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

ABSTRACT

Following heat acclimation (HA), endurance running performance remains impaired in hot vs temperate conditions. Combining HA with precooling demonstrates no additive benefit in intermittent sprint, or continuous cycling exercise protocols, during which heat strain may be less severe compared to endurance running. This study investigated the effect of short-term heat acclimation (STHA) combined with mixed-methods precooling, on endurance running performance and directly compared precooling and HA. Nine amateur trained runners completed 5 km treadmill time trials in the heat (32°C, 60% RH) under four conditions; no intervention (CON), precooling (PC), short-term heat acclimation (5 days - HA) and short-term heat acclimation with precooling (HA+PC). Mean (±SD) performance times were; CON 1476 (173) s, PC 1421 (146) s, HA 1378 (116) s and HA+PC 1373 (121) s. This equated to the following improvements versus CON; PC -3.7%, HA -6.6% and HA+PC -7.0%. Statistical differences were only observed between HA and CON ($p=0.004, d=0.68, 95\% CI [-0.27, 1.63]$) however, similar effect sizes were observed for HA+PC vs CON ($d=0.70, 95\% CI [-0.25, 1.65]$), with smaller effects between PC vs CON ($d=0.34, 95\% CI [-0.59, 1.27]$), HA vs PC ($d=0.33, 95\% CI [-0.60, 1.26]$) and HA+PC vs PC ($d=0.36, 95\% CI [-0.57, 1.29]$). Pilot testing revealed a time trial typical error of 16 s (1.2%). Precooling offered no further benefit to performance in the acclimated individual, despite modest alleviation of physiological strain. Maintenance of running speed in HA+PC, despite reduced physiological strain, may indicate an inappropriate pacing strategy therefore, further familiarisation is recommended to optimise a combined strategy. Finally, these data indicate HA, achieved through cycle training, yields a larger ergogenic effect than precooling on 5km running performance in the heat, although precooling remains beneficial when HA is not possible.
Combining heat acclimation and precooling for running in the heat.

Key words
Hyperthermia; endurance; lactate threshold; \( \dot{VO}_{2\text{max}} \); heat stress, thermoregulation.

INTRODUCTION

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

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

Acute, precooling techniques may be classified as internal (e.g. ice slurry ingestion) or external (e.g. ice vests, ice packs), depending upon how the cooling impulse is delivered. External precooling demonstrates larger effects on \(T_{\text{SKIN}}\) and thermal sensation than internal precooling\(^8\). External precooling presents a dose-dependent response, with a mixed-methods approach, involving multiple cooling garments and hand/forearm cold water immersion appearing preferable to singular cooling...
Combining heat acclimation and precooling for running in the heat.

garments, due to the greater cooled skin surface area\textsuperscript{9}. Accordingly, recent meta-analyses report large
effects on subsequent endurance performance when multiple cooling garments are worn either
alongside or following part-body cold water immersion (+7.3\%,  d =0.72 \textsuperscript{10},  d = 1.91 \textsuperscript{11}). Of note, the
practical mixed methods technique of Duffield et al. \textsuperscript{12}, involving ice towels, ice packs, ice vest and
hand immersion in cold water, ameliorates physiological and thermoregulatory strain during fixed
intensity endurance exercise in the heat \textsuperscript{8}, but has yet to be evaluated during free-paced exercise,
where the influence of alterations in T\textsubscript{SKIN} and thermal perception may be most pronounced \textsuperscript{13}.

