Discipline of Exercise Science

Curtin School of Allied Health

Using Cooling to Enhance Heat Acclimation and Endurance Performance

Julian Andro Potestades Ramos

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Author Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Numbers HRE2020-0685, HRE2021-0398, and HRE2022-0638.

Acknowledgement of Country

I acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. I wish to pay my deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. My passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work I do, reflective of my institutions’ values and commitment to the role as leaders in the Reconciliation space in Australia.

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Statement of Contribution

This thesis contains works in preparation for publication, all of which have been co-authored. Using the CRediT (Contributor Roles Taxonomy) author statement, the candidate’s individual contribution is recognised and described for each of the following chapters in this thesis.

Term	**Candidate Individual Contribution**
Conceptualisation	Formulation of thesis and individual study question, aims and hypotheses
Methodology	Development or design of methodology, and thesis document
Formal analysis	Synthesis and analysis of study data
Investigation	Database searching; data collection
Resources	Procurement and maintenance of required equipment and facilities, and recruitment of participants
Writing – Original Draft	Preparation, creation and presentation of the work, specifically writing the initial draft
Writing – Review and Editing	Preparation, creation and presentation of the work, specifically writing the initial draft
Visualisation	Preparation, creation and presentation of the work, and publication preparation
Project administration	Management and coordination of responsibility for the research activity planning and execution

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Thesis Abstract

Self-paced endurance exercise in heat can be negatively affected by increased thermal strain. Acute heat alleviation techniques before (i.e., pre-cooling; PreC) and/or during (i.e., per-cooling; PerC) exercise, along with chronic heat alleviation strategies such as heat acclimation/acclimatisation (HA) can enhance performance in heat by reducing/blunting an individual's thermal strain. However, further investigation is warranted exploring the most beneficial environmental stress for PreC and the effect of cooling on thermal strain and performance during HA and for heat-acclimated/acclimatised (Heat_{Acc}) individuals. The overarching aim of this thesis was to explore the use of cooling to enhance HA and exercise performance, consisting of a literature review, four experimental studies, and an opinion piece.

The literature review (chapter 2): i) briefly reviews the effect of heat on autonomic and behavioural thermoregulation, ii) discusses the effect of cooling and HA methods on exercise performance in different environmental conditions, and iii) explores current knowledge regarding the combination of cooling and HA to improve endurance performance in heat.

Study one (chapter 3) explored the effects of PreC on 20-km cycling time-trial (TT) performance in three different environmental conditions; hot-dry, moderately hot-humid and hot-humid using apparent temperature (AT) to stratify environmental stress. Faster 20-km cycling TT performance in hot-humid conditions preceded by PreC, whereas no difference or slower finish times were observed in moderately hot-humid, and hot-dry environments, respectively. These results support the use of PreC prior to 20-km cycling TT conducted in hot-humid conditions. Alternatively, PreC may not be a priority or

is not recommended prior to 20-km cycling TT in moderately hot-humid or hot-dry conditions.

Study two (chapter 4) investigated the effects of single session intermittent heat exposure (IHE) with more frequent and shorter cooling breaks on physiological, perceptual, and self-paced maximal cycling performance, compared to continuous heat exposure in the same hot-humid conditions as study one. Improved self-paced cycling performance during IHE compared to continuous heat exposure was observed. Additionally, despite the addition of PerC, the necessary stimulus and physiological responses for effective HA was not compromised. Therefore, IHE may be preferred when both HA stimulus and training quality holds equal priority. However, further research is required to determine whether the addition of cooling in Heat_{Acc} individuals will result in further benefits to self-paced endurance performance in hot-humid conditions. Additionally, single session IHE needs to be investigated as a full HA program and determine whether it results in HA.

Thus, study three (chapter 5) investigated the effects of PreC on 20-km cycling TT in Heat_{Acc} individuals. Our main findings showed insufficient evidence to support a meaningful change in performance when PreC was applied prior to 20-km cycling TT in hot-humid conditions in Heat_{Acc} individuals. This may indicate that PreC may not be a priority prior to self-paced maximal endurance performance in hot-humid conditions for Heat_{Acc} individuals.

