



Effect of regular precooling on adaptation to training in the heat

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Abstract

Purpose This study investigated whether regular precooling would help to maintain day-to-day training intensity and improve 20-km cycling time trial (TT) performed in the heat. Twenty males cycled for 10 day × 60 min at perceived exertion equivalent to 15 in the heat (35 °C, 50% relative humidity), preceded by no cooling (CON, $n = 10$) or 30-min water immersion at 22 °C (PRECOOL, $n = 10$).

Methods 19 participants ($n = 9$ and 10 for CON and PRECOOL, respectively) completed heat stress tests (25-min at 60% $\dot{V}O_{2peak}$ and 20-km TT) before and after heat acclimation.

Results Changes in mean power output (Δ MPO, $P = 0.024$) and heart rate (Δ HR, $P = 0.029$) during heat acclimation were lower for CON (Δ MPO $-2.6 \pm 8.1\%$, Δ HR -7 ± 7 bpm), compared with PRECOOL (Δ MPO $+2.9 \pm 6.6\%$, Δ HR -1 ± 8 bpm). HR during constant-paced cycling was decreased from the pre-acclimation test in both groups ($P < 0.001$). Only PRECOOL demonstrated lower rectal temperature (T_{re}) during constant-paced cycling ($P = 0.002$) and lower T_{re} threshold for sweating ($P = 0.042$). However, skin perfusion and total sweat output did not change in either CON or PRECOOL (all $P > 0.05$). MPO ($P = 0.016$) and finish time ($P = 0.013$) for the 20-km TT were improved in PRECOOL but did not change in CON ($P = 0.052$ for MPO, $P = 0.140$ for finish time).

Conclusion Precooling maintains day-to-day training intensity and does not appear to attenuate adaptation to training in the heat.

Keywords Exercise performance · Heat acclimation · Heat adaptation · Cold water immersion

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Abbreviations

Δ	Delta change
CON	Control
CVC	Cutaneous vascular conductance
CWI	Cold water immersion
HR	Heart rate
LSR	Local sweat rate
MPO	Mean power output
PRECOOL	Precooling
PU	Perfusion units
RH	Relative humidity
RPE	Rating of perceived exertion
T_{arm}	Forearm skin temperature
T_{ca}	Calf skin temperature
T_{re}	Rectal temperature
T_{sk}	Weighted mean skin temperature
T_{st}	Sternal skin temperature
TT	Time trial
T_{th}	Thigh skin temperature
USG	Urine specific gravity

$\dot{V}O_{2\max}$	Maximal oxygen uptake
$\dot{V}O_{2\text{peak}}$	Peak oxygen uptake
WBS	Whole body sweat loss

Introduction

Heat acclimation (Castle et al. 2011; Schmit et al. 2017a) and precooling (Choo et al. 2019; Schmit et al. 2017b) are strategies commonly adopted to mitigate the adverse effect of heat stress on performance during exercise in the heat. The physiological adaptations to heat include increased sweat rate, enhanced cardiovascular capacity (e.g., lower heart rate and increased stroke volume) and lower core temperature (Castle et al. 2011; Fujii et al. 2012; Lorenzo and Minson 2010). These physiological adaptations are believed to alleviate cardiovascular strain and can improve exercise performance in the heat (Périard et al. 2016). A heat acclimation programme typically involves 10–14 day \times 90–120 min of low-intensity exercise ($\leq 50\% \dot{V}O_{2\max}$) (Pandolf et al. 1988; Poirier et al. 2015) or intermittent exercise to maintain a target core temperature, i.e., controlled-hyperthermia (Patterson et al. 2004). Such protocols require more time commitment, may compromise training intensity, and have less training specificity. Meeting specific energetic demands during heat acclimation may also be important for exercise performance gains (Wingfield et al. 2016); however, training at higher intensities in the heat may increase the likelihood of overreaching despite some heat adaptation (Reeve et al. 2019; Schmit et al. 2017a). Indeed, Schmit et al. (2017a) observed greater perceived fatigue and detrimental effects on 20-km time trial (TT) performance in triathletes who trained at high intensity in the heat for five consecutive days, compared with counterparts who trained at low intensity. Hence, there is a need to explore methods that can help to maintain the quality of training in the heat without risks of maladaptation.

Precooling aims to reduce body temperature prior to exercise and is another common adopted method to enhance exercise performance in the heat (Choo et al. 2017). The mechanisms and ergogenic effects of different precooling techniques have been reviewed elsewhere (Bongers et al. 2015; Choo et al. 2017). Although these reviews show that precooling can be beneficial for performance or work capacity during exercise in the heat, it is acknowledged that the efficacy of different cooling techniques can be influenced by factors including the environmental conditions and the nature of the exercise tasks. For instance, cold water immersion (CWI) has been shown to be less beneficial for intermittent sprint performance due to its drastic muscle cooling effect (Skein et al. 2012). Interestingly, regular CWI following high-intensity cycling in the heat for 3–5 consecutive

days has been shown to result in better maintenance of day-to-day sprint performance across the days (Stanley et al. 2013; Vaile et al. 2008). While these studies highlight the benefits of CWI on day-to-day training performance, they were not designed to examine the interaction between precooling and heat adaptation. To the best of our knowledge, no study has extensively examined the influence of regular precooling on day-to-day training performance and heat adaptation. Cooling prior to training in the heat may seem incongruous with the aim of heat acclimation, in which a certain level of thermal stimulus is essential for heat adaptation. Yet, it is plausible that precooling may help to maintain the quality of training in the heat and minimise the risks of maladaptation.