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

Recently Schmit et al.\textsuperscript{17} investigated national-level triathletes wearing ice vests at rest and during
the warm-up prior to a 20 km cycling time trial, following 10-days of acclimatisation. Although the
addition of precooling did not improve performance above acclimatisation alone, transient, beneficial
pacing alterations were observed during the first half of the trial, alongside improved perceived
thermal strain, following precooling. Therefore, a more potent precooling strategy e.g. mixed
methods\textsuperscript{12}, may magnify or prolong this transient benefit. This transient benefit may also be more
impactful in a shorter event than the ~32 min trial of Schmit et al.\textsuperscript{17}, as the effects of precooling will
Combining heat acclimation and precooling for running in the heat.
be experienced for a greater proportion of the event duration before dissipating. A further
consideration is the type of exercise undertaken, with exercise such as running, that yields a significant
metabolic heat production (MHP) appearing best suited to combining interventions, given heat strain
can be mitigated by STHA alone during intermittent sprinting whilst cycling\textsuperscript{15}. Running elicits a greater
MHP than cycling and provides less convective cooling\textsuperscript{18,19}, which collectively expedites heat strain,
relative to cycling\textsuperscript{20}. Therefore, when STHA is adopted, affording partial heat adaptation, an additive
effect from precooling may be observed when heat strain remains high during exercise such as
endurance running. However, no investigations have combined precooling and HA prior to endurance
running.

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

**METHODS**

**Experimental approach to the problem**

A repeated measures design was adopted, with individuals completing two 5 km treadmill time
trials and a graded exercise test (GXT) before and after 5 days of STHA, as shown in Figure 1. Each GXT
was ordered immediately pre and post HA training, whilst time trials (TT) with either precooling (PC)
or a no intervention control trial (CON) were completed in a counterbalanced order, prior to ST HA.
Experimental trials occurred at least 10 days after instrumented familiarisations of the GXT and TT in
the heat. Following STHA, a TT was completed without precooling (HA) and another with precooling
(HA+PC), again counterbalanced (Figure 1). Trials occurred at a similar time of the day to minimise
fluctuation in thermoregulatory responses from circadian variation\textsuperscript{21}. 
Combining heat acclimation and precooling for running in the heat.

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

Subjects

Nine amateur, club runners (8 male, 1 female), who had trained at least three times per week for the previous 2 months, volunteered for this study (mean [±SD]: age 32 [16] years, stature 175 [7] cm, mass 71.9 [8.8] kg, sum of four skinfolds 25.4 [3.8] mm, VO$_2$max 59.1 [6.9] mL.kg$^{-1}$.min$^{-1}$ recent 5km time: 20:44 [1:44] min). Participants were recruited as part of a larger study on heat acclimation. All participants had completed a sub-22 min 5km or sub 45 min 10km race in the previous 2 months and had never previously undertaken HA. The female participant completed pre-tests and training during the follicular phase of the menstrual cycle, with post-tests during the first 5 days of luteal phase.

Participants were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study. Ethical approval was issued in accordance with the Declaration of Helsinki (2013). Participants arrived hydrated, having refrained from intense exercise for 48 hours, and avoided alcohol and caffeine for 24 hours. Participants completed a 24-hour food diary prior to each test and indicated sleeping hours, motivation, muscle soreness and stress on 5-point Likert scales upon arrival.
Combining heat acclimation and precooling for running in the heat.

Procedures

Precooling

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

Heat acclimation

Heat acclimation involved five, 90 min daily training sessions in the heat (~37°C, ~60% relative humidity [RH]) using controlled hyperthermia and permissive dehydration23,24. Participants exercised on cycle ergometers (Monark, e724, Sweden) at an intensity initially prescribed relative to body mass, at 2.7 W.kg⁻¹ 25 and subsequently adjusted to maintain the maximum tolerable power to achieve the target T\textsubscript{CORE} (38.5°C) within 30 min. Upon T\textsubscript{CORE} reaching 38.5°C, exercise was completed intermittently to maintain T\textsubscript{CORE} above 38.5°C for 60 min 23,24. Throughout the training session, exercise intensity was adjusted in 5 min blocks. Therefore, the typical work pattern was 30 min of continuous cycling, before a further 5 min of exercise every 25 min. The initial prescription of exercise based on power output, relative to body mass, as opposed to %\textsubscript{VO2}\textsubscript{max} 14,26, removes the necessity for an initial cycling \textsubscript{VO2}\textsubscript{max} test and maintains the relative exercise intensity across training days, independent of adaptation. Furthermore, cycling training controlled for performance that could arise from increased training volume were participants to acclimate through running. Training occurred at the same time of day, predominantly in the morning (07:00-10:00 h) and one participant in the evening (18:00-20:00 h). No fluid intake was permitted during training 27.
Combining heat acclimation and precooling for running in the heat.