Study four (chapter 6) investigated the effects of IHE with PerC as an alternative HA method for non-Heat_{Acc} endurance athletes. Our main findings showed IHE with PerC resulted in a lack of attainment of hallmark adaptations to heat. Despite this, within-session performance post-HA was improved. The combination of PerC and HA may allow for greater work done within a session, particularly beneficial when high training volume is required with a limited timeframe.

I composed an opinion paper (chapter 7) which proposed the use of an internal-to-external training load ratio to objectively evaluate the effectiveness of self-paced HA sessions and programs. Currently, there is a lack of consensus regarding a method that objectively assess self-paced HA effectiveness or monitor training stimulus during such sessions. Our proposal advocates for the use of internal-to-external load ratios to address this gap, showing that similar within-session ratios indicate a maintained heat stimulus throughout the session. Alternatively, reductions or failure to maintain ratios may indicate an ineffective heat stimulus throughout the session. When comparing ratios between sessions, higher subsequent session ratios are the goal, which indicate an appropriate progressive overload stimulus.

In conclusion, this thesis outlined the importance of applying PreC prior to self-paced maximal 20-km endurance performance in hot-humid conditions. Alternatively, the application of PreC in less thermally stressful conditions holds less priority. In addition, when training quality and heat stimulus hold equal importance during a HA program, my acute observations indicate that IHE with PerC may be a viable method of HA, as shown by similar physiological responses as traditional continuous heat exposure. However, when applied over a full HA program, I observed a lack of attainment of hallmark adaptations to heat, but exercise performance was maintained compared to HA alone.

Publications Arising from Thesis

- Ramos, J. A. P., Brade, C. J., Ducker, K. J., Landers, G. J., & Girard, O. (2021). The internal-to-external load ratio: A tool to determine the efficacy of heat acclimation/acclimatization using self-paced exercise. *Frontiers in Sports and Active Living*, **3**:830378. DOI: 10.3389/fspor.2021.830378. See chapter 7.
- Ramos, J. A. P., Ducker, K. J., Riddell, H., Girard, O., Landers, G. J., Brade, C. J. (2024). Mixed-method precooling enhances self-paced 20-km cycling time-trial performance when apparent temperature is >46°C but may not be a priority in <46°C. *International Journal of Sports Physiology and Performance*, 1-8. DOI: 10.1123/ijspp.2023-0331. See chapter 3.
- Ramos, J. A. P., Ducker, K. J., Riddell, H., Girad, O., Landers, G. J., and Brade, C. J. (2024). Single session intermittent heat exposure with more frequent and shorter cooling breaks facilitates greater training intensity and elicits comparable physiological responses to continuous heat exposure. *International Journal of Sports Physiology and Performance*, in press. See chapter 4.
- Ramos, J. A. P., Ducker, K. J., Riddell, H., Girad, O., Landers, G. J., and Brade, C. J. Pre-cooling does not improve 20-km self-paced maximal cycling time-trial performance in heat acclimated endurance athletes. Submitted to Journal of Science and Medicine in Sport, *under review.* See chapter 5.
- Ramos, J. A. P., Ducker, K. J., Riddell, H., Girad, O., Landers, G. J., and Brade, C. J. Intermittent-heat exposure with per-cooling as a heat

acclimation training protocol does not induce heat adaptations. Submitted to Scandinavian Journal of Medicine & Science in Sports, *under review. See chapter 6.*

Conference Proceedings Arising from Thesis

- **Ramos, J. A. P.,** Brade, C. J., Girard, O., Landers, G. J., & Ducker, K. J. Mixed-method pre-cooling and self-paced cycling performance in the heat: Effects of apparent temperature. *Exercise Sport Science Australia (ESSA) Research to Practice 2022 (oral – online).*
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- **Ramos, J. A. P.,** Ducker, K. J., Riddell, H., Girard, O., Landers, G. J., & Brade, C. J. Acute intermittent heat exposure with more frequent and shorter cooling breaks enhances performance and elicits comparable physiological responses to continuous heat exposure. *Exercise Sport Science Australia Research to Practice 2024. Sydney (Australia), 2-4th May 2024 (oral). Awarded the Young Investigator Award for Sports Science at ESSA Research to Practice 2024.*

