TITLE PAGE

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

Original Investigation

Gyan A. Wijekulasuriya[1], Vernon G. Coffey[1], Luke Badham[1], Fergus O'Connor[1], Avish P. Sharma[2,3], Gregory R. Cox[1,2]

[1]Bond Institute of Health and Sport and Faculty of Health Sciences and Medicine, Bond University, Queensland, Australia; [2]Triathlon Australia, Queensland, Australia; [3]Griffith Sports Physiology and Performance, School of Allied Health Sciences, Griffith University, Queensland, Australia

Corresponding Author:

A/Professor Gregory R. Cox

Faculty of Health Sciences and Medicine

Bond University, Gold Coast, QLD 4226 Australia.

Email: gcox@bond.edu.au

Ph: +61-7-5595 3539

Running head: Acetaminophen and performance in heat

Word Count Abstract: 246

Word Count Manuscript: 3 235

Figures: 4

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ABSTRACT

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

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

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

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

KEYWORDS

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

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INTRODUCTION

It is well known that hot and humid environmental conditions reduce endurance exercise performance in trained individuals[1,2]. The impaired performance is typically related to a combination of factors including increased cardiovascular strain, decreased neural drive, as well as dehydration during prolonged exercise bouts[3]. Accordingly, numerous studies have investigated the efficacy of various cooling strategies to mitigate the detrimental effects of hot environmental conditions on endurance exercise performance. The methods of cooling can be categorized as interventions before (pre-cooling) or during (per-cooling) exercise and have been shown to improve prolonged aerobic exercise in the heat[4,5].

Effective pre- and per-cooling techniques generally aim to reduce core and/or skin temperature, decreasing the net heat storage in the body and increasing the magnitude of heat production required to upregulate autonomic thermoregulatory mechanisms[6].The most effective methods include cold water immersion, wearing iced garments and ice-slurry ingestion which have been shown to reduce pre-exercise core and skin temperatures and increase exercise performance in the heat[7]. In spite of this, implementing pre-cooling methods can present logistical challenges in the field and effects can be transient especially during endurance sports[5,8,9]. Therefore pharmaceutical compounds may represent alternate, less onerous, long-lasting ergogenic aids for endurance athletes participating in major events in hot conditions. Acetaminophen (ACT), also known as paracetamol, is an ergogenic aid that has been associated with increased cycling performance in trained and recreationally active humans but its potential to improve endurance performance in hot conditions is unknown[10,11].

Acetaminophen is a widely used medication with antipyretic and analgesic properties and is commonly used to reduce fever and pain during illness[12]. An aceteminophen dose of 20 $mg \cdot kg^{-1} \cdot body\ mass^{-1}$ has been shown to maximise plasma acetaminophen concentration ~2 h post ingestion[13], reduce core temperature when resting[13] and exercising[10,11] and increase moderate

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intensity cycling performance by 17% in hot conditions (~30°C, ~50% RH) for recreationally active individuals compared to placebo[11]. Conversely, studies have also shown only modest physiological or ergogenic effects of acetaminophen during 30-60 min steady state endurance exercise bouts in similar populations[14,15]. Therefore, while there may be an apparent ergogenic effect of acetaminophen, it is unclear whether its ingestion improves performance of trained endurance athletes undertaking a cycling bout in hot and humid conditions. The aim of the present study was to determine the effect of acetaminophen ingestion on cycling time trial performance in endurance trained athletes in hot and humid environmental conditions. We hypothesized that core (gastrointestinal) temperature, skin temperature and thermal sensation would be reduced by acetaminophen and enhance endurance cycling performance in a thermally challenging environment.

METHODS

Participants

Thirteen endurance trained (level 3) triathletes[16] volunteered to take part in the study (sex: 9 male/4 female, age: 29.2 ± 8.4 years, stature: 175.3 ± 7.6 cm, body mass 67.2 ± 9.0 kg, peak oxygen uptake ($\dot{V}O_{2peak}$): 64.5 ± 8.5 $mL \cdot kg^{-1} \cdot min^{-1}$, peak power output: 338.3 ± 61.6 Watts, body surface area: 1.8 ± 0.2 m^2). Participants were excluded if they had a recent injury and illness or were in recovery and if they were on medication which affected thermoregulatory autonomic responses or analgesia. A within-participant randomized double-blind crossover study design was employed with trials undertaken after either oral ingestion of acetaminophen or a placebo. Due to injury and illness, n = 2 did not complete experimental trials. Written informed consent was obtained from all participants prior to commencement of the study and

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

Preliminary Testing

Participants were instructed to refrain from ingesting analgesic agents (e.g. non-steroidal anti-inflammatory drugs) for 48 h before each laboratory visit and abstained from caffeine and alcohol consumption for the 24 h prior to each experimental trial. Prior to commencing experimental trials participants attended a preliminary testing session undertaken in temperate conditions (23.4 ± 1.2 °C, 63.1 ± 7.6% relative humidity). Participants arrived at the laboratory a minimum two hours prior to the initial trial commencing after an overnight fast and voided their bladder. Body mass was determined using electronic scales (WM204, Wedderburn, Australia) and height using a wall-mounted stadiometer (Harpenden stadiometer, Holtain Limited, UK). Dual energy X-ray absorptiometry (DXA; Lunar Prodigy DXA machine, GE Healthcare, USA) was performed by a licensed practitioner using methods described previously[17] to determine body composition.