**Exercise trials**

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

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

Prior to completing any experimental TT, participants underwent a familiarisation trial under the same circumstances. During the familiarisation, starting speed was determined based upon recent 5 km performance. For each experimental TT, following cooling and/or rest phases, participants completed a self-selected 5 min warm-up, replicated across all trials, on a motorised treadmill (Woodway ELG2, Germany). Standardised instructions were given at the start and nothing thereafter; ‘give your all’, ‘pace yourself throughout the trial’ and ‘adjust speed as you see fit’ as per similar studies. Participants straddled the treadmill belt, increased to the individual’s average pace from the familiarisation, to maintain a consistent blinded starting speed. Treadmill speed adjustment was permitted ad libitum (increment 0.2 km.h⁻¹), with the distance completed continuously displayed. Participants were blinded to all other feedback. Elapsed time was recorded every km.
Combining heat acclimation and precooling for running in the heat.

**Physiological measures**

During all trials, hydration was assessed upon arrival, whereby euhydration represented urine osmolality and specific gravity below 700 mOsmol.kg$^{-1}$ H$_2$O and 1.020, respectively. Pre and post nude body mass were recorded to estimate sweat loss (GFK150 scales, AE Adam, UK). A single-use rectal probe (Henleys Medical, UK) connected to a meter logger (Model 401, Yellow Springs Instruments, USA), was inserted 10 cm beyond the anal sphincter for $T_{CORE}$ measurement. Telemetry thermistors (U-Type connected to Gen II GD38 transmitter, Eltek, UK) were attached to the mid-belly of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius. Local skin temperatures were derived through a datalogger (RX250AL 1000 series Wireless Squirrel Logger, Eltek) as per James et al. in order to determine mean $T_{SKIN}$. Heart rate was monitored continuously using a Polar 810i heart rate monitor (Kempele, Finland).

During the GXTs, HR, $T_{CORE}$, $T_{SKIN}$, rating of perceived exertion (RPE) and thermal sensation (TS, 0=unbearably cold to 8=unbearably hot) were noted at the end of each stage. The following physiological responses were calculated; running speeds at blood lactate concentrations of 2 and 4 mmol.l$^{-1}$, running economy (RE), $\dot{VO}_{2\text{max}}$ and velocity at $\dot{VO}_{2\text{max}}$ ($\nu\dot{VO}_{2\text{max}}$) as per James et al. Derivative calculations included mean $T_{SKIN}$, Physiological Strain Index (PSI) and change in plasma volume. During the 5 km time trial, HR, $T_{CORE}$, $T_{SKIN}$, RPE and TS were recorded every km.

**Statistical analyses**

All outcome variables were assessed for normality and sphericity prior to further analysis. Where assumptions of ANOVA were not met, non-parametric statistics were adopted. Exercise data from both the GXTs and TTs were analysed using two-way, repeated measures ANOVA (Trial*Time) where data comprised repeated time points, with post hoc Bonferroni adjusted pairwise comparisons used where significant main or interaction effects occurred. During the time trials, all average, finishing and delta change data from physiological and performance variables were analysed with One-way repeated measures ANOVA. Where the use of repeated measures ANOVA was precluded through the
Combining heat acclimation and precooling for running in the heat.

violation of parametric assumptions, such as TT performance, Friedman’s ANOVA, with Wilcoxon follow up tests, were used to analyse these data. Singular data, that did not have repeated measures within the GXT, were analysed using Paired samples t-tests. Effect sizes for main effects and interaction effects are presented as partial eta squared (partial $\eta^2$), differences between related samples were evaluated through Cohen’s $d_{av}$ ($d_{av}$). Data were analysed using SPSS (Version 21, SPSS Inc., USA) with statistical significance set at $p<0.05$ and data presented as means and standard deviation (±SD).