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List of Abbreviations

AT	Apparent temperature
BM	Body mass
CI	Confidence interval
CON	Control
CTT	Cycling time-trial
CWI	Cold-water immersion
ES	Effect size
HA	Heat acclimation/acclimatisation
H_b	Haemoglobin
H_{ct}	Haematocrit
$Heat_{Acc}$	Heat-acclimated/acclimatised
HR	Heart rate
HST	Heat stress test
ICC	Intraclass correlation coefficient
IHE	Intermittent-heat exposure
ISAK	International Society for the Advancement of Kinanthropometry
PerC	Per-cooling
PO	Power output
PreC	Pre-cooling
PV	Plasma volume

RH	Relative humidity
RPE	Rating of perceived exertion
SD	Standard deviation
T_b	Body temperature
T_c	Core temperature
Th_c	Thermal comfort
Th_s	Thermal sensation
T_g	Core-to-skin temperature gradient
T_{re}	Rectal temperature
T_{sk}	Skin temperature
TTE	Time-to-exhaustion
TT	Time-trial
USG	Urine specific gravity
$\dot{V}O_{2max}$	Maximum aerobic capacity

Chapter 1: General Introduction

Endurance events are held globally under challenging hot environmental conditions. Compared to cool (i.e., 20 – 25°C) conditions, competing in both hot-dry (≥30°C, ≤40% relative humidity [RH]) and hot-humid conditions (≥30°C, >40% RH) likely results in slower finish times, lower sustained power output (PO), and higher risk of experiencing exertional heat illness (Gonzalez-Alonso et al., 2008, Mora-Rodriguez, 2012, Périard et al., 2021, Wendt et al., 2007). One method of combatting the negative effect of heat on self-paced endurance performance is the application of acute heat alleviation techniques before (i.e., PreC) or during (i.e., PerC) exercise (Bongers et al., 2017). These methods can increase heat storage capacity and/or attenuate the rise in core temperature (T_c; Bongers et al., 2017, Gibson et al., 2020). Improvements in endurance exercise performance (~30–90 min) after PreC were observed in environmental conditions exceeding 27°C and RH above 50% (Faulkner et al., 2018, Maia-Lima et al., 2017, Wegmann et al., 2012). Conversely, no performance improvement was noted in less thermally challenging conditions (≤24°C, ≤68% RH; ~55–60 min; Faulkner et al., 2018, Maia-Lima et al., 2017). Due to high variability in ambient temperatures and RH used in PreC literature, direct comparisons are difficult when two environmental constructs need considering and overlook how the environment feels. Apparent temperature (Steadman, 1994), which represents temperature in terms of how it is perceived using both RH and ambient temperature (e.g., 40°C, 40% RH feels like 46°C), is a potential solution to utilise when exploring the effectiveness of PreC in differing environmental conditions. However, researchers have yet to determine the specific AT conditions necessary for PreC to have the largest positive effect on endurance performance.

In addition to acute techniques, chronic heat alleviation strategies such as HA, result in adaptations advantageous to exercise in heat (Armstrong & Maresh, 1991, Périard et al., 2021). Typically HA is achieved by performing continuous exercise at low-to-moderate intensities for a duration of ~≥60 min per day for 10 – 14 days (Périard et al., 2021). While a HA program can yield physiological adaptations to heat, it also results in a high internal training load and fatigue in athletes, thus potentially compromising training quality and leading to overreaching. A potential method of reducing the internal training load and thus fatigue of athletes during HA training may be using an IHE protocol. This approach involves exposing athletes to heat during work (exercise) intervals and then intermittently resting passively in thermoneutral conditions. This method may blunt increases in T_c and skin temperature (T_{sk}), and improve thermal comfort (Th_c), and sensation (Th_s), and perceived effort, thus improving performance outcomes (Bongers et al., 2017). Although seeming contradictory to the aims of HA training, another potential method to decrease internal load may be the application of PreC and/or PerC during HA training sessions. Choo et al. (2020) found that using PreC (30 min of ~22°C cold-water immersion) prior to 10 HA sessions (60 min cycling at a rating of perceived exertion [RPE] of 15 [6-20 Borg scale] in ~35°C and ~53% RH) maintained mean PO over the 10-day period compared to no PreC (+2.9 *vs.* -2.6%). During a post-acclimation heat stress test (25 min cycling at 60% peak oxygen uptake, followed by 20-km cycling TT), PreC HA resulted in greater mean PO (~9% *vs.* ~8%) and faster finish times (~-2% *vs.* ~-3%) during the cycling TT compared to the no PreC HA group.To the best of our knowledge, the use of IHE as a method of HA has not been previously explored nor the effect of different IHE set structures with PerC on physiological, perceptual and performance responses compared to a traditional continuous exposure HA session.

