

1 **Short-term heat acclimation and precooling, independently and**  
2 **combined, improve 5 km running performance in the heat.**

3

4 **ABSTRACT**

5 Following heat acclimation (HA), endurance running performance remains impaired in hot vs  
6 temperate conditions. Combining HA with precooling demonstrates no additive benefit in  
7 intermittent sprint, or continuous cycling exercise protocols, during which heat strain may be less  
8 severe compared to endurance running. This study investigated the effect of short-term heat  
9 acclimation (STHA) combined with mixed-methods precooling, on endurance running performance  
10 and directly compared precooling and HA. Nine amateur trained runners completed 5 km treadmill  
11 time trials in the heat (32°C, 60% RH) under four conditions; no intervention (CON), precooling (PC),  
12 short-term heat acclimation (5 days - HA) and short-term heat acclimation with precooling (HA+PC).  
13 Mean ( $\pm$ SD) performance times were; CON 1476 (173) s, PC 1421 (146) s, HA 1378 (116) s and HA+PC  
14 1373 (121) s. This equated to the following improvements versus CON; PC -3.7%, HA -6.6% and  
15 HA+PC -7.0%. Statistical differences were only observed between HA and CON ( $p=0.004$ ,  $d=0.68$ ,  
16 95% CI [-0.27, 1.63]) however, similar effect sizes were observed for HA+PC vs CON ( $d=0.70$ , 95% CI  
17 [-0.25, 1.65]), with smaller effects between PC vs CON ( $d=0.34$ , 95% CI [-0.59, 1.27]), HA vs PC  
18 ( $d=0.33$ , 95% CI [-0.60, 1.26]) and HA+PC vs PC ( $d=0.36$ , 95% CI [-0.57, 1.29]). Pilot testing revealed a  
19 time trial typical error of 16 s (1.2%). Precooling offered no further benefit to performance in the  
20 acclimated individual, despite modest alleviation of physiological strain. Maintenance of running  
21 speed in HA+PC, despite reduced physiological strain, may indicate an inappropriate pacing strategy  
22 therefore, further familiarisation is recommended to optimise a combined strategy. Finally, these  
23 data indicate HA, achieved through cycle training, yields a larger ergogenic effect than precooling on

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1 5km running performance in the heat, although precooling remains beneficial when HA is not  
2 possible.

### 3 **Key words**

4 Hyperthermia; endurance; lactate threshold;  $\dot{V}O_{2max}$ ; heat stress, thermoregulation.

## 5 **INTRODUCTION**

6 Strategies to alleviate the deleterious effect of hyperthermia on endurance performance  
7 habitually adopt a uni-dimensional approach, with athletes advised to either precool or undertake  
8 heat acclimation (HA) <sup>1</sup>. This dichotomous practice persists despite a dearth of direct comparisons  
9 between acute and chronic strategies, that would indicate the most effective approach.

10 From a chronic perspective, HA is habitually classified as either short (STHA, <7 days), medium  
11 (MTHA, 8-14 days) or long term (LTHA, >15 days) <sup>2</sup>. Heat acclimation induces observable and  
12 prominent adaptations including decreased resting and exercising, core ( $T_{CORE}$ ) and skin ( $T_{SKIN}$ )  
13 temperatures, alongside a reduction in exercising heart rate (HR), which likely arises through an  
14 expanded plasma volume <sup>3</sup>. Typical ergogenic effects of STHA on endurance performance are  
15 reported to be 2.4% <sup>4</sup>. Such adaptations help mitigate against an accentuated cardiovascular  
16 challenge during exercising under heat stress, which notably reduces maximal oxygen uptake  
17 ( $\dot{V}O_{2max}$ ) as a consequence of thermoregulatory cutaneous vasodilation impeding venous return and  
18 cardiac filling <sup>5</sup>. However, evidence demonstrates both endurance performance <sup>6</sup> and  $\dot{V}O_{2max}$  <sup>7</sup> remain  
19 impaired in the heat following HA, relative to cooler conditions (13°C vs 38°C <sup>6</sup>, 21°C vs 49°C <sup>7</sup>),  
20 highlighting not only the persistence of heat strain, but a need to further improve endurance  
21 performance in the acclimated individual.

22 Acute, precooling techniques may be classified as internal (e.g. ice slurry ingestion) or external  
23 (e.g. ice vests, ice packs), depending upon how the cooling impulse is delivered. External precooling  
24 demonstrates larger effects on  $T_{SKIN}$  and thermal sensation than internal precooling <sup>8</sup>. External

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1 precooling presents a dose-dependent response, with a mixed-methods approach, involving multiple  
2 cooling garments and hand/forearm cold water immersion appearing preferable to singular cooling  
3 garments, due to the greater cooled skin surface area<sup>9</sup>. Accordingly, recent meta-analyses report  
4 large effects on subsequent endurance performance when multiple cooling garments are worn  
5 either alongside or following part-body cold water immersion (+7.3%,  $d = 0.72$ <sup>10</sup>,  $d = 1.91$ <sup>11</sup>). Of  
6 note, the practical mixed methods technique of Duffield et al.<sup>12</sup>, involving ice towels, ice packs, ice  
7 vest and hand immersion in cold water, ameliorates physiological and thermoregulatory strain  
8 during fixed intensity endurance exercise in the heat<sup>8</sup>, but has yet to be evaluated during free-paced  
9 exercise, where the influence of alterations in  $T_{SKIN}$  and thermal perception may be most pronounced  
10<sup>13</sup>.

11 Despite individual strategies failing to maintain endurance performance in the heat relative to  
12 normothermic conditions, the benefit of combining interventions is yet to be fully elucidated. Castle  
13 et al.<sup>14</sup> reported no additional benefit from quadriceps precooling during intermittent sprint-cycling,  
14 following LTHA. Results indicated LTHA alone sufficiently negated heat strain during this type of  
15 activity. Consequently, Brade et al.<sup>15</sup> investigated precooling following STHA, which affords only  
16 partial heat adaptation in comparison to LTHA<sup>24</sup>. However, no additive effect was observed, with  
17 STHA again mediating heat strain during intermittent sprinting sufficiently such that precooling was  
18 unwarranted and thus ineffective. Conversely, continuous running or cycling endurance exercise in  
19 the heat confers a large and consistent physiological strain<sup>16</sup>, which may therefore require a more  
20 potent intervention than HA alone to ameliorate declines in performance. This notion is reinforced  
21 by larger effects of precooling observed on endurance performance, compared with intermittent  
22 sprinting<sup>11</sup>.