RESULTS

Heat acclimation training

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

Graded exercise tests

Statistically significant reductions in resting (-0.15°C, $p=0.01$, $d_{av}=0.45$) and exercising TCORE (-0.21°C, $p=0.04$, $d_{av}=0.54$) were observed, alongside a reduced exercising (-3 b.min$^{-1}$, $p=0.02$, $d_{av}=0.26$), but not resting HR (-2 b.min$^{-1}$, $p=0.115$, $d_{av}=0.36$). Total sweat loss did not change following STHA (Pre 1.35 [0.3] L, Post 1.39 [0.39] L, $p=0.503$, $d_{av}=0.13$), but occurred alongside a smaller change in TCORE ($\Delta$ Pre 1.26 [0.27] °C, $\Delta$ Post 1.00 [0.28] °C, $p=0.006$, $d_{av}=0.91$) indicating increased sudomotor sensitivity.

A 5.7% increase ($p=0.03$, $d_{av}=1.06$) in blood plasma volume was also observed. No changes were observed in mean RPE ($p=0.342$) or TS ($p=0.262$), although there was a reduced change in thermal sensation ($p=0.04$, $d_{av}=0.86$). For complete STHA results please see James et al. 22.
Combining heat acclimation and precooling for running in the heat.

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

**Time trial performances**

Environmental conditions (WBGT) did not differ between trials; CON=27.4 (0.7)°C, PC=26.9 (0.5)°C, HA=27.5 (0.9)°C, HA+PC=27.0 (0.8)°C ($p=0.246$, partial $\eta^2=0.156$). Self-reported motivation, muscle soreness and stress responses did not differ between trials for the female participant or for the group as a whole ($p>0.05$). Friedman’s ANOVA revealed a difference in TT performance between trials ($p=0.001$). However, Wilcoxon tests with Bonferroni correction (whereby significance = $p<0.008$), only indicated a significant difference between the control trial and HA+PC. Group mean (±SD) performance times were; CON 1476 (173)s, PC 1421 (146)s, HA 1378 (116)s and HA+PC 1373 (121)s. Relative to control, a large mean improvement was observed in HA and HA+PC, with a modest improvement following PC (Table 1), although neither reached statistical significance. Compared with CON, nine participants ran faster in HA, whilst eight ran faster in HA+PC and PC trials. There was no observable difference in running performance between HA and HA+PC trial, whilst the observed effect sizes and mean difference indicate modest improvements in HA and HA+PC compared with PC (Table 1). In HA+PC, eight participants ran faster than in PC, with six participants performing better in HA than PC. Figure 2 displays the kilometre splits for each trial.
Table 1: Relative difference in 5 km time trial performance between trials. *Corrected statistical significance level for Wilcoxon signed-rank test post hoc $p<0.008$. Previously established typical error following 5 days high intensity normothermic training = 16 s, 1.2%. Data are: mean change (s), percentage change (%), statistical significance ($p$) and effect size ($d$) with 95% confidence intervals (CI). +/- represents the performance difference (s) relative to the trial in that row.

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<tr>
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<th>Time trial time</th>
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<th>Precooling</th>
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<td><strong>Control</strong></td>
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<td><strong>Precooling</strong></td>
<td>1421 (146) s</td>
<td>-55 s (3.7%)</td>
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<td><strong>Heat acclimation</strong></td>
<td>1378 (116) s</td>
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<td><strong>Heat acclimation + precooling</strong></td>
<td>1373 (121) s</td>
<td>-103 s (7.0%)</td>
<td>-48 s (3.4%)</td>
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Figure 2: Mean (±SD) kilometre split times during the 5 km time trial. Error bars represent one standard deviation. Error bars for control (CON) and heat acclimation + precooling (HA+PC) trials are omitted for clarity, but homogeneity of variance was present.

