLA: 157 ± 13 bpm)
223 and the time trial (ACT: 175 ± 12 bpm, PLA: 177 ± 12 bpm) with no difference between trials
224 (Figure 3C).

225 **Rating of Perceived Exertion, Thermal Sensation and Thermal Comfort**

226 There was no difference in perceived exertion between trials ($p=0.666$) despite small effects
227 for increased RPE during steady state cycling in the acetaminophen trial from 20 until 60 min
228 (0.5 - 0.8 AU, CI = -1.8 to 3.4 AU, $d=0.23$ - 0.37 , Figure 4A). There was a main effect of time
229 for RPE (main effect: $p<0.001$) increasing above pre-exercise levels throughout steady state

230 cycling (ACT:15.4±2.1AU, PLA:14.6±1.4AU) with a further increase during the time trial
231 (ACT:18.2±2.1AU, PLA:18.3±1.9AU). Thermal sensation was also not different between
232 trials ($p = 0.681$) but there was a small effect for reduced thermal sensation at commencement
233 of the ACT trial compared with PLA (-0.4AU, CI = -2.2 to 1.4AU, $d=-0.35$) and an increase
234 after 40 min of steady state cycling (0.3AU, CI = -0.8 to 1.3AU, $d=0.34$; Figure 4B). There
235 was a main effect of time for thermal sensation and comfort (main effect: $p<0.001$) with similar
236 maximum thermal comfort values that were not different between the ACT (3.5±0.5AU) and
237 PLA trials (3.7±0.5AU; Figure 4C).

238 **Fluid Balance**

239 Participants were euhydrated upon arrival for all except two experimental trials (ACT:
240 USG=1.015±0.003; PLA: USG=1.016±0.004, $p=0.636$). There was a similar reduction
241 ($p=0.990$) in body mass in both conditions (ACT:-1.8%, CI = 1.3 to 2.4%, $d =-2.2$, $p < 0.001$;
242 PLA:1.8%, CI = 1.1 to 2.5%, $d=-1.8$, $p<0.001$). Fluid ingestion (ACT:1.69±0.36L, PLA:
243 1.73±0.42 L, $p=0.505$) and WBSR (ACT:1.9±0.5L·h⁻¹, PLA:1.9±0.6L·h⁻¹, $p=0.930$) were not
244 different between the acetaminophen and placebo trials.

245

246 **DISCUSSION**

247 This study determined the effect of acetaminophen ingestion on physiological responses and
248 cycling time trial performance of endurance trained athletes in hot and humid conditions. The
249 major findings of the present study were that acetaminophen ingestion had no meaningful
250 impact on gastrointestinal or skin temperature, psychometric ratings of RPE, thermal sensation
251 and thermal comfort. Moreover, there was a moderate effect for poorer endurance performance
252 in the heat with acetaminophen ingestion. Accordingly, we suggest that ingestion of
253 acetaminophen in close temporal proximity to exercise may be ineffectual and does not appear
254 to have any practical ergogenic utility to improve endurance cycling performance in hot and
255 humid conditions in trained participants.

256 There is a paucity of data available on the effect of acetaminophen on exercise capacity and
257 performance. Intuitively, the antipyretic and/or analgesic effects of acetaminophen might be
258 expected to ameliorate the detrimental physiological and/or perceptual responses associated
259 with exercise in the heat²⁴. A purported mechanism of action of acetaminophen is the inhibition
260 of cyclooxygenase (COX) enzymes and subsequent reduction in prostaglandin release, fever
261 and pain signals associated with the inflammatory response^{25,26}. Inhibition of COX-2 has been
262 shown to reduce core temperature and skin temperature during steady-state cycling²⁷ however
263 evidence for the effect of acetaminophen on core and skin temperatures in hot and
264 normothermic conditions show no difference^{14,15}. We provide new data that supports and
265 extends the contention that acetaminophen has little antipyretic effects during exercise by
266 showing acetaminophen has no influence on gastrointestinal or skin temperature in afebrile
267 endurance trained individuals during steady-state and high intensity endurance exercise in hot
268 conditions.

269 The analgesic effect of acetaminophen acts to attenuate perceptions of pain^{10,11} which could
270 suppress perceptions of central fatigue and/or discomfort during high intensity exercise for

271 improved performance. Few studies have determined the effects of acetaminophen on exercise
272 performance and previous performance tests have had minimal practical application to
273 endurance events. Studies that employed a similar pre-exercise acetaminophen ingestion
274 protocol as the present study reported a ~17% improvement in time to exhaustion (~25 min) in
275 recreationally active individuals in hot conditions (~30°C and ~50% RH)¹¹ and 2%
276 improvement for trained individuals in a ~16 km time trial in thermoneutral conditions¹⁰. An
277 important distinction of the present study is the duration of heat exposure (~25 vs. ~120 min)
278 and exercise demands, with the self-paced cycling time trial in our study undertaken after an
279 initial ~75 min period in the heat mimicking the demands of the final leg of an Olympic-
280 distance triathlon in hot conditions. While differences in experimental design and training
281 status of participants may explain, at least in part, the lack of agreement in performance
282 outcomes between studies, the divergent thermal stress evidenced by higher mean
283 gastrointestinal temperature and maximum skin temperature in the present study is also a
284 significant mitigating factor¹¹. Moreover, we report no difference in perceptual measures of
285 RPE, thermal sensation and thermal comfort throughout the 1 h steady state cycling bout and
286 after completion of the time trial. This result was somewhat surprising when considering the
287 known analgesic effects of acetaminophen. Accordingly, our findings suggest that there is little
288 evidence supporting acetaminophen ingestion as an effective ergogenic aid in hot and humid
289 conditions when trained athletes undertake prolonged, high intensity exercise similar to the
290 demands of competition in cycling or triathlon.