23 Recently Schmit et al.<sup>17</sup> investigated national-level triathletes wearing ice vests at rest and  
24 during the warm-up prior to a 20 km cycling time trial, following 10-days of acclimatisation. Although  
25 the addition of precooling did not improve performance above acclimatisation alone, transient,  
26 beneficial pacing alterations were observed during the first half of the trial, alongside improved

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1 perceived thermal strain, following precooling. Therefore, a more potent precooling strategy e.g.  
2 mixed methods<sup>12</sup>, may magnify or prolong this transient benefit. This transient benefit may also be  
3 more impactful in a shorter event than the ~32 min trial of Schmit et al.<sup>17</sup>, as the effects of  
4 precooling will be experienced for a greater proportion of the event duration before dissipating. A  
5 further consideration is the type of exercise undertaken, with exercise such as running, that yields a  
6 significant metabolic heat production (MHP) appearing best suited to combining interventions, given  
7 heat strain can be mitigated by STHA alone during intermittent sprinting whilst cycling<sup>15</sup>. Running  
8 elicits a greater MHP than cycling and provides less convective cooling<sup>18,19</sup>, which collectively  
9 expedites heat strain, relative to cycling<sup>20</sup>. Therefore, when STHA is adopted, affording partial heat  
10 adaptation, an additive effect from precooling may be observed when heat strain remains high  
11 during exercise such as endurance running. However, no investigations have combined precooling  
12 and HA prior to endurance running.

13 Therefore, the aim of this study was to investigate whether mixed methods external precooling  
14 following STHA provided greater ergogenicity for maintaining endurance running performance, than  
15 STHA alone, whilst providing a direct comparison between precooling and heat acclimation. It was  
16 hypothesised that combining STHA and precooling would improve time trial performance relative to  
17 STHA, whilst STHA would be more beneficial than precooling alone.

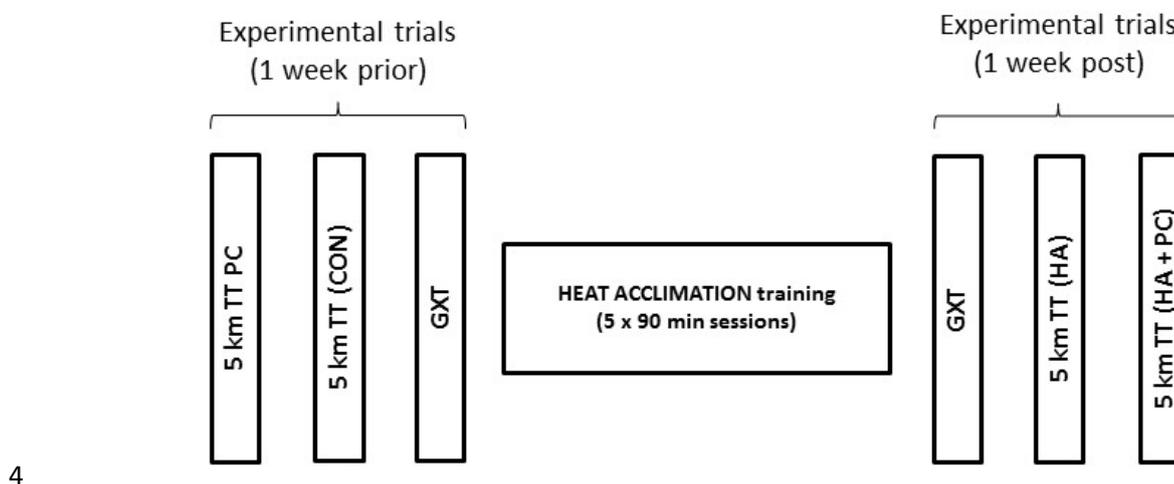
## 18 **METHODS**

### 19 **Experimental approach to the problem**

20 A repeated measures design was adopted, with individuals completing two 5 km treadmill time  
21 trials and a graded exercise test (GXT) before and after 5 days of STHA, as shown in Figure 1. Each  
22 GXT was ordered immediately pre and post HA training, whilst time trials (TT) with either precooling  
23 (PC) or a no intervention control trial (CON) were completed in a counterbalanced order, prior to  
24 STHA. Experimental trials occurred at least 10 days after instrumented familiarisations of the GXT

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1 and TT in the heat. Following STHA, a TT was completed without precooling (HA) and another with  
 2 precooling (HA+PC), again counterbalanced (Figure 1). Trials occurred at a similar time of the day to  
 3 minimise fluctuation in thermoregulatory responses from circadian variation <sup>21</sup>.



4  
 5 Figure 1: Overview of experimental design. 'GXT' = Graded exercise test. 'TT' = time trial. 'PC =  
 6 precooling. 'CON' = no intervention control. 'HA' = heat acclimation. 'HA+PC' = Heat acclimation and  
 7 precooling. All trials completed in the heat. Before training, five participants completed CON first  
 8 and four completed precooling first. After training, five completed HA first and four completed  
 9 HA+PC first.

## 10 Subjects

11 Nine amateur, club runners (8 male, 1 female), who had trained at least three times per week  
 12 for the previous 2 months, volunteered for this study (mean [±SD]: age 32 [16] years, stature 175 [7]  
 13 cm, mass 71.9 [8.8] kg, sum of four skinfolds 25.4 [3.8] mm,  $\dot{V}O_{2max}$  59.1 [6.9] mL.kg<sup>-1</sup>.min<sup>-1</sup>, recent  
 14 5km time: 20:44 [1:44] min). Participants were recruited as part of a larger study on heat acclimation  
 15 <sup>22</sup>. All participants had completed a sub-22 min 5km or sub 45 min 10km race in the previous 2  
 16 months and had never previously undertaken HA. The female participant completed pre-tests and  
 17 training during the follicular phase of the menstrual cycle, with post-tests during the first 5 days of  
 18 luteal phase. Participants were informed of the benefits and risks of the investigation prior to signing  
 19 an institutionally approved informed consent document to participate in the study. Ethical approval

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1 was issued in accordance with the Declaration of Helsinki (2013). Participants arrived hydrated,  
2 having refrained from intense exercise for 48 hours, and avoided alcohol and caffeine for 24 hours.  
3 Participants completed a 24-hour food diary prior to each test and indicated sleeping hours,  
4 motivation, muscle soreness and stress on 5-point Likert scales upon arrival.

## 5 **Procedures**

### 6 **Precooling**

7 A mixed-methods, external precooling technique was adopted, as per Duffield et al.<sup>12</sup> and James  
8 et al.<sup>8</sup>. This involved wet, iced towels covering the head and neck, forearm and hand immersion in  
9 cold water (9°C), an ice vest (Artic Heat, Australia) on the torso and ice packs affixed to the  
10 quadriceps using cooling shorts, across a 20 min seated period. Towels were changed after 10 min  
11 and hand immersion water temperature was actively maintained throughout.