Previous studies have shown that active secretion is obligatory for the adaptation of sweat glands (Buono et al. 2009; Collins et al. 1966; Fox et al. 1964); hence, cooling techniques that suppress sweat secretion during heat exposure may be antagonistic to heat adaptation. Local sweating response has been shown to be mediated by skin temperature and skin blood flow independently (Wingo et al. 2010). Conversely, CWI has been shown to substantially reduce skin temperature and skin blood flow (Choo et al. 2016). It is hence plausible that precooling by CWI during heat acclimation may have an inhibitory effect on the adaptation of sweat glands. However, we have previously demonstrated that precooling by CWI (22 °C \times 30 min) increases total work output at a given perceived exertion (Choo et al. 2019). Furthermore, although we found that CWI delayed onset of sweating, local sweat rate increased rapidly during exercise in the heat leading to similar sweat rate between CWI and a non-cooling control condition at the end of the exercise (Choo et al. 2019).

Elevation and earlier onset of cutaneous vasodilation during exercise facilitate heat transfer to the skin and increases cutaneous water vapour pressure for evaporative cooling (Taylor and Cotter 2006). Interestingly, skin blood flow changes following heat acclimation appear to be unclear with some studies reporting unchanged (Nielsen et al. 1997; Regan et al. 1996) or decreased skin blood flow (Chen et al. 2013). On the other hand, enhanced skin vascular sensitivity to acetylcholine (Lorenzo and Minson 2010) and lower temperature threshold for cutaneous vasodilation during passive lower body heating (Amano et al. 2015) have been observed after 5–10 days of heat acclimation. As mentioned previously, CWI reduces skin blood flow; hence, whether precooling by CWI will inhibit heat acclimation in terms of sudo- and vaso-motor responses during exercise in the heat warrants investigation.

Accordingly, this study aimed to examine the effects of precooling by CWI (PRECOOL) on day-to-day training intensity during heat acclimation and changes in endurance performance (i.e., 20-km TT) in the heat. It was hypothesised

that PRECOOL would result in better maintenance of the training intensity and improve endurance performance to a greater extent, compared with a non-cooling control condition (CON). The secondary hypothesis was that if training intensity was better maintained during heat acclimation, regular precooling by CWI would minimise adverse effects on the sudo- and vaso-motor adaptations.

Methods

Participants

This study was approved by Edith Cowan University Human Research Ethics Committee and Institutional Review Board of the Singapore Sport Institute. Experimental procedures were communicated to the participants before obtaining signed consent and were conducted in accordance with the standards set by Declaration of Helsinki.

Sample size calculation (G*Power 3.1.9.2) was performed using the mean within-group change in power output ($28 \text{ W} \pm 19 \text{ W}$) from an 8-days heat acclimation study (Schmit et al. 2017b). For one-tailed t-test with $\beta=0.20$ and $\alpha=0.05$, a sample size of six participants was calculated to detect a meaningful change in MPO during a 20-km TT. To account for attrition and to ensure sufficient power for other variables (e.g., core body temperature), 22 recreational cyclists and triathletes who were residents of the study location (Singapore) at the time of the study were recruited. Participants were considered at least partially heat acclimated given their self-reported heat exposure were 3–5 h per week. The data were collected from August 2018 to March 2019 in Singapore. Two participants did not complete the acclimation programme due to personal reasons. Hence, 20 participants were matched for $\dot{V}O_{2\text{peak}}$, associated peak aerobic power, and study enrolment dates ($n=10$ and 10 for CON and PRECOOL, respectively). Only 19 participants ($n=9$ and 10 for CON and PRECOOL, respectively) completed the pre- and post-acclimation tests, as one participant withdrew from the study after completing the acclimation programme due to illness unrelated to this study. Physical characteristics and baseline performance of the cohort are presented in Table 1. Self-reported heat exposure was determined through a questionnaire and included activities such as sauna or hot water immersion (temperature $> 35 \text{ }^\circ\text{C}$) and any form of physical activities or work in hot environments (e.g., poorly ventilated room, heated room or under the hot sun).

Experimental design

During a preliminary session, participants completed an incremental test to determine $\dot{V}O_{2\text{peak}}$ (TrueOne 2400,

Table 1 Baseline physical characteristics of participants undergoing heat acclimation preceded by either no cooling (CON) or 30 min of cold water immersion (PRECOOL)

	CON	PRECOOL
Age (years)	40 ± 9	37 ± 8
Body mass (kg)	74 ± 10	71 ± 5
Height (cm)	174 ± 3	172 ± 4
Sum of seven skinfolds (mm)	104 ± 46	107 ± 25
Body surface area (m ²)	1.88 ± 0.11	1.83 ± 0.07
$\dot{V}O_{2\text{peak}}$ (mL kg ⁻¹ min ⁻¹)	47.1 ± 7.7	50.4 ± 9
Prorated peak power (W)	312 ± 40	314 ± 48
Relative power (W kg ⁻¹)	4.2 ± 0.6	4.5 ± 0.8
Training history (h week ⁻¹)	8.4 ± 4.6	11.6 ± 3.3
Heat exposure (h week ⁻¹)	2.8 ± 1.6	4.3 ± 2.8
Training load (AU)	2481 ± 1356	3074 ± 3756
Baseline 20-km TT MPO (W)	180 ± 42	177 ± 50
Baseline 20-km TT relative MPO (W kg ⁻¹)	2.4 ± 0.6	2.5 ± 0.8
Baseline 20-km TT finish time (min)	38.4 ± 4.4	38.9 ± 4.4
Baseline 20-km TT WBS (%)	2.3 ± 0.5	2.2 ± 0.4

$\dot{V}O_{2\text{peak}}$ peak oxygen uptake, TT time trial, MPO mean power output, WBS (%) whole body sweat loss expressed as percentage of pre-exercise body mass. Data are presented as mean ± SD within each group ($n=10$ for each group, except for the baseline 20-km TT data, where $n=9$ for CON). Significance level was accepted at $P \leq 0.05$

ParvoMedics, Utah, USA) and a 20-km TT as familiarisation on a Velotron cycle ergometer (Racermate, Seattle, WA, USA). The incremental test was commenced at 100 W for 4 min and increased by 25 W min⁻¹ until volitional exhaustion. Participants then completed two heat stress tests and 10 heat acclimation sessions. At ≥ 24 h following pre-acclimation tests, participants completed 10 acclimation sessions within 14 days. With the exception of one participant who performed the post-acclimation heat stress test 8 days after the last heat exposure due to personal commitment, the second heat stress test was performed ≥ 48 h (range 2–5 days) after the last acclimation session. During the 24 h period before each test, participants maintained similar diets facilitated by 1-day food records and refrained from strenuous exercise and alcohol. Participants were instructed to maintain their usual dietary habits throughout the study period.

