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Effect of a moderate caffeine dose on endurance cycle performance and thermoregulation during prolonged exercise in the heat

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## 1 Abstract

2 *Objectives:* This study investigated the influence of a moderate caffeine dose on endurance cycle  
3 performance and thermoregulation during prolonged exercise in high ambient temperature.

4 *Design:* Double-blind cross-over study.

5 *Methods:* Eight healthy, recreationally active males (Mean  $\pm$  SD; age:  $22 \pm 1$  y; body mass:  $71.1 \pm 8.5$   
6 kg;  $VO_{2peak}$ :  $55.9 \pm 5.8$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ;  $W_{max}$ :  $318 \pm 37$  W) completed one  $VO_{2peak}$  test, one  
7 familiarisation trial and two experimental trials. After an overnight fast, participants ingested a  
8 placebo or a 6 mg $\cdot$ kg $^{-1}$  caffeine dose 60 min before exercise. The exercise protocol consisted of 60  
9 min of cycle exercise at 55%  $W_{max}$ , followed by a 30 min performance task (total kJ produced) in  
10 30°C and 50% RH.

11 *Results:* Performance was enhanced (Cohen's  $d$  effect size=0.22) in the caffeine trial ( $363.8 \pm 47.6$  kJ)  
12 compared with placebo ( $353.0 \pm 49.0$  kJ;  $p=0.004$ ). Caffeine did not influence core ( $p=0.188$ ) or skin  
13 temperature ( $p=0.577$ ) during exercise. Circulating prolactin ( $p=0.572$ ), cortisol ( $p=0.842$ ) and the  
14 estimated rates of fat ( $p=0.722$ ) and carbohydrate oxidation ( $p=0.454$ ) were also similar between trial  
15 conditions. Caffeine attenuated perceived exertion during the initial 60 min of exercise ( $p=0.033$ ),  
16 with no difference in thermal stress across trials ( $p=0.911$ ).

17 *Conclusions:* Supplementation with 6 mg $\cdot$ kg $^{-1}$  caffeine improved endurance cycle performance in a  
18 warm environment, without differentially influencing thermoregulation during prolonged exercise at a  
19 fixed work-rate versus placebo. Therefore, moderate caffeine doses which typically enhance  
20 performance in temperate environmental conditions also appear to benefit endurance performance in  
21 the heat.

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23 Key words: Stimulants; supplements; core temperature; exercise; fatigue; substrate oxidation

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## 25 Introduction

26 Caffeine (1,3,7-trimethylxanthine) is a well-established ergogenic aid commonly consumed by  
27 endurance athletes.<sup>1</sup> Intakes of low to moderate doses (3-6 mg·kg<sup>-1</sup>) consistently enhance performance  
28 in temperate environmental conditions (~20°C), especially when exercise is performed for 30 min or  
29 longer.<sup>2</sup> Few studies have investigated the ergogenic effects of caffeine in the heat, with some,<sup>3,4</sup> but  
30 not all,<sup>5, 6, 7</sup> reporting improved performance following caffeine ingestion. Hence, from the limited  
31 data available, it is unclear whether caffeine benefits endurance performance in the heat, despite a  
32 high prevalence of intake among athletes competing in warm environments<sup>1</sup>.

33 The progressive impairment in endurance capacity with increasing ambient temperature is well-  
34 documented.<sup>8</sup> Several explanations for this deterioration in performance have been proposed,  
35 including an increased physiological burden to dissipate heat via the skin and an elevated core  
36 temperature<sup>9</sup>. The resulting hyperthermia and increased brain temperature reduce central drive to  
37 continue exercise, thus precipitating the onset of fatigue.<sup>10</sup> During prolonged exercise in the heat,  
38 caffeine has elicited higher core temperatures than placebo.<sup>5,6,11</sup> Therefore, these perturbations to  
39 thermoregulation might explain the lack of performance benefit in the heat after caffeine intake.<sup>5</sup>  
40 Interestingly, larger caffeine doses ( $\geq 9$  mg·kg<sup>-1</sup>) consistently induce elevations in core and body  
41 temperature during exercise in the heat.<sup>6,11</sup> Hence, the provision of smaller doses (~6 mg·kg<sup>-1</sup>), which  
42 typically improve performance in temperate conditions,<sup>2</sup> might prove a more useful strategy to  
43 enhance performance in the heat.

44 Supplementation with 6 mg·kg<sup>-1</sup> caffeine enhanced maximal voluntary contraction of the quadriceps  
45 after prolonged cycle exercise in a hot (36°C) environment.<sup>4</sup> However, during exercise under the same  
46 environmental conditions, the same caffeine dose co-administered with carbohydrates elicited a  
47 higher core temperature than isolated carbohydrate intake.<sup>12</sup> To date, only two laboratory-based  
48 studies have examined the influence of 6 mg·kg<sup>-1</sup> caffeine on endurance cycle performance in the heat  
49 without additional carbohydrates.<sup>5,3</sup> Roelands et al. (2011)<sup>5</sup> reported no ergogenic effect of caffeine  
50 but an increase in core temperature during prolonged exercise at a fixed work-rate, while Ganio et al.

51 (2011)<sup>3</sup> observed an improvement in endurance cycle performance but no thermogenic effects. Hence,  
52 it is unclear whether moderate caffeine doses influence endurance cycle performance or  
53 thermoregulation during prolonged exercise in the heat. Given the widespread intake of caffeine by  
54 athletes,<sup>1</sup> it would be of interest to determine whether moderate doses which consistently enhance  
55 performance in temperate conditions,<sup>2</sup> also confer performance benefits in the heat.

56 Consequently, the aim of this study was to examine the performance and thermoregulatory responses  
57 to prolonged exercise in the heat following the ingestion of a 6 mg·kg<sup>-1</sup> caffeine dose versus a placebo  
58 condition.