### 12 **Heat acclimation**

13 Heat acclimation involved five, 90 min daily training sessions in the heat (~37°C, ~60% relative  
14 humidity [RH]) using controlled hyperthermia and permissive dehydration<sup>23,24</sup>. Participants exercised  
15 on cycle ergometers (Monark, e724, Sweden) at an intensity initially prescribed relative to body  
16 mass, at 2.7 W.kg<sup>-1</sup><sup>25</sup> and subsequently adjusted to maintain the maximum tolerable power to  
17 achieve the target T<sub>CORE</sub> (38.5°C) within 30 min. Upon T<sub>CORE</sub> reaching 38.5°C, exercise was completed  
18 intermittently to maintain T<sub>CORE</sub> above 38.5°C for 60 min<sup>23,24</sup>. Throughout the training session,  
19 exercise intensity was adjusted in 5 min blocks. Therefore, the typical work pattern was 30 min of  
20 continuous cycling, before a further 5 min of exercise every 25 min. The initial prescription of  
21 exercise based on power output, relative to body mass, as opposed to % $\dot{V}O_{2max}$ <sup>14,26</sup>, removes the  
22 necessity for an initial cycling  $\dot{V}O_{2max}$  test and maintains the relative exercise intensity across training  
23 days, independent of adaptation. Furthermore, cycling training controlled for performance that  
24 could arise from increased training volume were participants to acclimate through running. Training

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1 occurred at the same time of day, predominantly in the morning (07:00-10:00 h) and one participant  
2 in the evening (18:00-20:00 h). No fluid intake was permitted during training <sup>27</sup>.

3

4

## 5 **Exercise trials**

6 During all trials, participants initially rested in the hot environment (32°C, 60% RH) for 10 mins,  
7 before a 20 min period for cooling, or additional rest, as appropriate. Therefore, the entire protocol  
8 occurred within a thermostatically controlled environmental chamber (WatFlow control system TISS,  
9 UK) within which conditions were continuously monitored throughout the trial using a heat stress  
10 meter (HT30, Extech Instruments, USA), which provided indoor wet bulb globe temperature (WBGT).

11 A GXT in the heat, split into two parts; GXT 1 and GXT 2, was adopted similar to that  
12 described by Jones <sup>28</sup> and as previously implemented <sup>8,29</sup>. The test comprised two parts; GXT 1, a  
13 submaximal incremental speed protocol, followed by GXT 2; an incremental gradient protocol to  
14 volitional exhaustion. During GXT 1 each participant completed a minimum of six stages, using speed  
15 increments of 1 km.h<sup>-1</sup>. The initial treadmill speed was based on the familiarisation trial, which in  
16 turn was determined from recent 5 km time. Following a 10 min rest, GXT 2 began at a speed 2 km.h<sup>-1</sup>  
17 <sup>1</sup> below the previous final speed with gradient increasing by 1% each min, continuing until volitional  
18 exhaustion <sup>28</sup>. Participants were not permitted to drink and were blinded to all forms of feedback  
19 throughout the duration of the trial.

20 Prior to completing any experimental TT, participants underwent a familiarisation trial under the  
21 same circumstances. During the familiarisation, starting speed was determined based upon recent 5  
22 km performance. For each experimental TT, following cooling and/or rest phases, participants  
23 completed a self-selected 5 min warm-up, replicated across all trials, on a motorised treadmill  
24 (Woodway ELG2, Germany). Standardised instructions were given at the start and nothing

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1 thereafter; 'give your all', 'pace yourself throughout the trial' and 'adjust speed as you see fit' as per  
2 similar studies<sup>30</sup>. Participants straddled the treadmill belt, increased to the individual's average pace  
3 from the familiarisation, to maintain a consistent blinded starting speed. Treadmill speed adjustment  
4 was permitted *ad libitum* (increment 0.2 km.h<sup>-1</sup>), with the distance completed continuously  
5 displayed. Participants were blinded to all other feedback. Elapsed time was recorded every km.

## 6 **Physiological measures**

7 During all trials, hydration was assessed upon arrival, whereby euhydration represented  
8 urine osmolality and specific gravity below 700 mOsmol.kg<sup>-1</sup> H<sub>2</sub>O and 1.020, respectively<sup>31</sup>. Pre and  
9 post nude body mass were recorded to estimate sweat loss (GFK150 scales, AE Adam, UK). A single-  
10 use rectal probe (Henleys Medical, UK) connected to a meter logger (Model 401, Yellow Springs  
11 Instruments, USA), was inserted 10 cm beyond the anal sphincter for T<sub>CORE</sub> measurement. Telemetry  
12 thermistors (U-Type connected to Gen II GD38 transmitter, Eltek, UK) were attached to the mid-belly  
13 of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius. Local skin temperatures  
14 were derived through a datalogger (RX250AL 1000 series Wireless Squirrel Logger, Eltek) as per  
15 James et al.<sup>32</sup> in order to determine mean T<sub>SKIN</sub><sup>33</sup>. Heart rate was monitored continuously using a  
16 Polar 810i heart rate monitor (Kempele, Finland).

17 During the GXTs, HR, T<sub>CORE</sub>, T<sub>SKIN</sub>, rating of perceived exertion (RPE<sup>34</sup>) and thermal sensation (TS,  
18 0=unbearably cold to 8=unbearably hot)<sup>35</sup> were noted at the end of each stage. The following  
19 physiological responses were calculated; running speeds at blood lactate concentrations of 2 and 4  
20 mmol.l<sup>-1</sup>, running economy (RE),  $\dot{V}O_{2max}$  and velocity at  $\dot{V}O_{2max}$  ( $v\dot{V}O_{2max}$ ) as per James et al.<sup>29</sup>.  
21 Derivative calculations included mean T<sub>SKIN</sub><sup>33</sup>, Physiological Strain Index (PSI)<sup>36</sup> and change in plasma  
22 volume<sup>37</sup>. During the 5 km time trial, HR, T<sub>CORE</sub>, T<sub>SKIN</sub>, RPE and TS were recorded every km.

## 23 **Statistical analyses**

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1 All outcome variables were assessed for normality and sphericity prior to further analysis. Where  
2 assumptions of ANOVA were not met, non-parametric statistics were adopted. Exercise data from  
3 both the GXTs and TTs were analysed using two-way, repeated measures ANOVA (Trial\*Time) where  
4 data comprised repeated time points, with post hoc Bonferroni adjusted pairwise comparisons used  
5 where significant main or interaction effects occurred. During the time trials, all average, finishing  
6 and delta change data from physiological and performance variables were analysed with One-way  
7 repeated measures ANOVA. Where the use of repeated measures ANOVA was precluded through  
8 the violation of parametric assumptions, such as TT performance, Friedman's ANOVA, with Wilcoxon  
9 follow up tests, were used to analyse these data. Singular data, that did not have repeated measures  
10 within the GXT, were analysed using Paired samples t-tests. Effect sizes for main effects and  
11 interaction effects are presented as partial eta squared (partial  $\eta^2$ ), differences between related  
12 samples were evaluated through Cohen's  $d_{av}$  ( $d_{av}$ )<sup>38</sup>. Data were analysed using SPSS (Version 21,  
13 SPSS Inc., USA) with statistical significance set at  $p < 0.05$  and data presented as means and standard  
14 deviation ( $\pm$ SD).

## 15 RESULTS

### 16 Heat acclimation training

17 Mean exercising time during STHA training was 39 (6) min, completed at a relative exercise  
18 intensity of 2.7 (0.3)  $W \cdot kg^{-1}$  (201 [33] W). The training environmental conditions (36.6 [0.8] $^{\circ}C$ , 59  
19 [9]% RH) elicited a mean peak session HR of 176 (9)  $b \cdot min^{-1}$  and average session  $T_{CORE}$  of 38.5 (0.2) $^{\circ}C$ .  
20 The mean time  $T_{CORE}$  exceeded 38.5 $^{\circ}C$  during each session was 63 (5) min, with an average peak  
21 session  $T_{CORE}$  of 39.1 (0.2) $^{\circ}C$ . Mean sweat rate was 1.5 (0.5)  $L \cdot hr^{-1}$ , equating to 3.2 (1.1)% of body  
22 mass.