Previous research supports the use of HA or PreC to improve endurance performance in hot-humid conditions (Fox et al., 1967, Zimmermann et al., 2018). Both methods have been shown to reduce the thermal strain experienced by individuals, specifically through inducing heat adaptations (i.e., via HA), and increasing heat storage capacity and improving thermal perception (i.e., via PreC). Theoretically, combining both methods (i.e., applying PreC to heat acclimated individuals) may further decrease T_c and/or T_{sk}, augmenting endurance exercise performance in heat. However, previous research which applied PreC to heat acclimated individuals prior to intermittent exercise (i.e., intermittent-sprint or repeat sprint efforts) in heat found no further benefits to performance compared to no PreC (Brade et al., 2013, Castle et al., 2011). For instance, Castle et al. (2011) investigated the effects of PreC (ice packs on thighs) in heat acclimated individuals performing an intermittent cycling sprint protocol in heat (~34°C, ~52% RH). Their findings showed a ~2% increase in peak power output post-HA (60 min cycling at 50% maximum aerobic capacity; $\dot{V}O_{2max}$) compared to pre-HA. However, no additional benefit to peak PO was observed when PreC was applied compared no PreC (~1,091 *vs.* ~1,119 W; Castle et al., 2011). The absence of evidence of PreC providing additional benefit to exercise performance in heat in $Heat_{Acc}$ individuals may be attributed to insufficient severity of heat stress. This includes the combination of metabolic heat production (internal strain) and environmental conditions (external stress). However, further research is required to determine the conditions required for PreC to provide further benefits to performance in heat acclimated individuals compared to no PreC.

This PhD thesis investigates how cooling can be used to enhance endurance performance and HA protocols and proposes an objective method of determining the effectiveness of self-paced HA protocols. Above, I have outlined gaps in the literature which will be investigated through four experimental studies and one opinion paper. First, I conducted an experimental study (chapter 3) to determine what air temperature and humidity (apparent temperature) PreC provides beneficial effects on self-paced maximal endurance performance. I then investigated (chapter 4) the effects of single session IHE with more frequent and shorter cooling breaks compared to traditional continuous heat exposure. Findings from both studies informed the fourth and fifth study (e.g., the temperature from chapter 3 and most effective IHE structure from chapter 4 will be used in both chapter 5 and 6). I then determine the effects of PreC on cycling TT (chapter 5) in Heat_{Acc} (via IHE; chapter 6) individuals. Finally, I propose the use of an internal-to-external load ratio to determine the effectiveness of a self-paced HA session (chapter 7). Outcomes from this PhD have the potential to identify the specific conditions where cooling can provide the greatest benefit to self-paced endurance in heat. In addition to this, our findings could demonstrate the efficacy of combining cooling with HA to induce heat adaptations and improve self-paced endurance performance in heat. Additionally, utilising the internal-to-external training load ratio to measure and monitor training can benefit both training and endurance performance in heat.