59

#### 60 Methods

61 Eight healthy, recreationally active, low-caffeine consuming, non-heat acclimated males (116 ± 46  
62 mg·day<sup>-1</sup>; age: 22 ± 1 y; body mass: 71.1 ± 8.5 kg; height: 1.74 ± 0.08 m; VO<sub>2peak</sub>: 55.9 ± 5.8  
63 mL·kg<sup>-1</sup>·min<sup>-1</sup>; peak power output at VO<sub>2peak</sub> [*W*<sub>max</sub>]: 318 ± 37 W) took part in this investigation,  
64 which employed a double-blind, randomised, repeated-measures, cross-over design. Participants  
65 provided written informed consent and were free from chronic disease. The experimental protocol  
66 was approved by the Ethics Approvals (Human Participants) Sub-Committee of Loughborough  
67 University, UK (Ref: R15-P104).

68 All participants completed one maximal exercise test, one familiarisation trial and two experimental  
69 trials. The initial visit consisted of an incremental exercise test to volitional exhaustion conducted on  
70 an electronically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine *W*<sub>max</sub> and  
71 the power required to elicit 55% and 75% of *W*<sub>max</sub>. This test was performed in temperate conditions  
72 (~20°C). After a brief recovery period (15 min), participants completed the performance task used in  
73 the familiarisation and experimental trials to practice pacing and control of the ergometer. After 5-7  
74 days, the familiarisation trial was undertaken to ensure that participants became fully accustomed to  
75 the procedures employed during the investigation and to minimise any learning or anxiety effects.

76 This trial was performed in environmental conditions maintained at 30°C and 50% RH and was  
77 identical to the experimental trials in all respects, although no treatment was administered.

78 The familiarisation and experimental trials were separated by 7 to 10 days to minimise the  
79 development of heat acclimation. Additionally, all trials were performed at the same time of day to  
80 minimise circadian-type variance. Participants were instructed to record their dietary habits and  
81 physical activity patterns during the 24 hours before the familiarisation trial and to replicate this in the  
82 24 hours preceding each experimental trial. Furthermore, no strenuous exercise or caffeine intake was  
83 permitted during this period and participants were provided with a list of commonly consumed  
84 caffeinated foods and drinks to help achieve this. On the evening before each trial, participants  
85 ingested a radio-telemetry pill (CoreTemp, HQ Inc, Palmetto, Florida, USA) to enable the  
86 measurement of core temperature.

87 Participants arrived at the laboratory in the morning (8-9 am) after an overnight fast (10-12 hours)  
88 with the exception of ingesting 500 mL of plain water approximately 90 min before arrival. Post-void  
89 nude body mass was recorded upon arrival (Adam AFW-120, Milton Keynes, UK) and a heart rate  
90 telemetry band (Polar Beat, Kempele, Finland) was positioned. Skin surface thermistors (Grant  
91 Squirrel SQ800, Cambridgeshire, UK) were attached to four sites (chest, upper arm, thigh and calf)  
92 for the determination of weighted mean skin temperature.<sup>13</sup> Next, an indwelling 21 g cannula was  
93 inserted into an antecubital vein to enable repeated blood sampling; this was flushed with a small  
94 volume of saline after each sample to ensure patency. After 15 min of seated rest at room temperature  
95 (20°C), a baseline 7 mL venous sample was collected, following which participants ingested a capsule  
96 containing either 6 mg·kg<sup>-1</sup> of caffeine (Sigma-Aldrich, UK) or 250 mg of starch (placebo; BDH Ltd,  
97 Poole, UK) with 50 mL of plain water. All capsules were indistinguishable with regards to dimension,  
98 weight and colour. Participants then remained seated for a further 60 min at room temperature. After  
99 45 min, core and skin temperature and heart rate were recorded at 5 min intervals, with a second 7 mL  
100 venous sample collected at 60 min.

101 Participants then entered the climatic chamber (Weiss-Gallenkamp, UK) maintained at 30°C and 50%  
102 RH and began 60 min of cycle exercise at a workload corresponding to 55%  $W_{max}$ . During this period,  
103 core and skin temperature and heart rate were recorded every 5 min. Rating of perceived exertion  
104 (RPE)<sup>14</sup> and perceived thermal stress (using a 21 point scale ranging from -10, unbearable cold, to +10,  
105 unbearable heat) were recorded every 10 min. Expired gas samples (1 min) were collected every 30  
106 min using the Douglas bag method; these values were used to determine the rates of substrate  
107 oxidation during exercise.<sup>15</sup> Participants were provided with 150 mL of plain water (temperature: 20°C)  
108 every 15 min and a third 7 mL venous sample was collected at 60 min while participants remained  
109 seated on the ergometer.

110 Subsequently, there was a 2-3 min delay while the ergometer was programmed for the performance  
111 task. Participants were instructed to produce as much work (kJ) as possible within 30 min; this  
112 method is consistent with previous studies.<sup>6,3</sup> Before starting, all participants were encouraged to  
113 produce a maximal effort. The initial workload was set at 75%  $W_{max}$ , but participants were free to  
114 adjust their power output as desired from the outset. During this period, participants received  
115 information regarding time elapsed and cadence, but no other information or verbal encouragement  
116 was provided. Core and skin temperature and heart rate were recorded every 5 min. A final 7 mL  
117 venous sample was collected immediately after the performance task while participants remained  
118 seated on the ergometer. The cannula, telemetry band and skin thermistors were then removed and  
119 after a short rest period, nude body mass was recorded after participants towelled dry. The change in  
120 body mass, corrected for fluid intake, was used to estimate sweat rate.