### 23 Graded exercise tests

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1 Statistically significant reductions in resting ( $-0.15^{\circ}\text{C}$ ,  $p=0.01$ ,  $d_{av}=0.45$ ) and exercising  $T_{\text{CORE}}$  ( $-$   
 2  $0.21^{\circ}\text{C}$ ,  $p=0.04$ ,  $d_{av}=0.54$ ) were observed, alongside a reduced exercising ( $-3 \text{ b}\cdot\text{min}^{-1}$ ,  $p=0.02$ ,  
 3  $d_{av}=0.26$ ), but not resting HR ( $-2 \text{ b}\cdot\text{min}^{-1}$ ,  $p=0.115$ ,  $d_{av}=0.36$ ). Total sweat loss did not change  
 4 following STHA (Pre  $1.35 [0.3] \text{ L}$ , Post  $1.39 [0.39] \text{ L}$ ,  $p= 0.503$ ,  $d_{av}=0.13$ ), but occurred alongside a  
 5 smaller change in  $T_{\text{CORE}}$  ( $\Delta$  Pre  $1.26 [0.27] ^{\circ}\text{C}$ ,  $\Delta$  Post  $1.00 [0.28] ^{\circ}\text{C}$ ,  $p=0.006$ ,  $d_{av}=0.91$ ) indicating  
 6 increased sudomotor sensitivity. A 5.7% increase ( $p=0.03$ ,  $d_{av}=1.06$ ) in blood plasma volume was also  
 7 observed. No changes were observed in mean RPE ( $p=0.342$ ) or TS ( $p=0.262$ ), although there was a  
 8 reduced change in thermal sensation ( $p=0.04$ ,  $d_{av}=0.86$ ). For complete STHA results please see James  
 9 et al. <sup>22</sup>.

10 The GXT also revealed an enhanced  $\dot{V}\text{O}_{2\text{max}}$  following STHA ( $+4.0 [2.2] \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $+7.3 [4.0]\%$ ,  
 11  $p=0.003$ ,  $d_{av}=0.47$ ). A reduced respiratory exchange ratio ( $-0.08$ ,  $p=0.03$ ,  $d_{av}=0.59$ ) during exercise is  
 12 commensurate with improvements observed in both the LT ( $+0.4 [0.6] \text{ k}\cdot\text{hr}^{-1}$ ,  $+4.0 [6.0]\%$ ,  $p=0.073$ ,  
 13  $d_{av}=0.24$ ) and LTP ( $+0.3 [0.4] \text{ k}\cdot\text{hr}^{-1}$ ,  $+2.5 [2.9]\%$ ,  $p=0.022$ ,  $d_{av}=0.20$ ). No statistical difference was  
 14 observed in  $v\dot{V}\text{O}_{2\text{max}}$  ( $p=0.144$ ,  $d_{av}=0.24$ ), although the mean difference of  $0.5 (0.8) \text{ k}\cdot\text{hr}^{-1}$  ( $3.5 [5.3]\%$ )  
 15 is potentially meaningful given our laboratory typical error of 2.9% for this measure in a similarly  
 16 trained cohort. Finally, RE worsened, with a greater amount of oxygen consumed per kilometre  
 17 following heat acclimation ( $+7.3 [7.3] \text{ mL}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$ ,  $3.5 [3.5]\%$ ,  $p=0.017$ ,  $d_{av}=0.59$ ).

## 18 Time trial performances

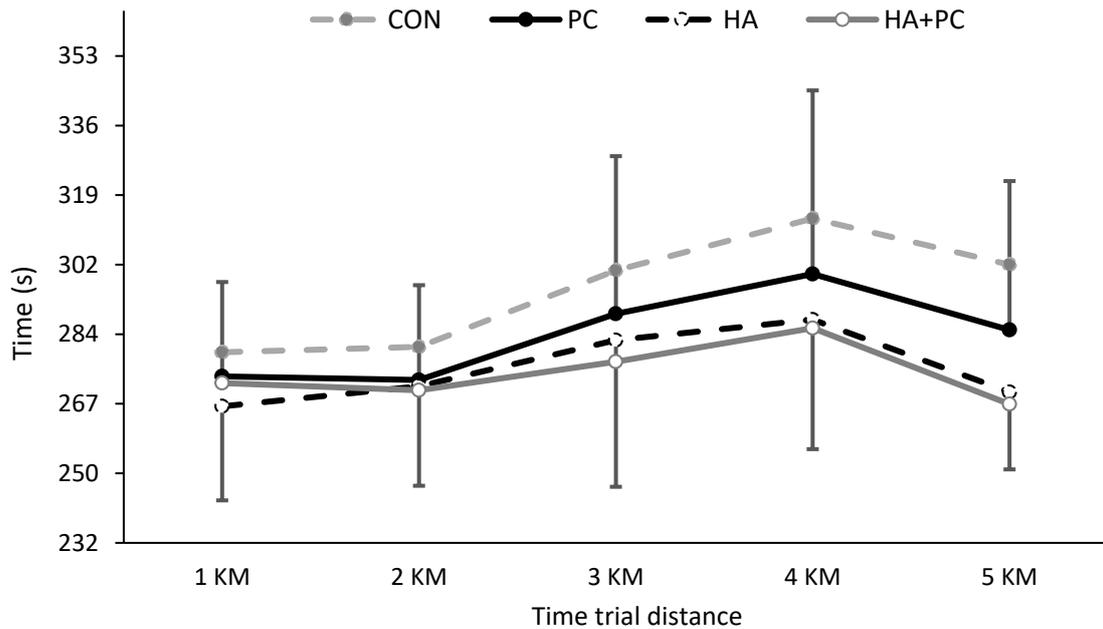
19 Environmental conditions (WBGT) did not differ between trials; CON= $27.4 (0.7)^{\circ}\text{C}$ , PC= $26.9$   
 20 ( $0.5$ ) $^{\circ}\text{C}$ , HA= $27.5 (0.9)^{\circ}\text{C}$ , HA+PC= $27.0 (0.8)^{\circ}\text{C}$  ( $p=0.246$ , partial  $\eta^2=0.156$ ). Self-reported motivation,  
 21 muscle soreness and stress responses did not differ between trials for the female participant or for  
 22 the group as a whole ( $p>0.05$ ). Friedman's ANOVA revealed a difference in TT performance between  
 23 trials ( $p=0.001$ ). However, Wilcoxon tests with Bonferroni correction (whereby significance =  
 24  $p<0.008$ ), only indicated a significant difference between the control trial and HA+PC. Group mean  
 25 ( $\pm$ SD) performance times were; CON  $1476 (173)\text{s}$ , PC  $1421 (146)\text{s}$ , HA  $1378 (116)\text{s}$  and HA+PC  $1373$

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1 (121)s. Relative to control, a large mean improvement was observed in HA and HA+PC, with a  
2 modest improvement following PC (Table 1), although neither reached statistical significance.  
3 Compared with CON, nine participants ran faster in HA, whilst eight ran faster in HA+PC and PC  
4 trials. There was no observable difference in running performance between HA and HA+PC trial,  
5 whilst the observed effect sizes and mean difference indicate modest improvements in HA and  
6 HA+PC compared with PC (Table 1). In HA+PC, eight participants ran faster than in PC, with six  
7 participants performing better in HA than PC. Figure 2 displays the kilometre splits for each trial.