Heat stress tests

During each test, nude body mass (Model Spider 2, Mettler-Toledo GmbH, Albstadt, Germany) and urine specific gravity ([USG], Atago Digital Refractometer PR-RI, Tokyo, Japan) were assessed before a 15-min seated rest in an environmental chamber ($35.3 \pm 0.2 \text{ }^\circ\text{C}$, $54 \pm 1\%$ relative humidity [RH]). Participants completed 25 min of constant-paced cycling at 60% $\dot{V}O_{2\text{peak}}$ followed by 20-km TT at

2.1 ± 0.3 min later. They were instructed to complete the TT as fast as possible and no feedback or verbal encouragement was provided except for the distance cycled. Airflow was provided at 28.8 km h^{-1} , placed 0.3 m away from the cycle ergometer. Water consumption ($22.0 \pm 1.1 \text{ }^\circ\text{C}$) was recorded for the purpose of calculating whole body sweat loss (WBS).

Temperature and sweat measurements

Rectal temperature (T_{re}) was monitored via a disposable thermistor (Monatherm Thermistor 400 Series, Mallinckrodt Medical, St. Louis, MO, USA) self-inserted 10 cm past the anal sphincter. Skin thermistors (YSI temperature probes 400 Series, Dayton, OH, USA) were attached to the mid-sternum (T_{st}), forearm (T_{arm}), thigh (T_{th}) and calf (T_{ca}) to calculate weighted mean skin temperature (T_{sk}) (Ramanaathan 1964). Local sweat rate ([LSR], $\text{mg cm}^{-2} \text{ min}^{-1}$) was assessed by attaching sweat capsules (5.31 cm^2) to the left forearm and the left thigh, 2 cm away from the skin thermistors. Influent anhydrous N_2 was supplied to the sweat capsules at 1.5 L min^{-1} (Kofloc RK1204, Kojima Instruments Inc., Tokyo, Japan), and water content of the effluent N_2 was measured (HMP60, Vaisala, Helsinki, Finland). Temperature and LSR data were recorded at 0.2 Hz (Squirrel 2020, Grant Instruments, Shepreth Cambridgeshire, UK). Sweating threshold and sensitivity were determined as previously described (Cheuvront et al. 2009). WBS was calculated from the change in nude body mass adjusted for volume of water ingested.

Skin perfusion, mean arterial blood pressure and HR

Skin perfusion (PU) was recorded at the left thigh (PeriFlux 5000, Perimed AB, Järfälla, Stockholm, Sweden) with the probe (Probe 457) placed 2 cm away from the skin thermistor. Mean arterial pressure (NOVA, Finapres Medical Systems©, Amsterdam, The Netherlands) was measured during constant-paced cycling only to minimise distraction to participants during the TT. Mean arterial pressure and PU were sampled at 10 Hz (Powerlab 16/30 ML 880/P, ADInstruments, New South Wales, Australia). Cutaneous vascular conductance (CVC) was calculated as PU/mean arterial pressure (PU-mmHg^{-1}). HR (S810i, Polar Electro Oy, Kempele, Finland), perceived exertion (RPE) (Borg 1982) and thermal sensation (Young et al. 1987) were recorded throughout the exercise.

Heat acclimation

Participants cycled at RPE 15 on Wattbike Pro cycle ergometers (Wattbike Ltd., Nottingham, UK) for 60 min in the heat ($35.3 \pm 0.3 \text{ }^\circ\text{C}$, $53 \pm 2\%$ RH). For PRECOOL, participants completed 30 min of mid-sternal CWI at

$21.9 \pm 0.5 \text{ }^\circ\text{C}$ (ambient conditions: $23.4 \pm 0.7 \text{ }^\circ\text{C}$, $78 \pm 7\%$ RH) and commenced exercise 12.3 ± 2.2 min later. HR was recorded continuously during the exercise and participants were reminded to maintain exercise intensity at RPE 15 every 10–15 min without feedback on HR and power output. Water consumption ($22.0 \pm 1.1 \text{ }^\circ\text{C}$) was recorded for the purpose of calculating WBS. The participants were instructed to record their training in addition to the heat acclimation sessions using the session-RPE method (Foster et al. 2001). Perceived well-being was recorded before each acclimation session (Ihsan et al. 2017). Specifically, participants were asked to rate their perceived general fatigue, sleep quality, general muscle soreness, stress levels and mood on a scale of one to five in increments of 0.5. Overall well-being was then determined by summing the scores from the five items. The aggregated score obtained prior to the first heat acclimation session was used as baseline and subsequent response was expressed as percentage change.

Statistical analysis

Data are expressed as mean \pm SD and statistical significance level was accepted at $P \leq 0.05$. Meaningfulness of the within-group difference in the 20-km TT performance was calculated using Hedges' g effect size and interpreted as $< 0.2 =$ negligible, $0.2\text{--}0.49 =$ small, $0.5\text{--}0.79 =$ medium, $\geq 0.8 =$ large (Cohen 1992). Within- and between-group difference in the physiological and performance data were analysed by linear mixed models fitted with restricted maximum likelihood (R v3.5.0, R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Initial models included a by-participant random intercept and time or/and group as fixed effects. Bonferroni adjustment for multiple comparisons was used for post-hoc analysis when appropriate.

Results

Participant characteristics and performance at baseline

A total of 20 participants ($n = 10$ and 10 for CON and PRECOOL, respectively) completed all 10 sessions within 14 days and were included for the analysis of the heat acclimation data. One participant from the CON group did not complete the post-acclimation heat stress test due to illness not related to this study. Therefore, 19 participants ($n = 9$ and 10 for CON and PRECOOL, respectively) were included for the analysis of the heat stress test data.