121 All venous samples were collected into dry syringes. A small volume (2 mL) was dispensed into tubes  
122 containing K<sub>2</sub>EDTA and duplicate 100 µL sub-samples were deproteinised in 0.3 M perchloric acid.  
123 These were centrifuged, and the resulting supernatant was used to determine plasma glucose  
124 concentrations using a commercially available assay (GOD-PAP, Randox Ltd, UK). Haemoglobin  
125 (cyanmethemoglobin method) and haematocrit (microcentrifugation) values were used to estimate  
126 percentage changes to blood and plasma volumes relative to the baseline sample.<sup>16</sup> The remaining 5  
127 mL was dispensed into tubes containing clotting activator and left for approximately 1 hour prior to

128 centrifugation at 1750 g for 10 min at 4°C. The resulting serum was stored at -21°C for the subsequent  
129 determination of cortisol and prolactin with ELISA (DRG diagnostic, Germany) and caffeine with  
130 reverse-phase HPLC.<sup>17</sup>

131 All data were analysed using IBM SPSS statistics version 22.0. Normality of distribution was  
132 determined using the Shapiro-Wilk test. Exercise performance, pre-exercise body mass, initial core  
133 temperature, fasting plasma glucose, and estimated sweat rates were examined using a paired *t*-test.  
134 Cohen's *d* effect size (ES) for differences in total work produced during the performance task was  
135 determined ( $[\text{mean } 1 - \text{mean } 2]/\text{pooled SD}$ ) and interpreted as trivial (0-0.19), small (0.2-0.49),  
136 medium (0.5-0.79) or large ( $\geq 0.8$ ) as described.<sup>18</sup> Variables measured throughout each trial were  
137 examined with a two-way (trial x time) repeated-measures ANOVA. The Greenhouse-Geisser  
138 correction was applied where the assumption of sphericity had been violated. Where a significant  
139 main effect or interaction was identified, Bonferroni adjusted paired *t*-tests for normally distributed  
140 data or Bonferroni adjusted Wilcoxon Signed Rank tests for non-normally distributed data were used.  
141 Data are presented as mean  $\pm$  SD throughout. Statistical significance was accepted at  $p < 0.05$ .

142

## 143 Results

144 Pre-exercise body mass ( $p=0.732$ ), initial core temperature ( $p=0.279$ ) and fasting plasma glucose  
145 ( $p=0.454$ ) were not different between trials, suggesting that participants began each trial in a similar  
146 physiological state.

147 All eight participants completed both trials, no adverse effects were reported. There was a small  
148 increase (ES=0.22) in total work produced during the caffeine trial ( $363.8 \pm 47.6$  kJ) than placebo  
149 ( $353.0 \pm 49.0$  kJ;  $p=0.004$ ). This represents a percentage increase in performance of  $3.2 \pm 2.4\%$  (range:  
150  $-0.4$  to  $7.7\%$ ; Figure 1). Post-study questionnaires revealed that three of the eight participants (37.5%)  
151 correctly identified the caffeine trial, thus blinding can be considered successful as these odds are less  
152 than what would be expected purely by chance.

153 Pre-exercise core temperature was similar between trials ( $p=0.718$ ; Figure 2A). There was a main  
154 effect of time during the initial 60 min of exercise ( $p<0.05$ ), but no main effect of trial ( $p=0.188$ ) or  
155 trial x time interaction ( $p=0.112$ ). There were main effects of time ( $p<0.05$ ) and trial ( $p=0.006$ ), as  
156 well as an interaction effect ( $p=0.005$ ) during the performance task. Higher values were recorded from  
157 20 to 30 min during the caffeine trial compared with placebo ( $p<0.05$ ; Figure 2A). Pre-exercise skin  
158 temperature was similar between trials ( $p=0.429$ ; Figure 2B). There was a main effect of time during  
159 the initial 60 min of exercise ( $p<0.05$ ), but no main effect of trial ( $p=0.577$ ) or trial x time interaction  
160 ( $p=0.116$ ). Similarly, during the performance task there was a main effect of time ( $p<0.05$ ), but no  
161 main effect of trial ( $p=0.970$ ) or interaction effect ( $p=0.311$ ; Figure 2B).

162 Heart rate (Figure 2C), RPE (Figure 2D), and perceived thermal stress (Figure 2E) all increased  
163 throughout the initial 60 min of exercise (all  $p<0.05$ ). There was also a main effect of trial for RPE  
164 ( $p=0.033$ ), but there were no other trial ( $p>0.644$ ) or interaction effects ( $p>0.253$ ) for these variables.  
165 During the performance task heart rate showed main effects of time ( $p<0.05$ ) and trial ( $p=0.011$ ), but  
166 no interaction effect ( $p=0.904$ ; Figure 2C).

167 Caffeine concentrations remained below the limit of quantification during the placebo trial and for the  
168 baseline sample during the caffeine trial, increasing to  $33.0 \pm 5.7$ ,  $35.3 \pm 10.9$ , and  $32.6 \pm 8.1$   $\mu\text{M}$  at  
169 60, 120 and 150 min post-capsule ingestion, respectively.

170 Serum cortisol and prolactin both showed main effects of time ( $p<0.05$ ), but no main effects of trial  
171 ( $p>0.572$ ) or interaction effects ( $p>0.148$ ; Table 1). Similarly, plasma glucose and the percentage  
172 change to blood and plasma volumes all showed main effects of time ( $p<0.05$ ), but no main effects of  
173 trial ( $p>0.056$ ) or trial x time interactions ( $p>0.111$ ) occurred (Table 1).