1 Table 1: Relative difference in 5 km time trial performance between trials. \*Corrected statistical significance level for Wilcoxon signed-rank test post hoc  
 2  $p < 0.008$ . Previously established typical error following 5 days high intensity normothermic training = 16 s, 1.2%. Data are; mean change (s), percentage  
 3 change (%), statistical significance ( $p$ ) and effect size ( $d$ ) with 95% confidence intervals (CI). +/- represents the performance difference (s) relative to the trial  
 4 in that row.

	<b>Time trial time</b>	<b>Control</b>	<b>Precooling</b>	<b>Heat acclimation</b>	<b>Heat acclimation + precooling</b>
<b>Control</b>	1476 (173) s	-	+55 s (3.7%) ( $p=0.039$ , $d=0.34$ ) 95% CI = -0.59, 1.27	+98 s (6.6%) ( $p=0.004^*$ , $d=0.68$ ) 95% CI = -0.27, 1.63	+103 s (7.0%) ( $p=0.012$ , $d=0.70$ ) 95% CI = -0.25, 1.65
<b>Precooling</b>	1421 (146) s	-55 s (3.7%) ( $p=0.039$ , $d=0.34$ ) 95% CI = -0.59, 1.27	-	+43 s (3.0%) ( $p=0.098$ , $d=0.33$ ) 95% CI = -0.60, 1.26	+48 s (3.4%) ( $p=0.023$ , $d=0.36$ ) 95% CI = -0.57, 1.29
<b>Heat acclimation</b>	1378 (116) s	-98 s (6.6%) ( $p=0.004^*$ , $d=0.68$ ) 95% CI = -0.27, 1.63	-43 s (3.0%) ( $p=0.098$ , $d=0.33$ ) 95% CI = -0.60, 1.26	-	+5 s (0.4%) ( $p=0.590$ , $d=0.04$ ) 95% CI = -0.88, 0.96
<b>Heat acclimation + precooling</b>	1373 (121) s	-103 s (7.0%) ( $p=0.012$ , $d=0.70$ ) 95% CI = -0.25, 1.65	-48 s (3.4%) ( $p=0.023$ , $d=0.36$ ) 95% CI = -0.57, 1.29	-5 s (0.4%) ( $p=0.590$ , $d=0.04$ ) 95% CI = -0.88, 0.96	-



1

2 Figure 2: Mean ( $\pm$ SD) kilometre split times during the 5 km time trial. Error bars represent one  
 3 standard deviation. Error bars for control (CON) and heat acclimation + precooling (HA+PC) trials are  
 4 omitted for clarity, but homogeneity of variance was present.

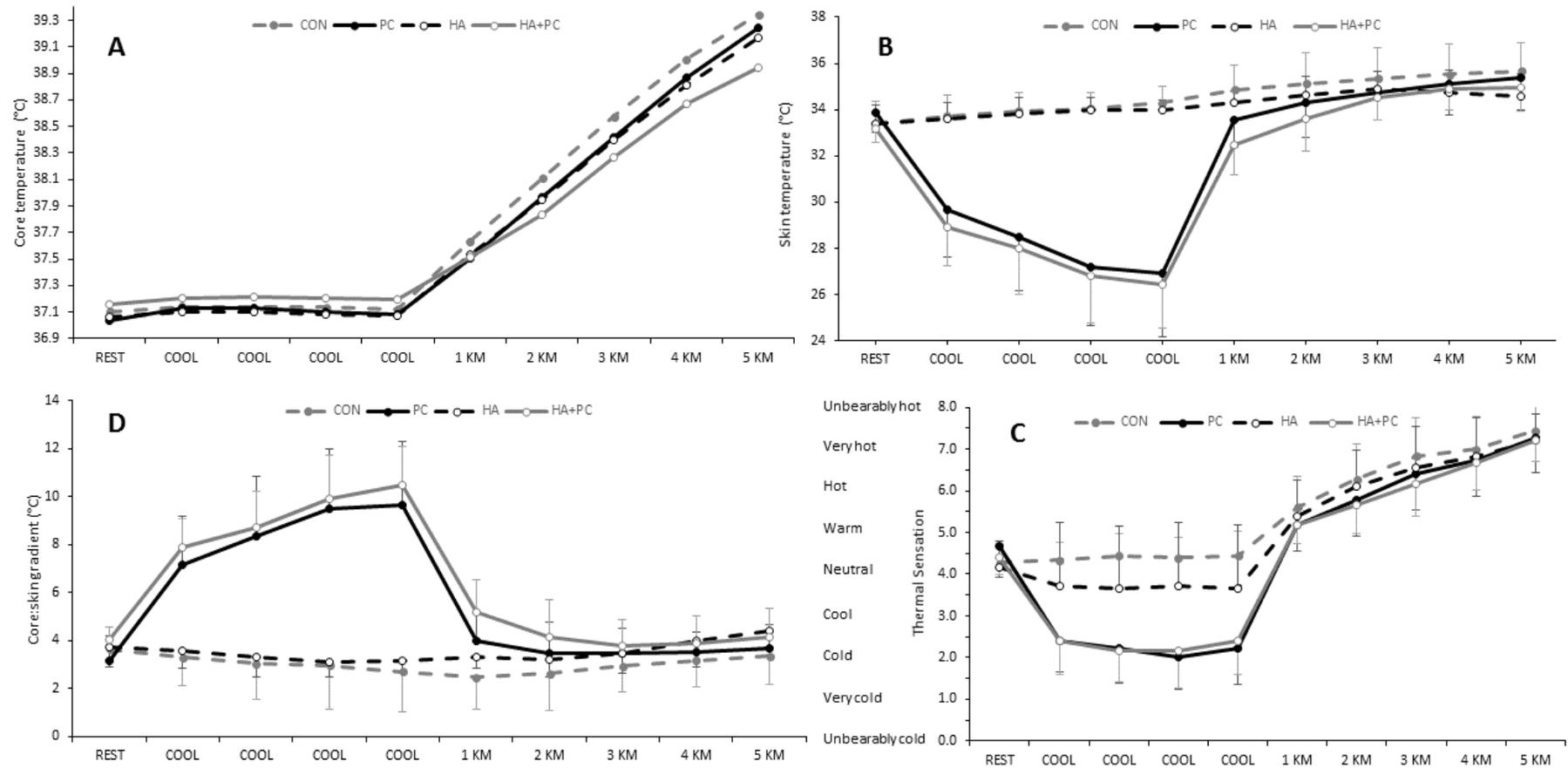
5 Following 20 min cooling there was no change in  $T_{CORE}$  ( $p=0.219$ , partial  $\eta^2=0.165$ ). Therefore,  
 6 starting  $T_{CORE}$  was not different between trials ( $p=0.697$ , partial  $\eta^2=0.075$ ); CON 37.12 (0.22) $^{\circ}$ C, PC  
 7 37.07 (0.30) $^{\circ}$ C, HA 37.07 (0.23) $^{\circ}$ C and 37.2 (0.22) $^{\circ}$ C during HA+PC. Large reductions in  $T_{SKIN}$  ( $p<0.001$ ,  
 8 partial  $\eta^2=0.906$ ) were observed across the cooling period in the trials containing precooling (-6.9  
 9 [2.7] $^{\circ}$ C PC; -6.8 [1.5] $^{\circ}$ C HA+PC), whilst  $T_{SKIN}$  was unchanged in non-precooling trials (CON +0.87  
 10 [0.50] $^{\circ}$ C; HA +0.58 [0.58] $^{\circ}$ C). Therefore, starting  $T_{SKIN}$  in PC (26.9 [2.8] $^{\circ}$ C) and HA+PC (26.4 [1.9] $^{\circ}$ C)  
 11 were lower ( $p<0.001$ , partial  $\eta^2=0.900$ ) than in non-precooled trials (34.3 [0.7] $^{\circ}$ C CON, 34.0 [0.4] $^{\circ}$ C  
 12 HA). This coincided with a reduced starting thermal sensation ( $p=0.002$ , partial  $\eta^2=0.907$ ) in PC (2.2  
 13 [0.8]) and HA+PC (2.4 [0.8]), compared with CON (4.4 [0.6] and HA (3.7 [1.5])). Finally, a greater  
 14 core:skin gradient ( $p<0.001$ , partial  $\eta^2=0.896$ ) was observed in PC (9.6 [2.6] $^{\circ}$ C) and HA+PC (10.5  
 15 [1.7] $^{\circ}$ C), compared with CON (2.7 [0.6] $^{\circ}$ C) and HA (3.2 [0.5] $^{\circ}$ C). Plots of thermoregulatory variables  
 16 during all trials are shown in Figure 3.