Participants were provided with a pre-exercise snack before beginning a cycling familiarization session. Subsequently, they completed a 10 min warm-up at self-selected intensity followed by a maximal incremental test to volitional fatigue as described previously[18]. Breath-by-breath respiratory gas data was averaged as 30 s epochs throughout the test via metabolic cart (Quark C-PET; Cosmed, Italy). Finally, after a ~5 min self-selected cooldown and ~5 min passive rest, participants completed a familiarization session which included a 10 min steady state ride at 63% peak power output (~70% $\dot{V}O_{2peak}$) followed by a short passive recovery (2 min), and the same 7 $kJ \cdot kg^{-1} \cdot BM^{-1}$ performance time trial used in the experimental trial (described subsequently). Participants were familiarized with perceptual scales[19,20] during the 10 min steady state cycling bout. All participants were endurance trained athletes experienced in

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

Experimental Trial

Dietary Standardization

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

Environmental Conditions

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

Steady State and Time Trial Cycling

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

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ingested gelatin capsules containing either 20mg·kg^{-1}·BM^{-1} acetaminophen (ACT) or a color-matched placebo (cornflour; PLA). Participants completed both steady state and time trial cycling on an electromagnetically braked ergometer (Lode Instruments; Groningen, The Netherlands) which was set up to replicate each participant's individual bicycle settings and facing wind speed ~1.5m·s^{-1} was facilitated using a fan. Initially participants entered the tent and rested passively in a seated position for 10 minutes before mounting the ergometer and beginning the cycling bout. Subjects had 5 min to progress mechanical work to the required 63% peak power output which they then maintained for 55 min. The steady state cycling was followed by a short passive recovery period (2 min). Participants then commenced the 7kJ·kg^{-1}·BM^{-1} time trial using a mode on the ergometer where power output is dependent on each participant's cadence and changes in a linear manner. This relationship was individualised using a linear factor that permits each participant to cycle at ~85% PPO at their preferred cadence similar to previous approaches[21]. Time to completion was recorded using a handheld stopwatch. Participants received no feedback or verbal encouragement during the time trial except notification of each 10% of work completed.

Gastrointestinal and Skin Temperature

Gastrointestinal temperature (T_{gi}) was measured as an indicator of core temperature. Participants ingested a thermistor pill ~9-10 h prior to arrival for each experimental trial (e-Celsius Performance; Bodycap, France). T_{gi} was recorded at 30s intervals which were subsequently converted to 60s epochs for analysis. Skin temperature (T_{sk}) was determined using a thermochron attached to the skin (iButton; Maxim Integrated, USA) with medical tape (Fixomull, BSN Medical, Germany). Thermochrons were placed on the back (infraspinale), mid-bicep, mid-thigh and medial mid-calf. Mean skin temperature was calculated using the unweighted mean of sites on the back, bicep, thigh, and calf[22].

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Heart Rate

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

Perceptual Measures

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

Fluid Balance

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

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

184 Equation 1

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

187 **Statistical Analyses**

188 Physiological and perceptual measures were analyzed between conditions using a repeated-
189 measures (condition × 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 Coorporation, Redmond, USA). Data are mean ± 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±326s, CON:
202 1923±257s, CI = -11.1 to 140.3s, d=0.57, p=0.086, Figure 2) and lower mean power output
203 (ACT: 246±44W, CON: 252±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).

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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: Δ1.5±0.4°C,
210 $p<0.001$; PLA: Δ1.6±0.4°C, $p<0.001$) and the time trial (ACT: Δ0.7±0.3°C, $p=0.002$; PLA:
211 Δ0.7±0.3°C, $p=0.005$). Similar gastrointestinal temperatures at the completion of steady state
212 cycling (ACT: 38.2±0.5°C, PLA: 38.3±0.4°C) and following the time trial (ACT: 38.9±0.5 °C,
213 PLA: 39.0±0.4 °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±0.8°C; PLA: 33.7±0.6°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

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

Fluid Balance

Participants were euhydrated upon arrival for all except two experimental trials (ACT: USG=1.015±0.003; PLA: USG=1.016±0.004, p=0.636). There was a similar reduction (p=0.990) in body mass in both conditions (ACT:-1.8%, CI = 1.3 to 2.4%, d =-2.2, $p < 0.001$; PLA:1.8%, CI = 1.1 to 2.5%, d=-1.8, $p<0.001$). Fluid ingestion (ACT:1.69±0.36L, PLA: 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 different between the acetaminophen and placebo trials.