174 There were no main effects of time ( $p>0.363$ ), trial ( $p>0.454$ ) or interaction effects ( $p>0.410$ ) for fat  
175 and carbohydrate oxidation and RER. Oxygen uptake showed a main effect of time ( $p=0.001$ ), but no  
176 main effect of trial ( $p=0.361$ ) or interaction effect ( $p=0.188$ ). Over the entire 90 min of exercise,  
177 estimated sweat rates were higher in the caffeine trial ( $2.31 \pm 0.43$  L) than placebo ( $2.20 \pm 0.37$  L;



178  $p=0.036$ ). Accordingly, percentage body mass loss after exercise was greater during the caffeine trial  
179 ( $2.30 \pm 0.36$ ) than placebo ( $2.16 \pm 0.31$ ;  $p=0.029$ ).

180

## 181 Discussion

182 This study investigated the performance and thermoregulatory effects of a  $6 \text{ mg}\cdot\text{kg}^{-1}$  caffeine dose  
183 during prolonged exercise in the heat. This caffeine dose consistently improves endurance  
184 performance in temperate environmental conditions,<sup>2</sup> yet there are conflicting reports when exercise is  
185 performed in the heat.<sup>5,3</sup> In the study by Roelands et al. (2011),<sup>5</sup> a  $6 \text{ mg}\cdot\text{kg}^{-1}$  caffeine dose  
186 administered 60 min before exercise failed to enhance time-trial performance but increased core  
187 temperature during exercise in  $30^{\circ}\text{C}$ . Conversely, Ganio et al (2011)<sup>3</sup> reported enhanced work  
188 production during a 15 min cycle performance task with no difference in core temperature  
189 between trials when  $3 \text{ mg}\cdot\text{kg}^{-1}$  caffeine was ingested 60 min before and 45 min during exercise in  
190  $33^{\circ}\text{C}$ . The results of the present study agree with the latter findings, as caffeine provided a small, but  
191 significant ergogenic effect (Figure 1), with no difference in core or skin temperature between trials  
192 (Figure 2A and B).

193 Several studies report no performance benefit in the heat after caffeine ingestion,<sup>5,6,7</sup> attributing this  
194 response to an elevation in core temperature during exercise.<sup>5</sup> However, even large caffeine doses ( $9$   
195  $\text{mg}\cdot\text{kg}^{-1}$ ) result in only mild thermogenic effects,<sup>6,11</sup> which is typically undetected by participants.<sup>11</sup> In  
196 addition, five days of controlled caffeine intake ( $3$  and  $6 \text{ mg}\cdot\text{kg}^{-1}$ ) did not influence the core  
197 temperature response during exercise in  $37^{\circ}\text{C}$ .<sup>19</sup> Alternatively, some researchers suggest that a high  
198 environmental temperature might negate the efficacy of caffeine.<sup>6</sup> These authors reported no  
199 performance benefit in  $40^{\circ}\text{C}$  after ingestion of  $9 \text{ mg}\cdot\text{kg}^{-1}$  caffeine. The lower environmental  
200 temperature and/or caffeine dose employed in the present study might account for these divergent  
201 findings. Additionally, 21 km race time in hot and humid conditions was not influenced by caffeine  
202 intakes of  $5$  or  $9 \text{ mg}\cdot\text{kg}^{-1}$ .<sup>7</sup> However, participants in this study became  $\sim 4\%$  dehydrated during  
203 exercise, thus it is unknown if caffeine would have enhanced performance if fluid-balance was

204 maintained. When hydration status is controlled across cool (12°C) and warm (33°C) environmental  
205 conditions, caffeine still improves endurance cycle performance.<sup>3</sup>

206 The ergogenic effect of caffeine was attributed to changes in fat metabolism during exercise, resulting  
207 in a glycogen sparing effect.<sup>20</sup> However, there is compelling evidence caffeine enhances performance  
208 through direct actions within the central nervous system.<sup>21</sup> Caffeine increases synaptic dopamine  
209 concentrations in exercising rats, although large doses (10-30 mg·kg<sup>-1</sup>) are required to induce this  
210 response.<sup>22</sup> Using positron emission topography, a moderate caffeine dose (300 mg) did not influence  
211 *in vivo* dopamine release in the human brain.<sup>23</sup> Attenuated prolactin concentrations would suggest an  
212 increase in dopamine,<sup>24</sup> but similar values were observed across trials (Table 1). Alternatively,  
213 caffeine influences key neuronal signaling proteins which mediate increases in physical activity and  
214 potentiates adenosine-dopamine receptor binding in striatum.<sup>25,26</sup> A reduced perception of effort is a  
215 common response to caffeine intake, which might account for approximately 29% of its ergogenic  
216 effect.<sup>27</sup> Participants in the present study reported lower RPE values during the initial hour of exercise  
217 with caffeine (Figure 2D), which is likely mediated by a reduced activity of cortical premotor and  
218 motor areas.<sup>28</sup>

219 Previous reports demonstrated that 6 mg·kg<sup>-1</sup> caffeine enhanced sweat-electrolyte losses in 36°C,<sup>12</sup>  
220 while 3 mg·kg<sup>-1</sup> augmented sweat rates during submaximal cycle exercise in 24°C.<sup>29</sup> In the present  
221 study, higher sweat rates were observed during the caffeine trial than placebo over the entire 90 min  
222 of exercise (2.31 ± 0.43 L vs. 2.20 ± 0.37 L; p=0.036). This small difference likely reflects the higher  
223 work rate during the performance task in the caffeine trial and the concomitant elevation in core  
224 temperature (Figure 2A). During prolonged exercise at a fixed work-rate, caffeine did not adversely  
225 influence fluid-balance, sweat rate or serum osmolality in cool (12°C) and warm (33°C)  
226 environmental conditions compared with placebo.<sup>3</sup> Additionally, there were no differences in fluid,  
227 electrolyte, or renal indices of hydration after 5 days of controlled caffeine intake (3 and 6 mg·kg<sup>-1</sup>)  
228 versus placebo.<sup>30</sup>