Heat acclimation

When MPO was expressed as percentage change from the first session (Δ MPO), there was a main group effect ($P=0.042$), and the overall Δ MPO for CON was 5.5% lower than PRECOOL (Fig. 1). However, there was no time ($P=0.361$) or group \times time interaction effect ($P=0.127$) for Δ MPO. Comparison of absolute MPO between the first and last sessions exhibited a group \times time interaction effect ($P=0.032$), resulting from a non-significant decrease in MPO in the CON group (session 1: 168 ± 35 W versus session 10: 157 ± 32 W, $P=0.062$), while absolute MPO was unchanged in the PRECOOL group (session 1: 166 ± 33 W versus session 10: 173 ± 41 W, $P=0.214$). However, absolute MPO was not different between the two groups during sessions 1 and 10 (both $P > 0.05$). Absolute MPO across the 10 sessions are presented as online-only supplementary material. On average, CON and PRECOOL cycled at power output equivalent to $61.7 \pm 6.1\%$ $\dot{V}O_{2peak}$ and $62.9 \pm 5.7\%$ $\dot{V}O_{2peak}$, respectively, during the ten heat acclimation sessions.

Group ($P=0.029$) and time ($P < 0.001$), but no interaction effects ($P=0.075$) were evident for HR expressed as absolute change from session 1 (Δ HR, Fig. 1). Overall Δ HR for CON and PRECOOL were -7 ± 7 bpm and -1 ± 8 bpm,

respectively. Δ HR during session 10 was decreased from session 1 ($P=0.008$). No group ($P=0.531$), time ($P=0.343$) or interaction effect ($P=0.249$) was observed for WBS expressed as percentage change from pre-exercise body mass (Fig. 1). No group ($P=0.372$), time ($P=0.638$) or interaction effect ($P=0.493$) was evident for well-being scores expressed as percentage change from session 1 (Fig. 1).

Heat stress tests

Hydration status, temperature, HR and perceptual measures

Participants were euhydrated (USG < 1.025) before exercise (Armstrong et al. 1994). For CON, there were no differences between pre- and post-acclimation tests for USG (Pre: 1.010 ± 0.006 g ml⁻¹, Post: 1.010 ± 0.007 g ml⁻¹, $P=0.896$) and body mass (Pre: 74.6 ± 10.4 kg, Post: 75.0 ± 10.6 kg, $P=0.138$). For PRECOOL, USG was lower at pre-acclimation tests when compared with post-acclimation tests (Pre: 1.008 ± 0.007 g ml⁻¹, Post: 1.017 ± 0.006 g ml⁻¹, $P=0.002$); however, there was no difference for body mass (Pre: 70.8 ± 5.4 kg, Post: 70.6 ± 4.8 kg, $P=0.484$).

Temperature, HR and perceptual responses during the heat stress tests are presented in Tables 2 and 3. There were

Fig. 1 Percentage change in mean power output (Δ MPO, a) and absolute change in heart rate (Δ HR, b), whole body sweat loss as percentage from pre-exercise body mass (WBS%, c) and well-being as percentage change from session 1 (Δ Well-being) during the 10 heat acclimation sessions for CON and PRECOOL. Data are mean \pm SD for all 20 participants, unless stated otherwise. †Significantly different from CON (main effect, $P \leq 0.05$, insets), *significantly different from session 1 (main effect, $P \leq 0.05$)

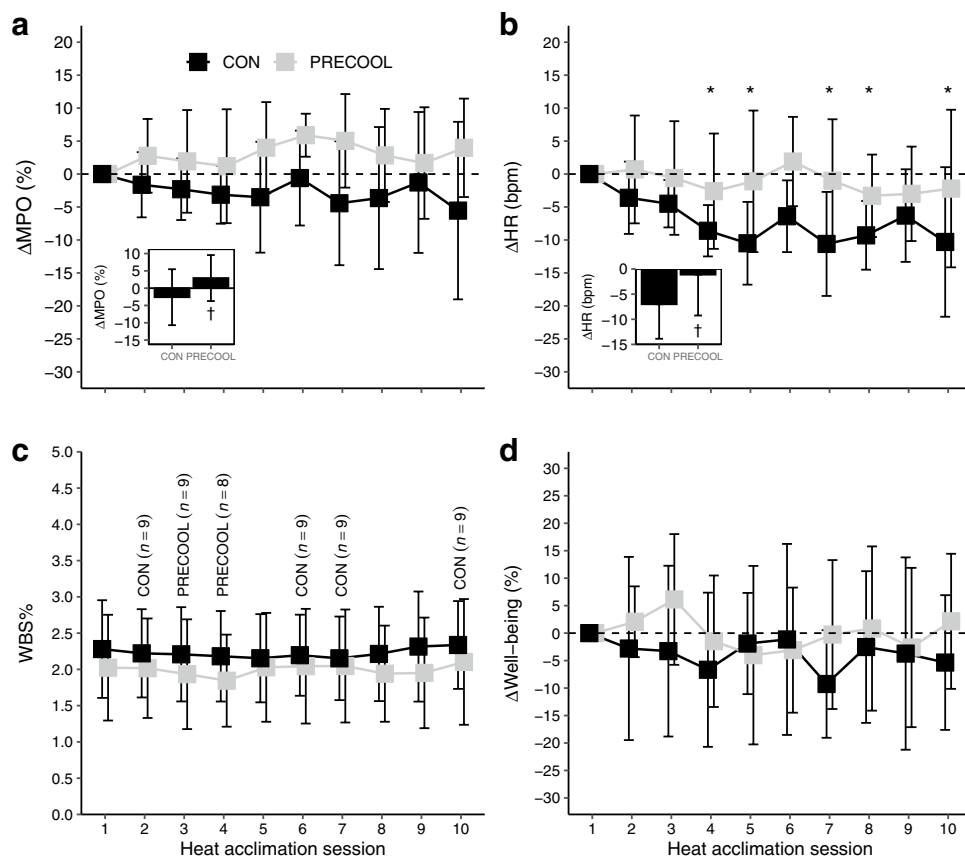


Table 2 Temperature, heart rate (HR) and perceptual responses during pre- and post-acclimation heat stress tests