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DISCUSSION

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

There is a paucity of data available on the effect of acetaminophen on exercise capacity and performance. Intuitively, the antipyretic and/or analgesic effects of acetaminophen might be expected to ameliorate the detrimental physiological and/or perceptual responses associated with exercise in the heat[24]. A purported mechanism of action of acetaminophen is the inhibition of cyclooxygenase (COX) enzymes and subsequent reduction in prostaglandin release, fever and pain signals associated with the inflammatory response[25,26]. Inhibition of COX-2 has been shown to reduce core temperature and skin temperature during steady-state cycling[27] however evidence for the effect of acetaminophen on core and skin temperatures in hot and normothermic conditions show no difference[14,15]. We provide new data that supports and extends the contention that acetaminophen has little antipyretic effects during exercise by showing acetaminophen has no influence on gastrointestinal or skin temperature in afebrile endurance trained individuals during steady-state and high intensity endurance exercise in hot conditions.

The analgesic effect of acetaminophen acts to attenuate perceptions of pain[10,11] which could suppress perceptions of central fatigue and/or discomfort during high intensity exercise for

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improved performance. Few studies have determined the effects of acetaminophen on exercise performance and previous performance tests have had minimal practical application to endurance events. Studies that employed a similar pre-exercise acetaminophen ingestion protocol as the present study reported a ~17% improvement in time to exhaustion (~25 min) in recreationally active individuals in hot conditions (~30°C and ~50% RH)[11] and 2% improvement for trained individuals in a ~16 km time trial in thermoneutral conditions[10]. An important distinction of the present study is the duration of heat exposure (~25 vs. ~120 min) and exercise demands, with the self-paced cycling time trial in our study undertaken after an initial ~75 min period in the heat mimicking the demands of the final leg of an Olympic-distance triathlon in hot conditions. While differences in experimental design and training status of participants may explain, at least in part, the lack of agreement in performance outcomes between studies, the divergent thermal stress evidenced by higher mean gastrointestinal temperature and maximum skin temperature in the present study is also a significant mitigating factor[11]. Moreover, we report no difference in perceptual measures of RPE, thermal sensation and thermal comfort throughout the 1 h steady state cycling bout and after completion of the time trial. This result was somewhat surprising when considering the known analgesic effects of acetaminophen. Accordingly, our findings suggest that there is little evidence supporting acetaminophen ingestion as an effective ergogenic aid in hot and humid conditions when trained athletes undertake prolonged, high intensity exercise similar to the demands of competition in cycling or triathlon.

The moderate effect for poorer time trial performance in the heat in the acetaminophen trial of the present study is difficult to reconcile. A limitation of our study was the lack of direct measurement of plasma acetaminophen concentration during the experimental trials. Consequently, the dose-dependent circulating acetaminophen levels are unknown. Foster, Mauger, Gorvus, Hewson & Taylor[13] have shown ingestion of 20 $mg \cdot kg^{-1} \cdot BM^{-1}$ of

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acetaminophen after a small, standardized meal induced peak plasma acetaminophen concentration (14ug·mL^{-1}) at rest between 100 and 120 min after ingestion which led to a subsequent reduction in gastrointestinal temperature of approximately ~0.2 °C. Our protocol was designed to ensure plasma acetaminophen was high during exercise by consuming it 30 min after a standardized meal (2 g CHO·kg^{-1}·BM^{-1}, 600 mL fluid) and ~120 min before the time trial to coincide with peak plasma concentration[13]. It is acknowledged that the 60 min steady state exercise bout may have affected plasma acetaminophen concentration however steady state exercise had little effect on acetaminophen pharmacokinetics in other studies[28].

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

PRACTICAL APPLICATION

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

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CONCLUSION

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

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ACKNOWLEDGEMENTS

GW was supported by an Australian Government Research Training Scholarship and this project received financial support from Queensland Academy of Sport's Sport Performance Innovation & Knowledge Excellence. The authors would like to thank Ashleigh Keefe, James Kleidon, and Amy-Lee Bowler for their technical assistance during experimental trials. The results of the study are presented honestly without fabrication, falsification, or inappropriate data manipulation.

DECLARATION OF INTEREST STATEMENT

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

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FIGURE CAPTIONS

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

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

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

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

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

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