229

## 230 Conclusion

231 In conclusion, supplementation with 6 mg·kg<sup>-1</sup> caffeine 60 min before prolonged exercise in 30°C and  
232 50% RH improved endurance cycle performance in non-heat acclimated participants, without any  
233 measureable change to thermoregulation versus placebo. There appeared to be a developing trend for  
234 core temperature during the initial 60 min of exercise (interaction effect, P=0.112), suggesting that a  
235 longer period of fixed-intensity might enable caffeine to elicit a greater increase in core temperature  
236 than placebo under these environmental conditions. However, the difference at the end of the preload  
237 was small (0.03°C, Figure 2A), which was also undetected by participants (Figure 4B). These data,  
238 together with previous reports,<sup>3</sup> suggest that moderate caffeine doses which typically improve  
239 endurance performance in temperate environmental conditions,<sup>2</sup> also benefit endurance cycle  
240 performance in the heat.

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242

## 243 Practical applications

- 244 • Moderate caffeine doses appear to be ergogenic to endurance cycle performance for  
245 recreationally active, non-heat acclimated, fasted individuals competing in the heat.
- 246 • Supplementation with 6 mg·kg<sup>-1</sup> caffeine does not significantly influence core or skin  
247 temperature up to 60 min of cycle exercise at a fixed work-rate.
- 248 • During prolonged fixed-intensity exercise in the heat, moderate caffeine intakes attenuate  
249 perceived exertion compared with placebo.

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251 Word count: 2,909

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254 *Acknowledgements*

255 The authors report no conflict of interest. The study did not receive any external financial support. We  
256 wish to thank the participants for their continued dedication, effort and motivation throughout their  
257 involvement in the study.

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## 274 References

- 275 1. Desbrow B, Leveritt MD. Awareness and use of caffeine by athletes competing at the 2005  
276 Ironman Triathlon World Championships. *Int J Sport Nutr Exerc Metab* 2006; 16(5):545-558.
- 277 2. Ganio MS, Klau JF, Casa DJ et al. Effect of caffeine on sport-specific endurance performance: a  
278 systematic review. *J Strength Cond Res* 2009; 23(1):315-324.
- 279 3. Ganio MS, Johnson EC, Klau JF et al. Effect of ambient temperature on caffeine ergogenicity  
280 during endurance exercise. *Eur J Appl Physiol* 2011; 111(6):1135-1146.
- 281 4. Del Coso J, Estevez E, Mora-Rodriguez R. Caffeine effects on short term performance during  
282 prolonged exercise in the heat. *Med Sci Sports Exerc* 2008; 40(4):744-751.
- 283 5. Roelands B, Buyse L, Pauwels F et al. No effect of caffeine on exercise performance in high  
284 ambient temperature. *Eur J Appl Physiol* 2011; 111(12):3089-3095.
- 285 6. Chevront SM, Ely BR, Kenefick RW et al. No effect of nutritional adenosine receptor antagonists  
286 on exercise performance in the heat. *Am J Physiol Regul Integr Comp Physiol* 2009; 296(2):R394-401.
- 287 7. Cohen BS, Nelson AG, Prevost M.C et al. Effects of caffeine ingestion on endurance racing in heat  
288 and humidity. *Eur J Appl Physiol* 1996; 73(3-4):358-363.
- 289 8. Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged  
290 cycle exercise in man. *Med Sci Sports Exerc* 1997; 29(9):1240-1249.
- 291 9. Chevront SM, Kenefick RW, Montain SJ et al. Mechanisms of aerobic performance impairment  
292 with heat stress and dehydration. *J Appl Physiol* 2010; 109(6):1989-1995.
- 293 10. Nybo L. CNS fatigue provoked by prolonged exercise in the heat. *Front Biosci (Elite Ed)* 2010;  
294 2:779-792.
- 295 11. Ely BR, Ely MR, Chevront SN. Marginal effects of a large caffeine dose on heat balance during  
296 exercise-heat stress. *In J Sport Nutr Exerc Metab* 2011; 21(1):65-70.

- 297 12. Del Coso J, Estevez E, Mora-Rodriguez R. Caffeine during exercise in the heat: thermoregulation  
298 and fluid-electrolyte balance. *Med Sci Sports Exerc* 2009; 41(1):164-173.
- 299 13. Ramanathan NL. A new weighting system for mean surface temperature of the human body. *J*  
300 *Appl Physiol* 1964; 19:531-533.
- 301 14. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14(5):377-381.
- 302 15. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci*  
303 1991; 16(1):23-29.
- 304 16. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells  
305 in dehydration. *J Appl Physiol* 1974; 37(2):247-248.
- 306 17. Holland DT, Godfredsen KA, Page T et al. Simple high-performance liquid chromatography  
307 method for the simultaneous determination of serum caffeine and paraxanthine following rapid  
308 sample preparation. *J Chromatogr B Biomed Sci Appl* 1998; **707**(1-2):105-110.
- 309 18. Cohen J. A power primer. *Psychol. Bull* 1992; 112(1):155-159.
- 310 19. Roti MW, Casa DJ, Pumerantz AC et al. Thermoregulatory responses to exercise in the heat:  
311 chronic caffeine intake has no effect. *Aviat Space Environ Med* 2006; 77(2):124-129.
- 312 20. Costill DL, Dalsky GP, Fink WJ. Effects of caffeine ingestion on metabolism and exercise  
313 performance. *Med Sci Sports* 1978; 10(3):155-158.
- 314 21. Fredholm BB, Bättig K, Holmén J et al. Actions of caffeine in the brain with special reference to  
315 factors that contribute to its widespread use. *Pharmacol Rev* 1999; 51(1):83-133.
- 316 22. Solinas M, Ferré S, You ZB et al. Caffeine induces dopamine and glutamate release in the shell of  
317 the nucleus accumbens. *J Neurosci* 2002; 22(15):6321-6324.
- 318 23. Volkow ND, Wang GJ, Logan J et al. Caffeine increases striatal dopamine D2/D3 receptor  
319 availability in the human brain. *Transl Psychiatry* 2015; **5**:e549.