	CON		P value	PRECOOL		P value
	Pre	Post		Pre	Post	
At rest						
T_{re} (°C)	37.3±0.2	37.2±0.3	0.213	37.6±0.4	37.3±0.4*	0.001
T_{sk} (°C)	34.3±0.7	34.3±0.6	0.889	34.7±0.4	34.5±0.4	0.315
HR (bpm)	76±8	76±9	0.973	74±10	72±5	0.323
Thermal sensation	4.7±0.5	4.4±0.5	0.139	4.4±0.4	4.3±0.5	0.726
Constant-paced cycling						
T_{re} (°C)	37.9±0.3	37.8±0.4	0.215	38.1±0.3	37.9±0.3*	0.002
T_{sk} (°C)	34.2±0.7	34.0±0.7	0.344	34.6±0.5	34.6±0.6	0.925
HR (bpm)	146±15	141±13*	0.033	146±11	140±11*	0.007
RPE	13.5±1.7	13.9±1.2	0.630	13.7±1.6	13.7±1.5	>0.90
Thermal sensation	5.0±0.6	4.9±0.9	0.545	5.2±0.5	5.0±0.4	0.269
20-km time trials						
T_{re} (°C)	38.8±0.6	38.7±0.5	0.553	38.8±0.5	38.7±0.3	0.650
T_{sk} (°C)	33.3±1.1	33.4±0.9	0.857	34.2±0.8	34.2±1.0	0.912
HR (bpm)	166±12	166±12	0.939	164±15	165±7	0.649
RPE	17.7±1.8	18.3±1.3	0.397	17.7±1.7	18.0±1.2	0.434
Thermal sensation	5.8±0.9	5.0±1.1*	0.005	5.6±0.5	5.3±0.5	0.081

End-exercise values are reported for constant-paced cycling and the 20-km time trials. T_{re} rectal temperature, T_{sk} weighted mean skin temperature, HR heart rate, RPE rating of perceived exertion. Data are mean ± SD for $n=9$ and 10 for CON and PRECOOL, respectively, except for T_{re} at rest and constant-paced cycling ($n=8$ for CON), and T_{re} during 20-km time trial ($n=8$ and 9 for CON and PRECOOL, respectively). *Significantly different from pre-acclimation test ($P \leq 0.05$)

Table 3 Change in the 20-km time trial performance and physiological outcomes during the heat stress tests for the CON and PRECOOL groups

	CON	PRECOOL	P value
Δ MPO for 20-km TT (W)	15±20	16±17	0.934
Δ finish time for 20-km TT (s)	-94±173	-101±103	0.921
ΔT_{re} at rest (°C)	-0.1±0.2	-0.3±0.2	0.088
ΔT_{re} during constant-paced cycling (°C)	-0.1±0.2	-0.3±0.2	0.100
Δ HR at rest (bpm)	0±6	-2±7	0.424
Δ HR during constant-paced cycling (bpm)	-4±4	-5±4	0.768

MPO mean power output, TT time trial, T_{re} rectal temperature, HR heart rate. Data are mean ± SD for $n=9$ and 10 for CON and PRECOOL, respectively, except for T_{re} at rest and constant-paced cycling ($n=8$ for CON). Significance level was accepted at $P \leq 0.05$

missing data for T_{re} due to probe damage and displacement (see Tables 2 and 3 for the exact sample size). For CON, there was no acclimation effect for T_{re} and T_{sk} , while PRECOOL demonstrated a significant 0.3 °C reduction in T_{re} at rest and during the constant-paced cycling from the first heat stress test. Both groups demonstrated lower HR during the constant-paced cycling after acclimation. Pooled analysis showed a decrease in T_{re} at rest (-0.2 °C, $P=0.001$) and during exercise (-0.2 °C, $P=0.002$). When analysed as a cohort, there was a decrease in HR during exercise (-5 bpm, $P<0.001$), while HR at rest was unchanged (-1 bpm, $P=0.419$). The absolute changes in T_{re} and HR at rest and during the constant-paced exercise were not different between the groups (Table 3). Thermal sensation was

lower during the 20-km TT after acclimation for CON but was unchanged for PRECOOL. There was no acclimation effect for RPE in either CON or PRECOOL group.

Skin perfusion and sweat responses

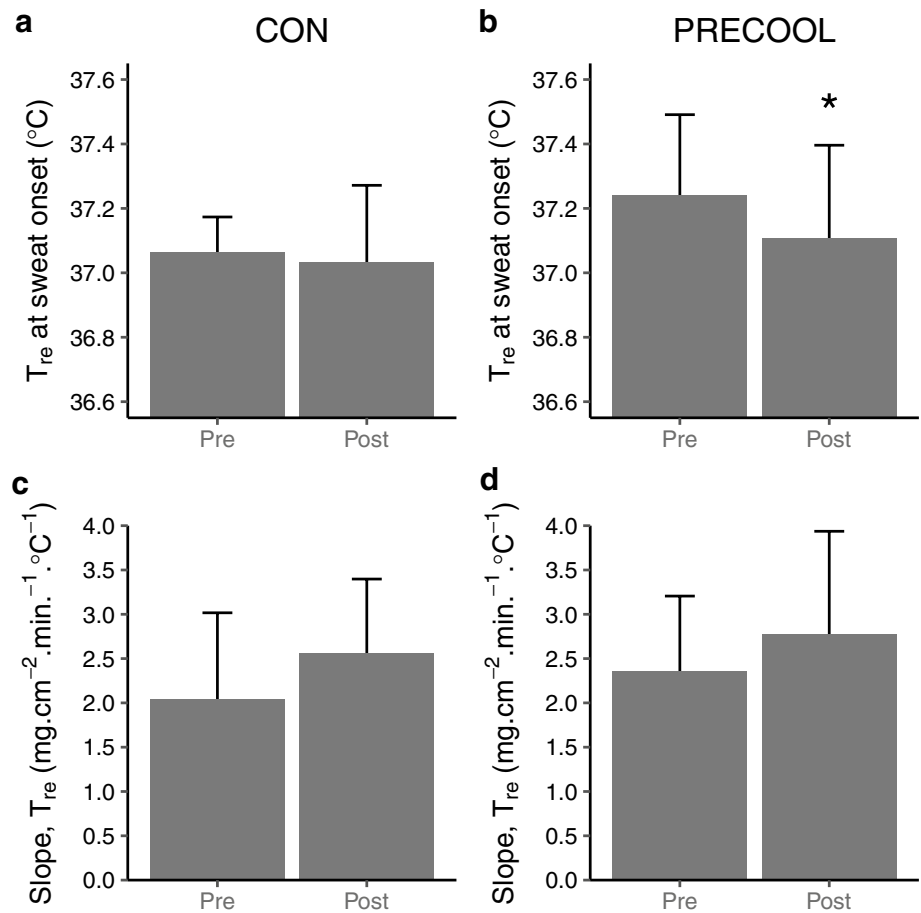
There was no acclimation effect for skin PU, CVC, LSR and WBS% for either CON or PRECOOL (Table 4). T_{re} at onset of sweating was lower for PRECOOL after heat acclimation (Fig. 2, $P=0.042$), while it was unchanged for CON ($P=0.642$). Sweat sensitivity against T_{re} was unaffected by heat acclimation for either CON ($P=0.181$) or PRECOOL ($P=0.232$).