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1 During the TTs, there was no difference in mean  $T_{\text{CORE}}$  between conditions ( $p=0.117$ , partial  
2  $\eta^2=0.273$ ), however the change ( $\Delta$ ) in  $T_{\text{CORE}}$  was different ( $p=0.044$ , partial  $\eta^2=0.776$ ) as shown in  
3 Figure 3. Finishing  $T_{\text{CORE}}$  differed between conditions ( $p=0.025$ , partial  $\eta^2=0.396$ ), with CON the  
4 warmest (39.34 [0.30] $^{\circ}\text{C}$ ), followed by PC (39.24 [0.51] $^{\circ}\text{C}$ ), HA (39.16 [0.44] $^{\circ}\text{C}$ ) and the lowest  
5 finishing  $T_{\text{CORE}}$  in HA+PC (38.96 [0.43] $^{\circ}\text{C}$ ).

6 The differences observed in starting  $T_{\text{SKIN}}$  continued during the respective TTs ( $p=0.010$ , partial  
7  $\eta^2=0.369$ ). Mean  $T_{\text{SKIN}}$  was highest during CON (35.3 [1.2] $^{\circ}\text{C}$ ), followed by HA (34.6 [0.7] $^{\circ}\text{C}$ ), PC (34.6  
8 [1.2] $^{\circ}\text{C}$ ) and the lowest was in HA+PC (34.1 [0.9] $^{\circ}\text{C}$ ) as shown in Figure 3. However, a statistical  
9 difference was only observed between CON and PC ( $p=0.029$ ,  $d=0.58$ ). A difference in finishing  $T_{\text{SKIN}}$   
10 was also apparent ( $p=0.037$ , partial  $\eta^2=0.293$ ), although only between CON and HA ( $p=0.026$ ,  
11  $d=0.48$ ). Finishing  $T_{\text{SKIN}}$  for each trial was; CON 35.1 (1.2) $^{\circ}\text{C}$ , PC 35.7 (1.2) $^{\circ}\text{C}$ , HA 34.6 (1.0) $^{\circ}\text{C}$  and  
12 HA+PC 34.9 (1.0) $^{\circ}\text{C}$ .

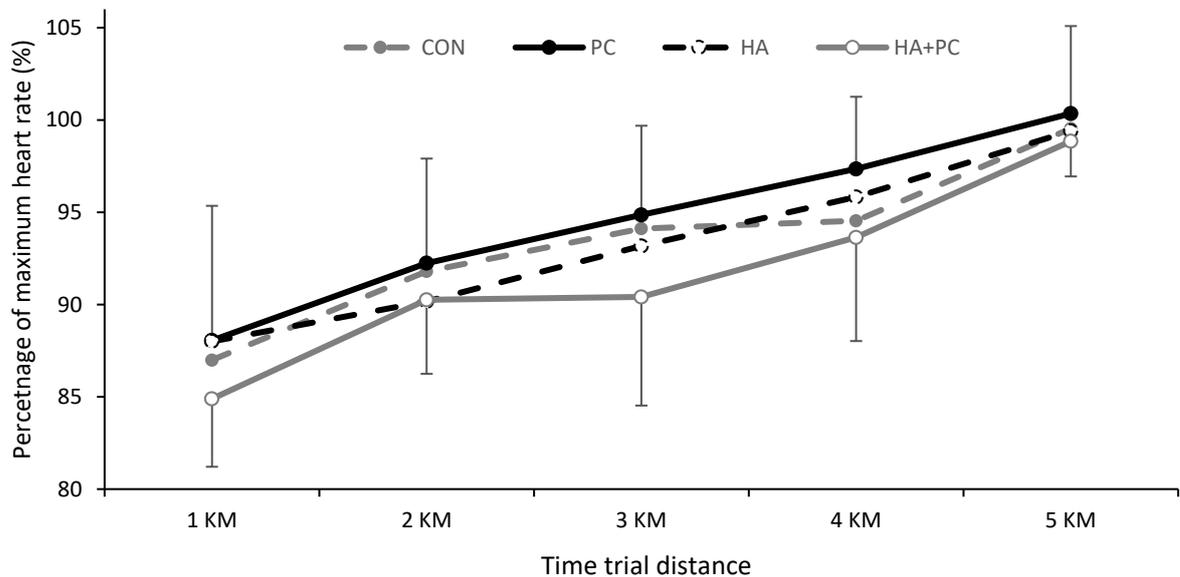
13 The mean core:skin gradient was also different between conditions ( $p=0.005$ , partial  $\eta^2=0.504$ ),  
14 as shown in Figure 3. The largest gradient was observed in HA+PC (4.2 [1.2] $^{\circ}\text{C}$ ), followed by HA (3.7  
15 [0.8] $^{\circ}\text{C}$ ), PC (3.6 [1.0] $^{\circ}\text{C}$ ) and CON (2.9 [0.9] $^{\circ}\text{C}$ ), with statistical differences between CON and HA+PC  
16 ( $p=0.034$ ,  $d=1.24$ ). There were different finishing core:skin gradients ( $p=0.028$ , partial  $\eta^2=0.388$ ),  
17 with the largest observed in HA (4.3 [1.1] $^{\circ}\text{C}$ ) and HA+PC (4.2 [1.2] $^{\circ}\text{C}$ ), followed by PC (3.7 [1.0] $^{\circ}\text{C}$ ) and  
18 CON (3.4 [1.0] $^{\circ}\text{C}$ ).