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Chapter 2: Literature Review

2.1 Introduction

Endurance events are held globally under challenging hot (combination of high radiant heat, presence/absence of air flow, air temperature and relative humidity [RH]) environmental conditions. For instance, hot-dry conditions (~39°C, ~30% RH) were reported during the 2018 Tour Down Under cycling race (Australian Bureau of Meteorology, 2018, Time and Date, 2018). Additionally, Kona Ironman World Championships in Hawaii regularly occur during hot-humid conditions (25 – 35°C, 40 – 80% RH; Dixon, 2019). Compared to thermoneutral (i.e., 20 – 25°C) conditions, competing in both hot-dry and hot-humid likely result in slower finish times, lower sustained power output (PO), and higher risk of experiencing exertional heat illness (Gonzalez-Alonso et al., 2008, Périard et al., 2021, Wendt et al., 2007). Decrements in exercise performance in heat result from changes in variables such as core (T_c) and skin (T_{sk}) temperatures, thermal comfort (Th_c), and thermal sensation (Th_s; Abbiss & Laursen, 2005, MacDougall et al., 1974, Nielsen et al., 1993). Thus, methods to manipulate these variables to result in improvement or maintenance of performance in the heat are of great importance for coaches, sport scientists and athletes a like.

Body temperature regulation during exercise is driven by autonomic and behavioural responses. Autonomic thermoregulation functions through involuntary physiological processes (e.g., sweating; Chiesa et al., 2019), whereas behavioural thermoregulation operates through conscious behavioural adjustments to achieve homeostasis (e.g., removing clothing or exercising in shaded areas to remain cool; Flouris, 2011). However, when exercise is performed in heat these regulatory mechanisms can be compromised and are thus unable to maintain homeostasis (e.g., T_c ~36.6°C; Périard et al., 2021) Therefore chronic and acute heat alleviation techniques can assist in maintaining body temperature.

Chronic heat alleviation (i.e., heat acclimation; HA) is achieved in the weeks or days prior to events to induce advantageous adaptations to the heat (e.g., increased sweat rate, and lower T_c and T_{sk}), which improves exercise performance capacity (Périard et al., 2021). Alternatively, acute heat alleviation techniques are typically used (30 – 60 minutes) before (i.e., pre-cooling; PreC) events to reduce starting T_c and/or attenuate the rise in T_c, and improve perception in heat. Moreover, they are also used during events (i.e., per-cooling; PerC) to increase the heat storage capacity of the body (Gibson et al., 2020, Périard et al., 2021). However, limited knowledge exists regarding which environment PreC has the greatest efficacy during endurance exercise efforts (i.e., hot-humid or hot-dry environments).

Combining chronic and acute heat alleviation techniques may further improve exercise performance in the heat by decreasing thermal strain (combination of high bodily temperatures and elevated levels of thermal discomfort) to a greater degree compared to when each technique is used in isolation (Castle et al., 2011). The consensus is that PreC induces no additional benefit to heat acclimated/acclimatised athletes, with PreC becoming beneficial only when external thermal stress experienced by individuals is high (Brade et al., 2013a, 2013b, Castle et al., 2011). It should be noted that environmental conditions used in both studies were similar (33-35°C, 50% RH), therefore whether similar observations will be seen in more thermally stressful environment (i.e., >35°C, >60% RH) or greater cooling intensities is unknown.

This review will seek to: i) briefly review knowledge of the effect of heat on autonomic and behavioural thermoregulatory processes, ii) discuss cooling and HA methods and their effects at different environmental conditions on self-paced endurance performance, and iii) explore the current literature regarding the combination of PreC and HA to improve endurance performance in the heat.

2.2 Impact of Heat on Endurance Performance

At rest, when air temperature exceeds 26°C, body heat dissipation occurs via convection, evaporation, conduction, and/or radiation (Ravanelli et al., 2019). However, when exercising in heat the main avenue of heat loss is evaporation of sweat (Chiesa et al., 2019, Flouris, 2019, Ravanelli et al., 2019). Greater strain is placed on these autonomic processes when exercising in hotter compared to cooler conditions as the additional metabolic heat production from exercise and heat gained from the environment when combined increase T_c and T_{sk}. Exercise induces peripheral vasodilation to redirect heat from the core to the skin to be cooled in hot-dry and hot-humid conditions. However, this may lead to a competition in blood flow between the working muscles, which require nutrients and oxygen, and the periphery for heat dissipation to occur via the evaporation of sweat (Flouris, 2019). When exercising in hot-humid environments (e.g., Kona Ironman World Championships, Hawaii) heat loss through the evaporation of sweat is impaired as the difference between the moisture in the air and skin surface is low (Bright et al., 2021, Maughan et al., 2012). This exacerbates the strain imposed on autonomic thermoregulatory processes and leads to increases in heat storage by the body, consequently increasing T_c and T_{sk}. In addition to this, excessive sweating during prolonged endurance events in the heat may lead to dehydration and/or increased levels of thermal discomfort and ultimately limits exercise performance (Nybo et al., 2014).