Following 20 min cooling there was no change in $T_{\text{CORE}}$ ($p=0.219$, partial $\eta^2=0.165$). Therefore, starting $T_{\text{CORE}}$ was not different between trials ($p=0.697$, partial $\eta^2=0.075$); CON 37.12 (0.22)$^\circ$C, PC 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 $T_{\text{SKIN}}$ ($p<0.001$, partial $\eta^2=0.906$) were observed across the cooling period in the trials containing precooling (-6.9 [2.7]$^\circ$C PC; -6.8 [1.5]$^\circ$C HA+PC), whilst $T_{\text{SKIN}}$ was unchanged in non-precooling trials (CON +0.87 [0.50]$^\circ$C; HA +0.58 [0.58]$^\circ$C). Therefore, starting $T_{\text{SKIN}}$ in PC (26.9 [2.8]$^\circ$C) and HA+PC (26.4 [1.9]$^\circ$C) 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 HA). This coincided with a reduced starting thermal sensation ($p=0.002$, partial $\eta^2=0.907$) in PC (2.2 [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 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 [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 during all trials are shown in Figure 3.
Combining heat acclimation and precooling for running in the heat.

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

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

The mean core:skin gradient was also different between conditions ($p=0.005$, partial $\eta^2=0.504$), as shown in Figure 3. The largest gradient was observed in HA+PC (4.2 [1.2]$^\circ$C), followed by HA (3.7 [0.8]$^\circ$C), PC (3.6 [1.0]$^\circ$C) and CON (2.9 [0.9]$^\circ$C, with statistical differences between CON and HA+PC ($p=0.034$, $d=1.24$). There were different finishing core:skin gradients ($p=0.028$, partial $\eta^2=0.388$), with the largest observed in HA (4.3 [1.1]$^\circ$C) and HA+PC (4.2 [1.2]$^\circ$C), followed by PC (3.7 [1.0]$^\circ$C) and CON (3.4 [1.0]$^\circ$C).
Figure 3: Clockwise from top left: Mean (±SD) core temperature (A), skin temperature (B), thermal sensation (C) and core:skin gradient (D) during rest, 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 cooling finished. Error bars represent one standard deviation, with core temperature error bars omitted for clarity. Participants completed additional rest in CON and HA trials during the ‘cooling’ phase.
No differences were observed in the mean TS ($p=0.066$, partial $\eta^2=0.255$) or RPE ($p=0.213$, partial $\eta^2=0.168$) between conditions. Neither mean HR ($p=0.252$, partial $\eta^2=0.154$) or finishing HR ($p=0.734$, partial $\eta^2=0.051$), differed between conditions. Similarly, mean HR as a percentage of maximum HR (%HRmax) was not different between conditions ($p=0.089$, partial $\eta^2=0.234$), as shown 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)% and HA+PC 91.6 (3.1)%. Sweat loss was different between trials ($p=0.008$, partial $\eta^2=0.386$), with the largest fluid loss in HA (2.5 [0.5] l.hr$^{-1}$), compared with CON (2.2 [0.8] l.hr$^{-1}$), PC (1.7 [0.5] l.hr$^{-1}$) and HA+PC (2.3 [0.6] l.hr$^{-1}$). Pairwise comparisons revealed a difference between PC and HA ($p=0.006$, $d=1.50$), but not other conditions.