Table 4 Skin blood flow and sweat responses during pre- and post-acclimation heat stress tests

	CON		P value	PRECOOL		P value
	Pre	Post		Pre	Post	
At rest						
Skin perfusion (PU)	24±9	28±16	0.483	31±15	26±10	0.627
CVC (PU mm Hg ⁻¹)	0.31±0.13	0.36±0.18	0.558	0.39±0.18	0.32±0.11	0.176
Constant-paced cycling						
Skin perfusion (PU)	99±28	99±13	0.937	85±18	90±39	0.263
CVC (PU mm Hg ⁻¹)	1.15±0.34	1.14±0.20	0.807	0.96±0.25	0.99±0.45	0.879
LSR	0.94±0.47	0.91±0.48	0.645	1.00±0.31	0.89±0.33	0.388
20-km time trials						
Skin perfusion (PU)	91±26	93±15	0.804	84±22	88±29	0.207
LSR	1.15±0.64	1.15±0.56	0.926	1.04±0.40	1.13±0.43	0.463
WBS%	2.3±0.5	2.3±0.4	0.858	2.2±0.4	2.3±0.5	0.461

Data are mean ± SD for *n* = 9 and 10 for CON and PRECOOL, respectively, except for skin perfusion and CVC (*n* = 9 and 8 for CON and PRECOOL, respectively). End-exercise values are reported for constant-paced cycling and the 20-km time trials. CVC cutaneous vascular conductance, LSR local sweat rate averaged from forearm and thigh, WBS% whole body sweat loss as percentage from pre-exercise body mass. Significance level was accepted at *P* ≤ 0.05

Fig. 2 Rectal core temperature (*T_{re}*, **a** and **b**) at onset of sweating, and slope of regression line for the increase in local sweat rate against *T_{re}* (**c** and **d**) for CON and PRECOOL. Data are mean ± SD for *n* = 9 and 8 for CON and PRECOOL, respectively. *Significantly different from pre-acclimation test based on within-group analysis (*P* ≤ 0.05)

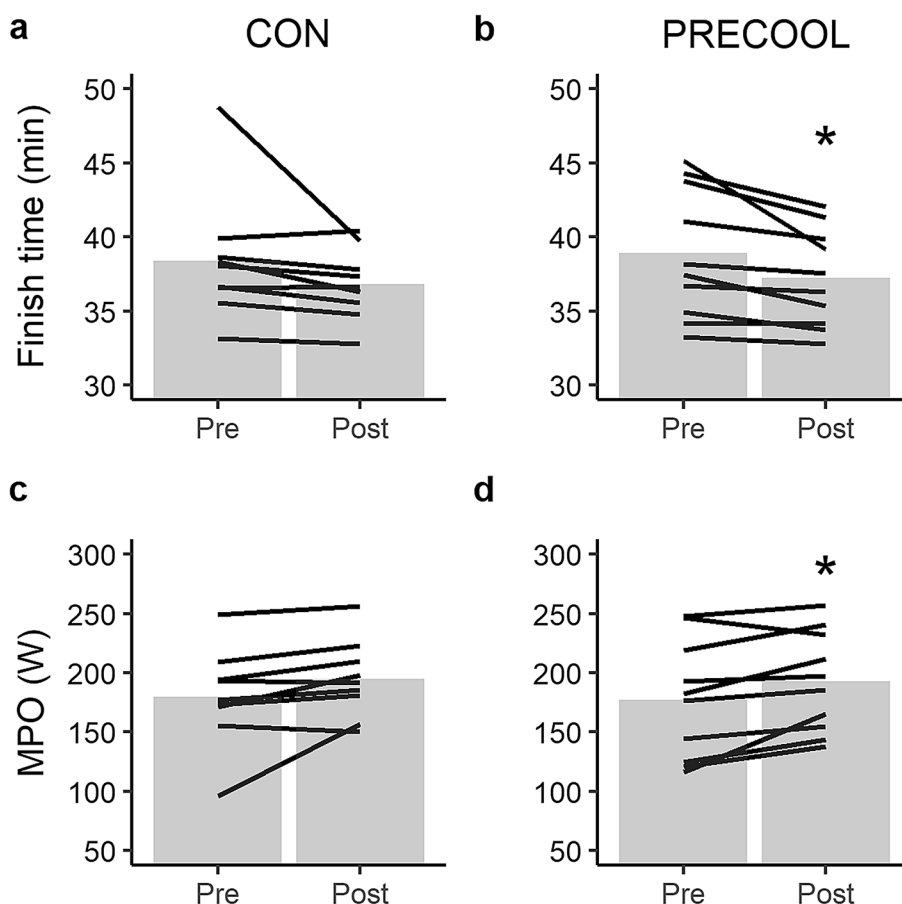


Twenty kilometre TT performance

Within-group analysis showed that PRECOOL improved MPO (*P* = 0.016, *g* = 0.90) and finish time (*P* = 0.013,

g = 0.93) during the second TT, with nine out of ten participants demonstrated improved performance (Fig. 3). For the CON group, TT performance was improved in seven out of nine participants, resulting in non-significant performance