1

2 Figure 3: Clockwise from top left: Mean ( $\pm$ SD) core temperature (A), skin temperature (B), thermal sensation (C) and core:skin gradient (D) during rest,  
 3 cooling and exercise phases of the time trial protocol. Each increment represents 5 min during rest and cooling phases. The time trial began 15 min after  
 4 cooling finished. Error bars represent one standard deviation, with core temperature error bars omitted for clarity. Participants completed additional rest in  
 5 CON and HA trials during the 'cooling' phase.

1 No differences were observed in the mean TS ( $p=0.066$ , partial  $\eta^2=0.255$ ) or RPE ( $p=0.213$ ,  
2 partial  $\eta^2=0.168$ ) between conditions. Neither mean HR ( $p=0.252$ , partial  $\eta^2=0.154$ ) or finishing HR  
3 ( $p=0.734$ , partial  $\eta^2=0.051$ ), differed between conditions. Similarly, mean HR as a percentage of  
4 maximum HR (%HRmax) was not different between conditions ( $p=0.089$ , partial  $\eta^2=0.234$ ), as shown  
5 in Figure 4. The mean %HRmax for each trial was; CON 93.4 (3.8)%, PC 94.6 (4.9)%, HA 93.3 (3.8)%  
6 and HA+PC 91.6 (3.1)%. Sweat loss was different between trials ( $p=0.008$ , partial  $\eta^2=0.386$ ), with the  
7 largest fluid loss in HA (2.5 [0.5] l.hr<sup>-1</sup>), compared with CON (2.2 [0.8] l.hr<sup>-1</sup>), PC (1.7 [0.5] l.hr<sup>-1</sup>) and  
8 HA+PC (2.3 [0.6] l.hr<sup>-1</sup>). Pairwise comparisons revealed a difference between PC and HA ( $p=0.006$ ,  
9  $d=1.50$ ), but not other conditions.



10

11 Figure 4: Mean ( $\pm$ SD) percentage of maximum heart rate maintained throughout each trial. Error  
12 bars represent one standard deviation. Error bars for control and heat acclimation trials are omitted  
13 for clarity, but homogeneity of variance was present.

14

Combining heat acclimation and precooling for running in the heat.

## 1 **DISCUSSION**

2 Our primary aim was to assess the efficacy of combining precooling and heat acclimation for  
3 improving endurance running performance in the heat. Our data reaffirm previous observations,  
4 with precooling offering no further benefit to performance in the acclimated individual, but  
5 demonstrate modest alleviation of physiological strain. The second aim was to directly compare the  
6 ergogenic potential of precooling and heat acclimation. Despite the lack of a statistical difference,  
7 these data indicate heat acclimation improves endurance running performance further than  
8 precooling.

### 9 **Combined heat acclimation and precooling**

10 In spite of the theoretical potential to improve running performance further by adding  
11 precooling, we did not observe a performance improvement in HA+PC above that of HA. The only  
12 prior study investigating HA+PC on endurance exercise, highlighted a potentially meaningful greater  
13 self-selected exercise intensity during the first half of the cycling time trial, alongside reduced  
14 thermal sensation<sup>17</sup>. However, the faster pace was not sustained, reducing alongside the dissipation  
15 of PC effects, with a comparable trend in the precooled trials completed prior to the heat  
16 acclimatisation camp. Our data, implementing a more potent cooling strategy in HA+PC, afforded  
17 greater differences in  $T_{CORE}$ ,  $T_{SKIN}$ , core:skin gradient and thermal sensation during the first half of the  
18 trial (Figure 3), but did not alter the initial pace. The reasons for this are unclear, but speculatively,  
19 may represent a different, and ultimately sub-optimal, pacing strategy being adopted in HA+PC.

20 Recent evidence indicates athletes target a more even pacing profile with familiarisation in the  
21 heat<sup>39</sup> and as shown in Figure 2, HA+PC displays the most even profile. It appears this was sub-  
22 optimal following HA+PC, given the transient benefit that precooling affords, an interpretation  
23 supported by a slightly lower %HRmax during HA+PC until 4 km into the trial (Figure 4). This indicates  
24 participants exercised at a lower relative intensity in HA+PC, despite relative intensity normally being  
25 maintained across individuals for a given event<sup>40</sup>. Therefore, both the mediated physiological and

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1 thermoregulatory strain afforded by HA+PC during the first half of the trial may not have been  
2 exploited, as individuals targeted an even pace. These observations are supported by participant  
3 feedback, indicating pacing may have been incorrect, either under/overestimating the effect of  
4 HA+PC, resulting in them beginning at too high or too low a pace. We therefore recommend further  
5 familiarisation is necessary when combining interventions. Future research should consider whether  
6 familiarisation to exercise in the heat is influenced by racing experience or performance standard  
7 and thus specific to the population used in a study.

8 Previous studies have suggested cardiovascular and thermoregulatory adaptations from HA may  
9 reduce the ergogenic effects of PC by influencing the same mechanisms, such as the enlarged  
10 core:skin gradient and reduced cardiovascular strain, creating an insensitivity or 'ceiling effect'<sup>14,17</sup>.  
11 However, when an aggressive precooling technique is adopted and heat strain remains severe, these  
12 data would contend otherwise, evidencing small beneficial changes, notably in %HRmax and T<sub>SKIN</sub>.  
13 Therefore, further familiarisation with HA+PC appears necessary to ensure pacing is optimised and  
14 future research should investigate this across a range of standards of athletes, including 5 km  
15 distance specialists.