- 320 24. Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. *Endocr Rev*  
321 2001;22(6):724-763.
- 322 25. Lindskog M, Svenningsson P, Pozzi L et al. Involvement of DARPP-32 phosphorylation in the  
323 stimulant action of caffeine. *Nature* 2002; 418(6899):744-748.
- 324 26. Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. *J Neurochem*  
325 2008; 105(4):1067-1079.
- 326 27. Doherty M, Smith PM. Effects of caffeine ingestion on rating of perceived exertion during and  
327 after exercise: a meta-analysis. *Scand J Med Sci Sports* 2005; 15(2):69-78.
- 328 28. de Morree HM, Klein C, Marcora SM. Cortical substrates of the effects of caffeine and time-on-  
329 task on perception of effort. *J Appl Physiol* 2014; 117(12):1514-1523.
- 330 29. Kim TW, Shin YO, Lee JB et al. Caffeine increases sweating sensitivity via changes in sudomotor  
331 activity during physical loading. *J Med Food* 2011; 14(11):1448-1455.
- 332 30. Armstrong LE, Pumerantz AC, Roti MW et al. Fluid, electrolyte, and renal indices of hydration  
333 during 11 days of controlled caffeine consumption. *Int J Sport Nutr Exerc Metab* 2005; 15(3):252-265.
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Table 1 Circulating concentrations of cortisol, prolactin and glucose and the percentage change to blood and plasma volumes during the experimental trials.

	Treatment	-60	0	60	90
Cortisol (nM)	placebo	449.1 ± 127.6	483.7 ± 115.3	519.4 ± 105.5	701.1 ± 130.4*
	caffeine	450.4 ± 140.7	458.4 ± 137.6	524.3 ± 135.0	734.8 ± 142.6*
Prolactin (mIU·L <sup>-1</sup> )	placebo	182.7 ± 73.1	152.8 ± 30.6	405.8 ± 61.8*	534.2 ± 105.6*
	caffeine	160.7 ± 38.9	146.6 ± 36.7	380.3 ± 71.1*	529.8 ± 126.7*
Glucose (mmol·mL <sup>-1</sup> )	placebo	4.33 ± 0.35	4.23 ± 0.39*	4.88 ± 0.36*	6.06 ± 0.17*
	caffeine	4.34 ± 0.38	4.25 ± 0.38*	4.88 ± 0.37*	6.17 ± 0.16*
Blood volume (%)	placebo	0.0 ± 0.0	0.19 ± 0.56	-1.67 ± 0.99*	-4.87 ± 2.45*
	caffeine	0.0 ± 0.0	0.13 ± 0.67	-2.02 ± 0.95*	-5.49 ± 1.95*
Plasma volume (%)	placebo	0.0 ± 0.0	0.20 ± 1.42	-3.29 ± 1.83*	-8.25 ± 2.67*
	caffeine	0.0 ± 0.0	0.02 ± 1.34	-3.88 ± 1.69*	-9.20 ± 2.67*

Values are mean ± SD. \*significant difference ( $P < 0.05$ ) from -60.



## 338 Figure Captions

339 Figure 1: Total kJ produced (bars) and individual responses (lines) during the experimental trials.

340 Figure 2: Core temperature (a), skin temperature (b), heart rate (c), RPE (d), and perceived thermal  
341 stress (e) during the experimental trials. \*denotes a significant difference ( $P < 0.05$ ) between trials.