Fig. 3 Finish time (a and b) and mean power output (MPO, c and d) during the 20-km time trials at pre- and post-acclimation heat stress tests. Lines represent individual participant performance and shaded bars show the mean group performance. Data are mean \pm SD for $n=9$ and 10 for CON and PRECOOL, respectively. *Significantly different from pre-acclimation test based on within-group analysis ($P \leq 0.05$)



gains for CON (MPO: $P=0.052$ and $g=0.72$, finish time: $P=0.140$ and $g=0.52$, Fig. 3). However, there was no difference in the absolute change in 20-km TT MPO or finish time between the CON and PRECOOL groups (Table 3).

Discussion

Meeting specific energetic demands during heat acclimation may be important for exercise performance gains. However, the challenge of training at moderate-to-high intensity in the heat without the risks of functional overreaching remains. Accordingly, the present study investigated the effects of precooling on the day-to-day exercise intensity and thermoregulatory adaptation during 10 days of training in the heat. The main findings were that: (1) overall training intensity expressed as percentage change from the first heat acclimation session was higher in the PRECOOL group when compared with the CON group; (2) only the PRECOOL group demonstrated a significant $0.3\text{ }^{\circ}\text{C}$ reduction in T_{re} , while the whole cohort exhibited a lower HR response during cycling at $60\% \dot{V}O_{2peak}$; however, there was no observable increase in sweat rate or skin blood flow; and (3) significant improvement in the 20-km TT was observed

in the PRECOOL group but not in the CON group, although mean performance change was not different between the two groups. Collectively, there is some evidence of heat adaptation in the PRECOOL group only, but there is insufficient evidence to support or refute the secondary hypothesis that regular precooling during heat acclimation would have any detrimental effect on the sudo- and vaso-motor adaptations.

Novel to this study, our results showed that precooling helped to maintain the training intensity at a given RPE during heat acclimation (Fig. 1), consistent with our previous observation that CWI resulted in a modest increase in MPO during 60 min of cycling at a constant RPE (Choo et al. 2019). Regular post-exercise CWI has been shown to improve sprint performance by 2% during 3–5 consecutive days of high-intensity cycling in the heat (Stanley et al. 2013; Vaile et al. 2008). However, to date, no studies have explored the possibility of combining precooling with heat acclimation. Our results showed that when $30\text{ min} \times 22\text{ }^{\circ}\text{C}$ CWI was used as cooling before training in the heat, day-to-day exercise intensity was better maintained when compared with no cooling, with an overall mean difference of 5.5% in Δ MPO between the CON and PRECOOL groups (Fig. 1). Average HR during heat acclimation was maintained for PRECOOL, whereas it was decreased for CON

(Fig. 1). Taken together with the decline in MPO during heat acclimation, it seems logical to suggest a decrease in the relative exercise intensity for CON. The authors nevertheless acknowledge that regular CWI may be logistically demanding to implement in a real-world scenario. Other more practical precooling modalities that target core and skin temperatures (e.g., ice slurry ingestion combined with cooling garments) may be of interest to practitioners. However, we have previously shown that the ergogenic effect of ice slushy is less effective when compared with 22 °C × 30 min CWI (Choo et al. 2019), and hence how comparable the current findings would be to such precooling modalities is unclear.

Hallmarks of heat adaptation include increased sweat rate (Poirier et al. 2016), lower core temperature (Poirier et al. 2015) and HR responses during exercise (Garrett et al. 2009). Except for a lower HR response during cycling at 60% $\dot{V}O_{2\text{peak}}$, minimal heat adaptations were observed for CON (Table 3). In contrast, the PRECOOL group exhibited significant reduction in HR (−5 bpm) and T_{re} (−0.3 °C) during the submaximal exercise, as well as a lower T_{re} at the onset of sweating (Fig. 2). A reduction of 0.3 °C in T_{re} has also been reported by previous studies using controlled-hyperthermic (Garrett et al. 2009), fixed-intensity (Castle et al. 2011; Poirier et al. 2015), or passive post-exercise heat acclimation protocols (Zurawlew et al. 2016). However, changes in LSR or WBS were not evident in both CON and PRECOOL groups in the present study. Interestingly, higher sweat loss at a given exercise bout following heat acclimation has been observed in some (Poirier et al. 2015, 2016; Schmit et al. 2017a), but not all studies (Castle et al. 2011; Reeve et al. 2019; Zurawlew et al. 2016). The variability in sudomotor adaptation may be in part related to the duration of heat exposure (Patterson et al. 2004) and the environmental conditions (Tebeck et al. 2019). It is also possible that prolonged residence in warm and humid climates has resulted in a negative adaptation in the sweat glands to conserve body fluid (Bae et al. 2006). Taken together, our findings do not demonstrate profound physiological changes typifying heat adaptation, particularly the CON group.