16

## 17 **Comparison of heat acclimation and precooling**

18 A secondary aim was to directly compare the effect of acute and chronic interventions on  
19 endurance running performance. Participants ran 43 s (3%) faster following HA than PC, which  
20 exceeds our typical error, established during pilot testing, of 16 s (1.2%). In-turn, PC afforded a 55 s  
21 (3.7%) improvement over CON, with eight participants improving more than our typical error. In HA,  
22 compared with CON, six participants improved more than the typical error, with a mean  
23 improvement of 98 s (6.6%), which was the only statistically significant difference. That no other  
24 comparisons were statistically different likely reflects a disparity in running performance within this  
25 cohort, as well as the adoption of a more conservative non-parametric statistical test, with both the

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1 mean differences and effect sizes (Table 1) indicative of meaningful changes between conditions.  
2 Indeed, six participants ran faster in HA than PC, with five improving more than the typical error.  
3 Elapsed time was similar between HA and PC at 2 km (PC; 547 [46] s, HA; 538 [45] s), before PC  
4 demonstrated a greater reduction in running speed between 2-4 km (elapsed time at 4 km PC; 1135  
5 [111] s, HA; 1108 [106] s). As shown in Figure 3, this reduction in running speed during PC coincides  
6 with the dissipation of a lower  $T_{SKIN}$  and core:skin gradient, relative to HA. It is possible the trial order  
7 may have contributed to the flatter pacing profile in HA, as PC was not randomised with HA, and  
8 both repeated trials <sup>41</sup> and familiarisation to the heat <sup>39</sup> may result in a flatter pacing profile.  
9 Therefore, it is more likely that the greater reduction in running speed in PC reflects greater heat  
10 strain, given the aforementioned dissipation of both a reduced  $T_{SKIN}$  and core:skin ratio.  
11 Concomitantly, this may result in a greater progressive reduction in  $\dot{V}O_{2max}$ , necessitating a reduced  
12 running speed to maintain relative intensity during PC.

13 The reduction in maximum aerobic capacity has been suggested to be the most plausible  
14 explanation for the decline in endurance performance under heat stress <sup>42</sup>, whilst the relative  
15 intensity that an event is completed at has been shown to be maintained across both hot, cold and  
16 hypoxic conditions <sup>41,43</sup>. Given the transient nature of the intervention, precooling does not provide  
17 prolonged, uniform alleviation of cardiovascular and thermoregulatory strain, as shown by the  
18 ineffectiveness of precooling on  $\dot{V}O_{2max}$  after approximately 30 min of exercise <sup>8</sup>. Conversely,  
19 meaningful improvements in  $\dot{V}O_{2max}$  in the heat were observed following HA, which may persist for  
20 5-14 days, depending on the HA protocol adopted, in accordance with HA decay <sup>44,45</sup> and would  
21 facilitate a greater maintained running speed, despite the inevitable progressive decline in  $\dot{V}O_{2max}$ .  
22 Enhanced  $\dot{V}O_{2max}$  (~7%) following HA is thought to arise primarily through an expanded plasma  
23 volume <sup>6</sup>, whilst endurance performance may also benefit from a slowed progressive decline in  
24  $\dot{V}O_{2max}$  during exercise, due to increased heat dissipation. A lower  $T_{SKIN}$  better maintains the core:skin  
25 gradient, thereby mediating cutaneous blood flow demands and preserving stroke volume and  
26  $\dot{V}O_{2max}$  <sup>46</sup>, as well as delaying exercise termination under heat stress <sup>47</sup>.

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1        Alongside improved maintenance of a runner's aerobic capacity in the heat, HA reduced the  
2        change in perceived thermal strain during the GXT and afforded reduced  $T_{SKIN}$  relative to PC during  
3        the second half of the TT (Figure 3), which is pertinent given elevated  $T_{SKIN}$  and thermal discomfort  
4        are associated with the voluntary reduction of exercise intensity in the heat <sup>13,48</sup>. Although, Ely et al.  
5        <sup>42</sup> suggest these effects may be subsidiary to the decrement in  $\dot{V}O_{2max}$  and subsequent increase in  
6        relative intensity at a fixed running speed, given the magnitude of  $\dot{V}O_{2max}$  impairment.

7        Relative intensity and perceived thermal strain alone cannot fully explain the differences  
8        between HA and PC, given the different pace after one kilometre. Speculatively, this could reflect a  
9        lower training status of the current cohort of runners, who began trials with a predetermined even-  
10       paced strategy, in comparison to the highly experienced cyclists in the study of Racinais et al. <sup>41</sup> who  
11       maintained a fixed relative intensity ( $\% \dot{V}O_{2max}$ ) from the start of the trial. Alternatively, naivety of the  
12       optimal pacing following precooling would also seem plausible. In HA+PC, the marked reduction in  
13        $T_{SKIN}$  that persists through the first half of the trial, differs from the afferent feedback participants are  
14       accustomed to, that determines self-selected running speed in the heat <sup>13</sup>. Indeed, anecdotally,  
15       participants highlighted ambiguity about how to maximise performance in PC, reinforcing the notion  
16       that pacing must be practiced, through repeated familiarisation, in advance of adopting PC in  
17       competition. Therefore, these data would appear to be the first to demonstrate a marked advantage  
18       from STHA over acute precooling in club runners, running in the heat.

19       Despite the sub-elite training status of this cohort, our design controls for an order effect  
20       because the number and scheduling of experimental trials was in keeping with their weekly training  
21       load, meaning familiarisations and pre/post trials are unlikely to have elicited changes in training  
22       status. Furthermore, participants also completed cycling training, rather than running, which helps  
23       to control for any mode-specific adaptations biasing our conclusions. Finally, whilst daily HA provides  
24       an increased training volume, this is an inherent part of this intervention. Passive heat acclimation  
25       protocols were not selected because we wanted to compare what we feel to be an optimal

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1 approach i.e. higher intensity exercise & a controlled hyperthermia model, as supported by recent  
2 literature<sup>23,24,27,49,50</sup>.

3 Despite the reduced performance compared with HA, these data reaffirm the potential for  
4 mixed methods, precooling to benefit endurance performance in the heat when HA is not possible.  
5 Whilst the use of external precooling for endurance performance is well supported<sup>10,11</sup>, this has not  
6 previously been assessed during free-paced endurance exercise. As per previous research that have  
7 used this technique<sup>8,9,12</sup>, PC did not elicit a reduction in  $T_{CORE}$  during the cooling phase. Similarly, an  
8 'after-drop' was not observed, whereby vasoconstriction dissipates and warm blood is subsequently  
9 cooled in the periphery<sup>51</sup>, which is likely a result of the significant and immediate metabolic heat  
10 production during treadmill running. A reduced rate of  $T_{CORE}$  increase may be inferred, given similar  
11 response to CON, but at higher running speeds.

12 It should be acknowledged that the lack of air-flow, as might be experienced outdoors, may  
13 over-estimate the magnitude of the reported PC effect<sup>52</sup>, although the influence will be less severe  
14 than in cycling due to the reduced air velocity during running. Another potential limitation is the  
15 failure to counterbalance the order of the pre and post training time trials, therefore the magnitude  
16 of improvement may be exaggerated. However, when compared against the typical error of 16 s  
17 (1.2%), the reported improvements all appear to represent true differences.

## 18 **Practical applications**

19 These results suggest athletes and coaches should prioritise a HA strategy where possible prior  
20 to endurance exercise in the heat. When this is not possible, a mixed methods precooling strategy,  
21 that cools a large surface area of skin, would appear to remain beneficial, although time should be  
22 taken to familiarise with pacing strategies. Combining HA and PC appears to elicit a better  
23 maintenance of the core:skin gradient, but this did not transfer into improved 5km time trial

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- 1 performance. Therefore, researchers, practitioners and coaches should consider familiarising
- 2 individuals with HA+PC to ensure pacing strategies maximise the alleviation of physiological strain.

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