291 The moderate effect for poorer time trial performance in the heat in the acetaminophen trial of
292 the present study is difficult to reconcile. A limitation of our study was the lack of direct
293 measurement of plasma acetaminophen concentration during the experimental trials.
294 Consequently, the dose-dependent circulating acetaminophen levels are unknown. Foster,
295 Mauger, Gorvus, Hewson & Taylor¹³ have shown ingestion of 20 mg·kg⁻¹·BM⁻¹ of

296 acetaminophen after a small, standardized meal induced peak plasma acetaminophen
297 concentration ($14\mu\text{g}\cdot\text{mL}^{-1}$) at rest between 100 and 120 min after ingestion which led to a
298 subsequent reduction in gastrointestinal temperature of approximately $\sim 0.2\text{ }^{\circ}\text{C}$. Our protocol
299 was designed to ensure plasma acetaminophen was high during exercise by consuming it 30
300 min after a standardized meal ($2\text{ g CHO}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$, 600 mL fluid) and ~ 120 min before the
301 time trial to coincide with peak plasma concentration¹³. It is acknowledged that the 60 min
302 steady state exercise bout may have affected plasma acetaminophen concentration however
303 steady state exercise had little effect on acetaminophen pharmacokinetics in other studies²⁸.

304 It is plausible that time trial performance was reduced with acetaminophen supplementation
305 during the present study as a result of attenuated sensory feedback leading to subsequent
306 reductions in the capacity to ‘self-pace’ during exercise. However, if altered perceptions of the
307 environment because of acetaminophen supplementation did influence subsequent exercise
308 performance, the perceived exertion and thermal sensation/comfort scales lacked the sensitivity
309 to identify a change in response between trials. Clearly, further work is needed to elucidate the
310 interactions between the environment, exercise task and pharmacological responses to
311 determine whether acetaminophen ingestion reduces performance capacity in endurance
312 trained athletes.

313 **PRACTICAL APPLICATION**

314 Our findings provide evidence that purported pharmaceutical ergogenic aids, specifically
315 acetaminophen, for elite performance may not be effective in an ecologically valid endurance
316 performance context. Therefore, we recommend that coaches and athletes continue to use
317 existing pre- and per-cooling strategies including ice baths, iced garments and ice-slurry
318 ingestion to mitigate heat stress and improve endurance exercise performance in the heat.

319 **CONCLUSION**

320 In conclusion, we propose that acetaminophen is not an effective ergogenic aid for endurance
321 exercise bouts in hot and humid conditions. Specifically, the antipyretic and analgesic effects
322 typically associated with acetaminophen did not reduce gastrointestinal temperature, skin
323 temperature, and perceptual measures of exertion or thermal stress, with no apparent beneficial
324 effect on steady state endurance cycling nor cycling time trial performance.

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419 **DECLARATION OF INTEREST STATEMENT**

420 The authors declare no conflicts of interest which could influence the work reported in this
421 paper.

422

423 **FIGURE CAPTIONS**

424 **Figure 1** Overview of an experimental trial (n=11 participants) showing the steady state
425 cycling and time trial, and timeline of dependent variable data collection to determine
426 physiological and perceptual responses. Arrows depict timepoints where data was collected for
427 each variable. The white box denotes the pre-testing phase, the light grey steady state cycling
428 and dark grey the cycling time trial. PPO, peak power out; BM, body mass.

429 **Figure 1** Time to completion of a $7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ time trial in hot and humid conditions
430 undertaken by endurance trained triathletes (n = 11) after consuming a $20 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ dose
431 of acetaminophen (ACT, $29.9\pm 0.7^\circ\text{C}$, $68.7\pm 2.7\%$ RH) or placebo (PLA, $29.7\pm 0.7^\circ\text{C}$,
432 $68.7\pm 2.8\%$ RH). Participants completed the time trial after cycling for ~60 min in hot and
433 humid conditions at 63% of their previously determined peak power output. Data are mean \pm
434 standard deviation and were analyzed using a paired t-test with alpha set at $P < 0.05$.

435 **Figure 3** Gastrointestinal temperature (A), skin temperature (B) and heart rate (C) responses
436 in hot and humid conditions of endurance trained triathletes (A: n = 11, B: n = 9, C: n = 10)
437 during ~60 min steady state cycling (63% peak power output) and a cycling time trial ($7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$)
438 after consuming $20 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ of acetaminophen (ACT, $29.9\pm 0.7^\circ\text{C}$, $68.7\pm 2.7\%$
439 RH) and placebo (PLA, $29.7\pm 0.7^\circ\text{C}$, $68.7\pm 2.8\%$ RH). Data are mean \pm standard deviation and
440 were analyzed using two-way repeated measures ANOVA (condition \times time) with post-hoc
441 Bonferroni procedure for multiple comparisons ($p < 0.05$).

442 **Figure 4** Rating of perceived exertion (A), thermal sensation (B) and thermal comfort (C) of
443 endurance trained triathletes (A: n = 10, B and C: n = 7) in hot and humid conditions during
444 ~60 min steady state cycling (63% peak power output) and $7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ time trial after
445 consuming a $20 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{BM}^{-1}$ dose of acetaminophen (ACT, $29.9\pm 0.7^\circ\text{C}$, $68.7\pm 2.7\%$ RH) or
446 placebo (PLA, $29.7\pm 0.7^\circ\text{C}$, $68.7\pm 2.8\%$ RH). Data are mean \pm standard deviation and were

447 analyzed using two-way repeated measures ANOVA (condition × time) with post-hoc
448 Bonferroni analyses for multiple comparisons with alpha set $P < 0.05$.