The present study used an RPE-based protocol during heat acclimation. While such protocols may have great ecological validity, participants were instructed to regulate exercise intensity based on a fixed RPE and T_{re} was not monitored during the heat acclimation sessions. Hence, it is possible that the imposed heat stimulus was insufficient to induce heat adaptation in the present study. This, however, seems unlikely as the exogenous heat load, relative exercise intensity (60% $\dot{V}O_{2\text{peak}}$) and total heat exposure (600 min) in the present study were comparable to studies that reported lower T_{re} and HR responses following heat acclimation (Castle et al. 2011; Fujii et al. 2012). We have previously demonstrated that 22 °C × 30 min CWI minimally reduces T_{re} , but significantly reduces T_{sk} and increases power

output during 60 min of cycling at RPE 15, compared with no cooling (Choo et al. 2019). In that study, T_{re} increased by 1.2–1.4 °C, which has been shown to be sufficient to induce heat acclimation after 6–10 days of heat exposure (Magalhães et al. 2010; Regan et al. 1996; Zurawlew et al. 2016). Indeed, some signs of heat acclimation were observed in the PRECOOL group as indicated by lower T_{re} (−0.3 °C) and lower HR (−5 bpm) during the constant-paced cycling. In contrast, T_{re} decreased by 0.1 °C ($P=0.215$) in the CON group. Taken together with the decline in HR and MPO during the acclimation period, it is, therefore, conceivable that a less optimal adaptation has occurred for CON. Previous research involving tropical natives has produced equivocal findings with regards to thermophysiological adaptation to heat exposure (Magalhães et al. 2010; Saat et al. 2005). For example, 14 day × 60 min cycling at 60% $\dot{V}O_{2\text{max}}$ in the heat has been shown to decrease exercising T_{re} and HR without concomitant changes in LSR for the upper back (Saat et al. 2005). Conversely, using a controlled-hyperthermic model (i.e., raising T_{re} by 1 °C) during 11 days of heat acclimation, increased LSR for multiple sites including chest and thigh have been observed (Magalhães et al. 2010). Common to these studies, resting T_{re} was unaffected by acclimation (mean change +0.12 °C to −0.21 °C), while lower HR during exercise was observed (Magalhães et al. 2010; Saat et al. 2005). Taken together, the plausible explanation for minimal adaptation to heat stimulus in tropical natives may be a combination of acclimated status and the effectiveness of different heat acclimation techniques.

Heat acclimation did not affect sweating sensitivity or skin blood flow response in the present study (Table 4 and Fig. 2). Earlier studies have also reported unchanged sweat gain against core temperature after 8–10 day × 60–100 min of heat acclimation (Pandolf et al. 1988; Patterson et al. 2004; Regan et al. 1996). Although continuous suppression of local sweating by CWI during heat acclimation has been shown to inhibit the adaptation in sweat glands (Collins et al. 1966; Fox et al. 1964), previous observation from our laboratory has shown that precooling by CWI delays sweat gland recruitment but minimally affects LSR (Choo et al. 2019). Additionally, the lack of skin vasomotor adaptation in the present study is consistent with some studies (Nielsen et al. 1997; Regan et al. 1996), but not others (Amano et al. 2015; Fujii et al. 2012; Notley et al. 2018). Lorenzo and Minson (2010) postulated that heat acclimation enhanced skin microvascular sensitivity to certain vasodilatory stimuli (e.g., acetylcholine) rather than inducing structural changes. However, a more recent study has suggested that cutaneous vasomotor adaptation is evident only under specific conditions, e.g., maintaining a constant level of thermal stimulus via a controlled-hyperthermic protocol (Notley et al. 2018). As mentioned, the variability in sudo- and vaso-motor adaptations may also be related to the total exposure and the

ambient conditions during heat acclimation. Collectively, there is no evidence that precooling by CWI attenuates the thermal stimulus necessary for sudo- and vaso-motor adaptations.

The ergogenic mechanisms of heat acclimation may be largely related to enhanced cardiovascular stability (Périard et al. 2016). In the present study, both CON and PRECOOL demonstrated lower exercising HR response after acclimation, but significant performance gains were evident in the PRECOOL group only (Fig. 3). The authors acknowledged that absence of a significant between-group difference in the mean performance change lessens the impact of our findings (Table 3), and the lack of significant improvement for CON may be due to a type II error as moderate effects were observed for MPO and finish time ($g=0.72$ and 0.52 , respectively). Training at high intensity in the heat, even for as little as 5 days, has been shown to result in functional overreaching and adversely affect 20-km TT performance by 1.7% (Schmit et al. 2017a) and cycling to exhaustion by 22 min (Reeve et al. 2019), despite signs of heat adaptation. There was no group or acclimation effect for perceived well-being (Fig. 1). However, as well-being or other related measures (e.g., heart rate variability or hormonal response) were not assessed during the heat stress tests and only one TT was performed following acclimation, there is a possibility of functional overreaching in some individuals in the CON group, resulting in a non-significant performance change.

Conclusions

The current findings suggest mild CWI ($22\text{ }^{\circ}\text{C}\times 30\text{ min}$) can help to maintain day-to-day exercise intensity during training in the heat and improves subsequent time trial performance in the heat. Given that exercise intensity is typically reduced when performed in the heat and could result in peripheral de-conditioning, the current study extends support for the use of precooling to maintain day-to-day intensity during heat acclimation. Athletes who live in tropical countries or undertaking heat acclimation ahead of competing in the heat could, therefore, include precooling strategies to preserve training quality. With regards to whether precooling might influence thermoregulatory adaptations, a significant reduction of $0.3\text{ }^{\circ}\text{C}$ in T_{re} was observed for PRECOOL only; however, as there was no observable increase in sweat rate, there is insufficient evidence to support or refute that regular precooling would have any detrimental effect on the sudo- and vaso-motor adaptations during training in the heat. As T_{re} was not monitored during the heat acclimation sessions, it is possible that the magnitude of heat stimulus was insufficient to induce heat adaptation in the present study. Additionally, training in the heat without precooling elicited similar decreases in the HR response during submaximal

cycling, but any ergogenic effect of heat acclimation may be offset by a less than optimal level of physiological stimulus and/or functional overreaching. The present study highlights the benefits of precooling on heat acclimation, but whether it will have similar effects on elite athletes who typically train at higher intensities requires further investigation.

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Compliance with ethical standards

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the authors.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of Edith Cowan University Human Research Ethics Committee (Reference number: 15078 CHOO) and Institutional Review Board of the Singapore Sport Institute (Reference number: PH-ECP-022) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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