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

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

Combined heat acclimation and precooling

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

Recent evidence indicates athletes target a more even pacing profile with familiarisation in the heat and as shown in Figure 2, HA+PC displays the most even profile. It appears this was sub-optimal following HA+PC, given the transient benefit that precooling affords, an interpretation supported by a slightly lower %HRmax during HA+PC until 4 km into the trial (Figure 4). This indicates participants exercised at a lower relative intensity in HA+PC, despite relative intensity normally being maintained across individuals for a given event. Therefore, both the mediated physiological and thermoregulatory strain afforded by HA+PC during the first half of the trial may not have been
Combining heat acclimation and precooling for running in the heat.

exploited, as individuals targeted an even pace. These observations are supported by participant feedback, indicating pacing may have been incorrect, either under/overestimating the effect of HA+PC, resulting in them beginning at too high or too low a pace. We therefore recommend further familiarisation is necessary when combining interventions. Future research should consider whether familiarisation to exercise in the heat is influenced by racing experience or performance standard and thus specific to the population used in a study.

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

Comparison of heat acclimation and precooling

A secondary aim was to directly compare the effect of acute and chronic interventions on endurance running performance. Participants ran 43 s (3%) faster following HA than PC, which exceeds our typical error, established during pilot testing, of 16 s (1.2%). In-turn, PC afforded a 55 s (3.7%) improvement over CON, with eight participants improving more than our typical error. In HA, compared with CON, six participants improved more than the typical error, with a mean improvement of 98 s (6.6%), which was the only statistically significant difference. That no other comparisons were statistically different likely reflects a disparity in running performance within this cohort, as well as the adoption of a more conservative non-parametric statistical test, with both the mean differences and effect sizes (Table 1) indicative of meaningful changes between conditions. Indeed, six participants
Combining heat acclimation and precooling for running in the heat.

ran faster in HA than PC, with five improving more than the typical error. Elapsed time was similar between HA and PC at 2 km (PC; 547 [46] s, HA; 538 [45] s), before PC demonstrated a greater reduction in running speed between 2-4 km (elapsed time at 4 km PC; 1135 [111] s, HA; 1108 [106] s).

As shown in Figure 3, this reduction in running speed during PC coincides with the dissipation of a lower TSKIN and core:skin gradient, relative to HA. It is possible the trial order may have contributed to the flatter pacing profile in HA, as PC was not randomised with HA, and both repeated trials 41 and familiarisation to the heat 39 may result in a flatter pacing profile. Therefore, it is more likely that the greater reduction in running speed in PC reflects greater heat strain, given the aforementioned dissipation of both a reduced TSKIN and core:skin ratio. Concomitantly, this may result in a greater progressive reduction in VO2max, necessitating a reduced running speed to maintain relative intensity during PC.

The reduction in maximum aerobic capacity has been suggested to be the most plausible explanation for the decline in endurance performance under heat stress 42, whilst the relative intensity that an event is completed at has been shown to be maintained across both hot, cold and hypoxic conditions 41,43. Given the transient nature of the intervention, precooling does not provide prolonged, uniform alleviation of cardiovascular and thermoregulatory strain, as shown by the ineffectiveness of precooling on VO2max after approximately 30 min of exercise 6. Conversely, meaningful improvements in VO2max in the heat were observed following HA, which may present for 5-14 days, depending on the HA protocol adopted, in accordance with HA decay 44,45 and would facilitate a greater maintained running speed, despite the inevitable progressive decline in VO2max. Enhanced VO2max (~7%) following HA is thought to arise primarily through an expanded plasma volume 6, whilst endurance performance may also benefit from a slowed progressive decline in VO2max during exercise, due to increased heat dissipation. A lower TSKIN better maintains the core:skin gradient, thereby mediating cutaneous blood flow demands and preserving stroke volume and VO2max 46, as well as delaying exercise termination under heat stress 47.
Combining heat acclimation and precooling for running in the heat.

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

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

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

i.e. higher intensity exercise & a controlled hyperthermia model, as supported by recent literature 23,24,27,49,50.

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

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

**Practical applications**

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

1 researchers, practitioners and coaches should consider familiarising individuals with HA+PC to ensure
2 pacing strategies maximise the alleviation of physiological strain.
Combining heat acclimation and precooling for running in the heat.

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