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358 Figure 1

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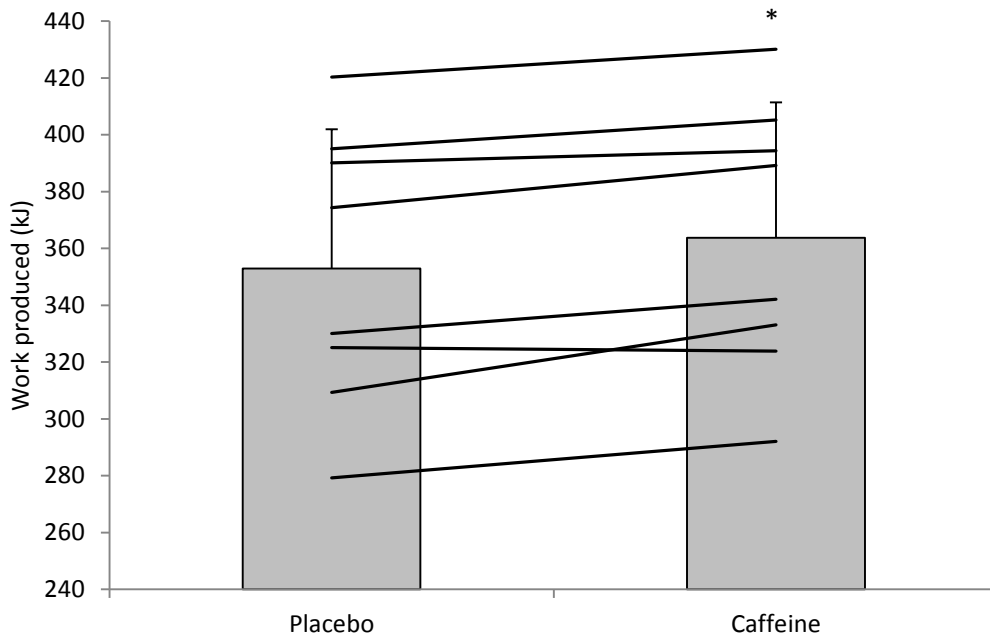
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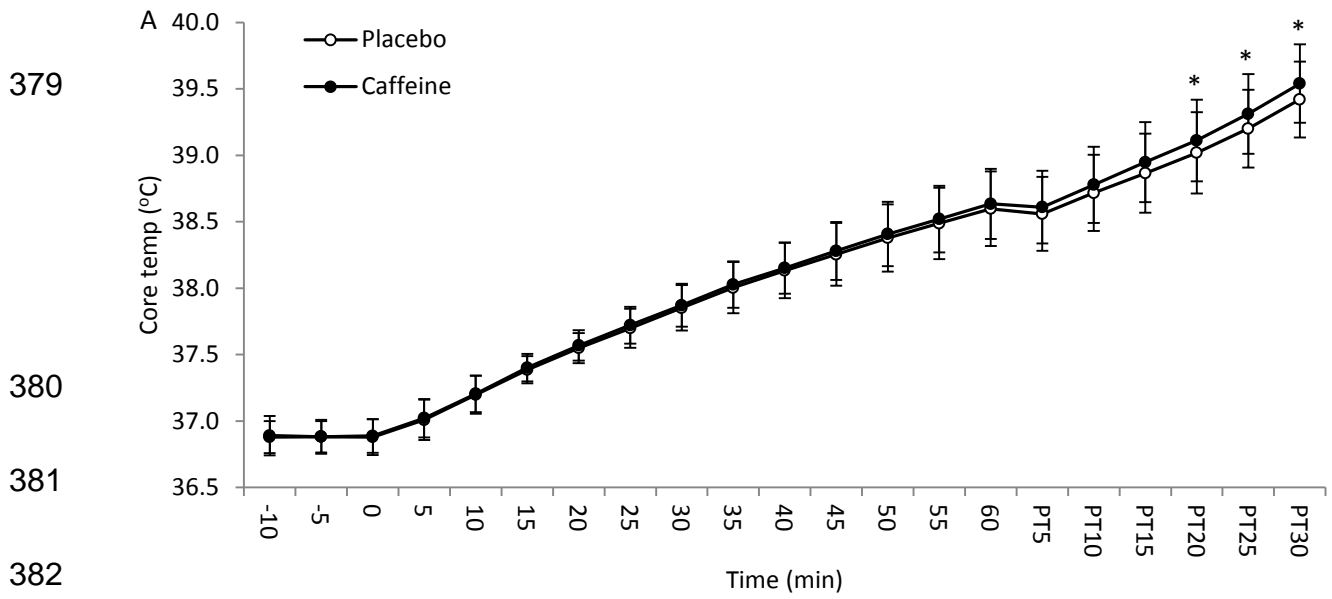
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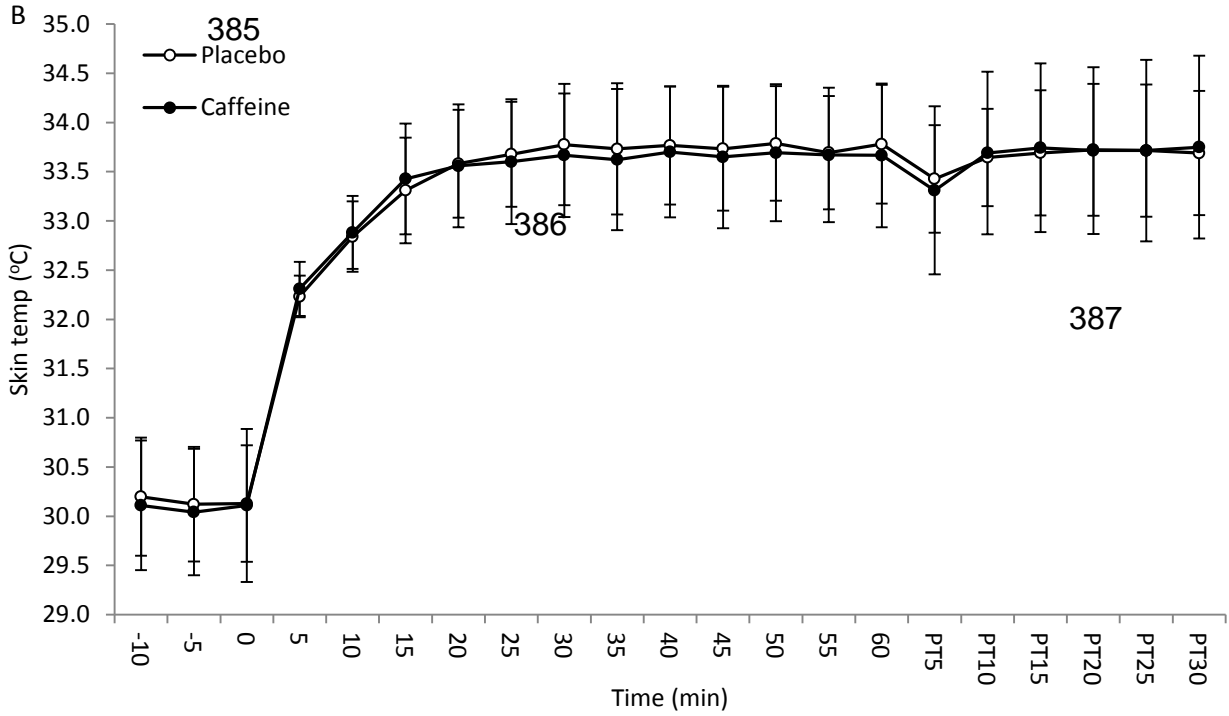