The negative effects of heat on exercise performance can be explained by central (e.g., central nervous system) and peripheral factors (e.g., muscles; Flouris & Schlader, 2015). MacDougall et al. (1974) proposed that exercise performance is terminated when a critical T_c is reached (~39.5°C). However, this theory has been rejected by studies who observed no exercise cessation at the proposed critical temperature (Cuddy et al., 2014, Ely et al., 2009, Racinais, Casa, et al., 2019). For example, although MacDougall et al. (1974) observed mean rectal temperatures (T_{re}) of 39.5°C at exhaustion, one individual still recorded a T_{re} of 40.3°C. Likewise, Racinais, Moussay, et al., 2019 reported no voluntary cessation of exercise or adverse effects on health despite observing a maximum of 41.5°C T_c and mean T_c of ~39.2 – 39.8°C in male cyclists competing in the Union Cycliste Internationale Road World Championships held in Doha, Qatar (37°C environment). This may be due to the level of the athletes recruited (athlete classification level of Tier 4; McKay et al., 2022). Indeed, athletes with higher levels of aerobic fitness can tolerate and perform longer at higher levels of hyperthermia compared to those with lower levels of aerobic fitness (Cheung & McLellan, 1998, Mora-Rodriguez, 2012, Mora-Rodriguez et al., 2010).

Endurance performance can be sustained even at T_c or T_{re} >40°C, assuming a large core-to-skin temperature gradient (T_g) exists, allowing heat loss to the environment (i.e., core-to-skin temperature theory; Cuddy et al., 2014, Ely et al., 2009). In a study of trained runners performing an 8-km running time-trial (TT) in cool (13°C) or warm (27°C) conditions, no difference in average running speed were reported when T_{re} reached >40°C compared to when T_{re} was <40°C (~279 vs. ~282 m·min-1; Ely et al., 2009). Moreover, participants were able to accelerate in the final 600-m of the TT despite T_{re} reaching the 'critical threshold' (39.4-40.9°C). These findings may be due to the larger T_g observed in the cool (8.5°C) and warm (5.2°C) conditions. Likewise, Cuddy et al. (2014) investigated the impact of T_g on performance during a running time-to-exhaustion trial in four different environmental conditions. They noted earlier task failure in the 49°C apparent temperature (AT) group (42°C, 40% RH; 51.3 ± 8.3 min) compared to the 19°C AT (18°C, 40% RH) and 26°C AT group (26°C, 40% RH; ~58.1 and ~62.6 min, respectively), although final T_c across all the conditions were less than the proposed critical threshold of 39.5°C (Cuddy et al., 2014). Their findings were attributed to lower T_g in the 49°C AT group (2.1°C) compared to the 19°C AT and 26°C AT conditions (3.3 – 3.5°C). Low T_g have also been observed in cooler ambient temperatures with high levels of humidity (i.e., 30°C, >50% RH). This was evident in individuals performing four cycling trials at 70% maximum aerobic capacity ($\dot{V}O_{2max}$) until volitional exhaustion in various levels of humidity (Maughan et al., 2012). It was observed that exercise termination occurred earlier in hot-humid (30°C, 60-80% RH, 38°C AT; ~46 min) environments compared to hot-dry (30°C, 24% RH, 29°C AT; ~68 min; Maughan et al., 2012). Moreover, although T_c at exhaustion was not different between conditions (~39.0 – 39.1°C), the rate of rise in T_c was significantly faster when RH was 80% (~0.5°C/10 min) compared to 24% (~0.3°C/10 min; Maughan et al., 2012). This indicates that the T_g may be one of the limiting factors to exercising in heat.