378 Figure 2A



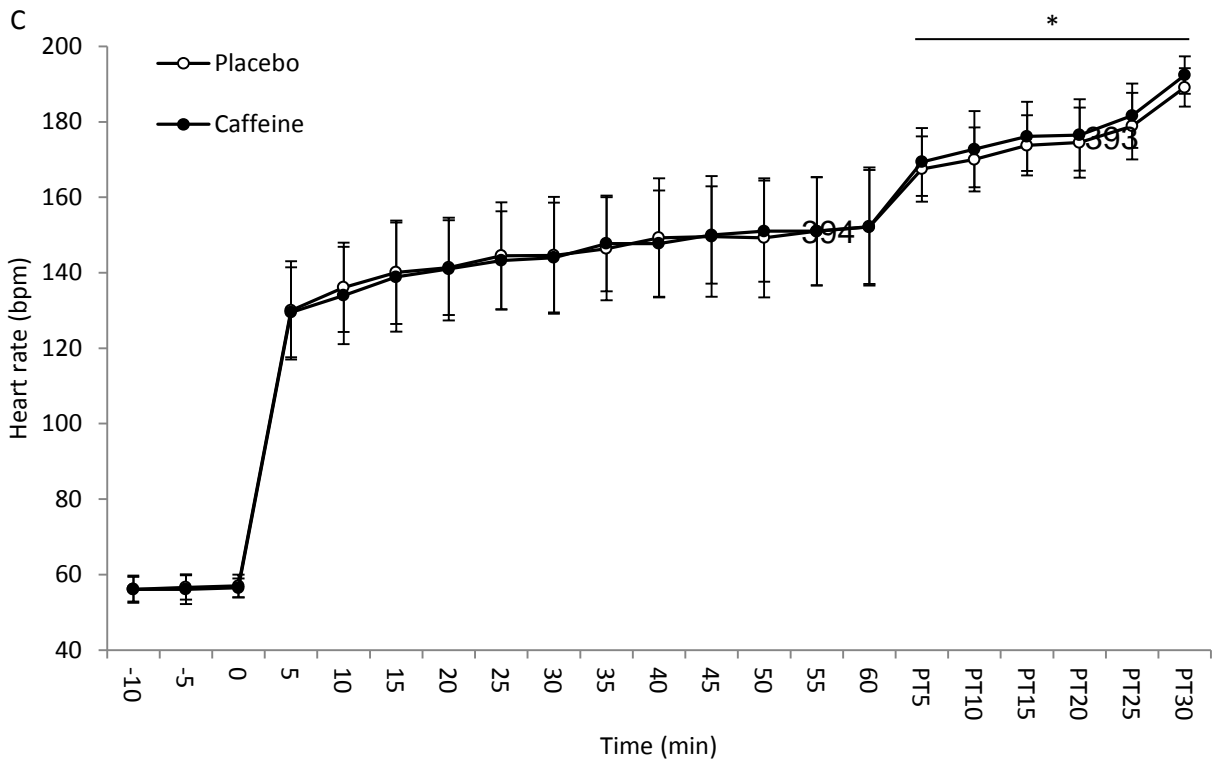
383 Figure 2

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391 Figure 2C

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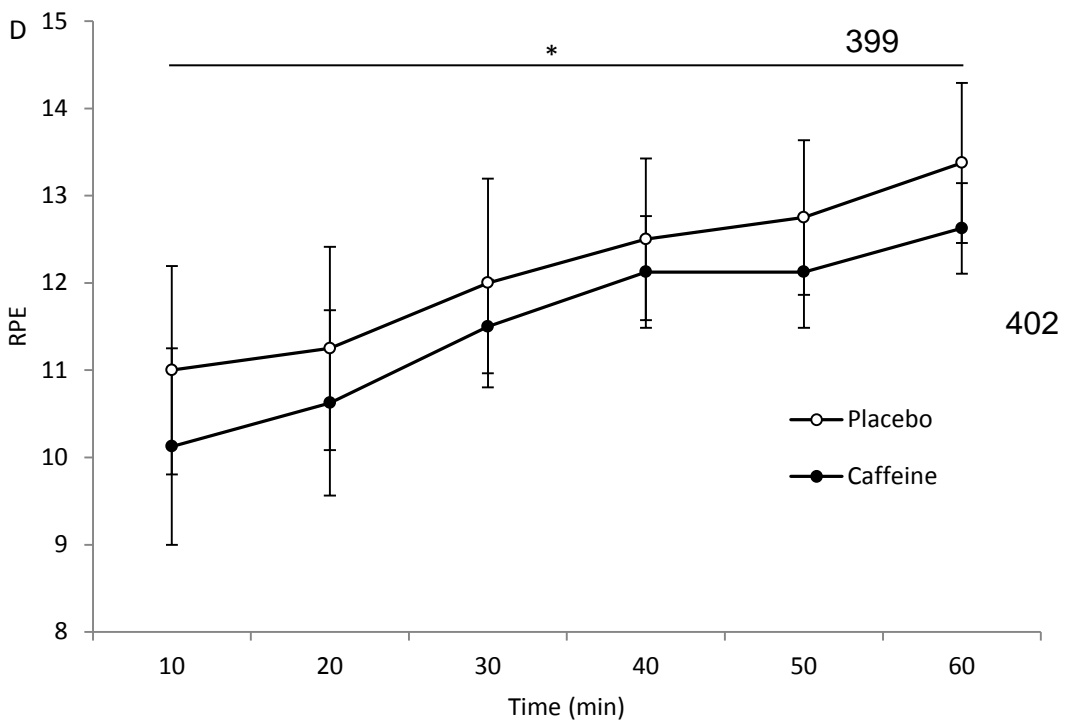


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397 Figure 2D

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