Self-paced exercise where athletes exercise at their own intensity (e.g., cycling TT), is also negatively affected when performed in hot environmental conditions (Gibson et al., 2020, Périard & Racinais, 2015). For instance, Périard and Racinais (2015) investigated the peak oxygen uptake utilised by trained cyclists during four consecutive 16.5 min cycling TT in hot (35°C, 60% RH, 42°C AT) and cooler conditions (18°C, 40% RH, 19°C AT). Their findings showed lower PO (~382 vs. ~395 W) and higher T_c (~39.4 vs. ~38.6°C) in hot compared to the cooler condition (Périard & Racinais, 2015). The negative effect on PO has been linked to higher T_c (or T_{re}) and T_{sk} in hot compared to cool conditions, which indicates increased stress placed upon cardiovascular and thermoregulatory systems (Galloway & Maughan, 1997, Schlader et al., 2011).

2.3 Behavioural Thermoregulation

Autonomic thermoregulatory processes (e.g., sweating) are complimented by conscious decisions made by individuals to maintain homeostasis (i.e., behavioural thermoregulation; Weiss & Laties, 1961). At rest in hot ambient temperatures, increased thermal discomfort (subjective indifference to the thermal environment; Mercer, 2001) occurs as a result of an increase in T_{sk} (Flouris, 2019, Flouris & Schlader, 2015). To regain Th_c, behavioural adaptations typically occur (e.g., removal of clothing or moving to shade; Flouris & Schlader, 2015). However, during exercise in heat, increased thermal discomfort leads to increased perceived exertion and fatigue, resulting in decreased exercise work rate (Flouris, 2019). To achieve Th_c, an anticipatory response to the heat occurs which prompts individuals to commence exercise with lower work rates, because of decreased neural drive to active musculature, to preserve energy to complete the entire bout (Cheung, 2007, Schlader et al., 2011, Tucker et al., 2004). Both processes occur to minimise metabolic heat production and maintain body temperature within homeostatic ranges (Cheung, 2007, Nybo & Nielsen, 2001, Tucker et al., 2004).

During exercise in heat, changes in neuromuscular activation have also been observed in order to reduce metabolic heat production (Cheung, 2007, Schlader et al., 2011). For example, Tucker et al. (2004) investigated whether recruitment of skeletal muscle motor units changes during 20-km cycling TT in hot (35°C) compared to cool (15°C) conditions. The integrated electromyographic activity of the vastus lateralis muscle was measured at 5%, 10%, 50%, 75% and 100% of distance completed during the 20-km cycling TT. Results showed lower mean PO in hot compared to cool (~255 vs. ~272 W), and lower integrated electromyographic activity in hot compared to cool at 50% and 100% of the distance completed (Tucker et al., 2004). Interestingly, these changes were observed prior to the attainment of a critical T_{re} (Tucker et al., 2004).

It has been previously suggested that T_{sk}, Th_c and Th_s (intensity of the temperature being sensed; Attia, 1984) levels are important in the self-selection of starting exercise intensity in the heat compared to T_c (Schlader et al., 2011). This was suggested as Schlader et al. (2011) observed greater mean PO (~258 vs. ~251 W) from the commencement of exercise during a 60 min cycling TT when T_{sk} (manipulated by a perfused-suit) changed from cold (~6.6°C) to hot (~61.4°C) compared to hot-to-cold, despite no difference in T_c between conditions. The lower initial work rates in the hot-to-cold condition were attributed to greater Th_c and Th_s (4-point scale; Gagge et al., 1967) when T_{sk} was high, leading to an anticipatory response resulting in a lower self-selected intensity to ensure exercise task completion.

Lower initial work rates have also been attributed to an anticipatory response, which reduces metabolic heat production to ensure the exercise bout is completed efficiently and within homeostatic T_c range of 37°C (i.e., anticipatory theory; Cheung, 2007, Tucker et al., 2006, Tucker et al., 2004). This observation is supported by Tucker et al. (2006) who examined male cyclists performing a cycling time-to-exhaustion (TTE) at an RPE of 16 in three different temperatures (Cool: 15.1 ± 0.3°C, 68 ± 4% RH, 15°C AT, Normal: 24.9 ± 0.4°C, 66 ± 4% RH, 28°C AT, Hot: 35.2 ± 0.6°C, 65 ± 3% RH, 43°C AT). It was observed that PO declined over the course of the TTE at a higher rate in hot (2.35 ± 0.73 W·min-1) compared to cool (1.63 ± 0.70 W·min-1) and normal (1.61 ± 0.80 W·min-1) conditions, well before a critical T_{re} threshold was attained (Tucker et al., 2006). This supports previous findings by Tucker et al. (2004), who observed lower PO (~255 vs. 273 W) in the heat (35°C, 60% RH, 42°C AT) compared to the cooler condition (15°C, 60% RH, 15°C AT) in 10 male cyclists performing a 20-km cycling TT. Moreover, PO in the latter stages of the cycling TT (80-100% of total duration of TT) was lower in the heat compared to the cooler condition which may explain the significantly slower finish times in the heat (Tucker et al., 2004). However, knowledge regarding the environmental conditions where cooling has the greatest benefit to thermal comfort is limited.

2.4 Acute Heat Alleviation

To further assist autonomic and behavioural thermoregulatory processes in thermally stressful environments, acute heat alleviation techniques applied prior (i.e., PreC) or during competition (i.e., PerC) may assist in cooling the body. For example, cooling can be applied externally on the skin by immersing in cold or ice water or wearing external cooling garments (e.g., ice or gel vests), internally by ingesting cold fluids or crushed-ice, or by using a combination of external and internal cooling methods termed mixed-method cooling (Bongers et al., 2015, Marino, 2002, Quod et al., 2006). Pre-cooling is the process of reducing T_c and/or T_{sk} prior to an exercise bout with the aim to increase the heat storage capacity of the body (Marino, 2002, Wegmann et al., 2012). Previous meta-analytical reviews by Wegmann et al. (2012) and Bongers et al. (2017) found that PreC improves finish time, work completed, TTE and PO by 5.7% - 6.6% (effect size [ES] = 0.44 – 0.62). Moreover, a recent systematic review by Alhadad et al. (2019) found that PreC (Hedge's g = 1.01, 95% confidence intervals [CI] = 0.85 – 1.17) was more effective at lowering T_c before exercise bouts compared to HA (0.72, 0.58 – 0.86). Alternatively, PerC is applied during exercise or a break in exercise to blunt any potential rise in T_c and T_{sk} and improve perception to the heat (e.g., Th_c and Th_s; Bongers et al., 2015, Stevens et al., 2017, Tyler et al., 2015) leading to improved TT and TTE performance by up to 9.3% (ES = 0.35; Bongers et al., 2017).

2.4.1 External Cooling

The aim of external cooling is to decrease T_{sk}, resulting in greater T_g, therefore mitigating the rate of increase in T_c, and improving thermal perception to the heat (Bongers et al., 2017, Cuddy et al., 2014). A recent systematic review by Rodriguez et al. (2020) reported that external cooling (e.g., cold-water immersion [CWI], ice packs, iced towels, cooling gloves and cooling vest) results in faster completion time (0.5 – 5.8%) and improvements in distance covered (3.6 – 13.1%), mean PO (0.9 – 4.5%) and work (0.1 – 8.5%) compared to when no-cooling is applied.

Another review by Bongers et al. (2017) considered CWI as the most powerful method of external PreC as it creates a large temperature gradient between the skin and environment therefore enhancing heat dissipation (Bongers et al., 2017, Gibson et al., 2020). Furthermore, it has been observed to decrease T_c by 0.23 – 0.80°C when used as a PreC method (Booth et al., 1997, Maia-Lima et al., 2017, Moss et al., 2021, Stevens et al., 2017, Wilson et al., 2002). Maia-Lima et al. (2017) investigated the effects of 60 min intermittent PreC (i.e., 3 min CWI at 24°C followed by 3 min in a 25°C environment) on 30-km self-paced cycling performance in trained cyclists in the heat (35°C, 68% RH, 44°C AT). Their findings showed that a decrease in T_c and T_{sk} (-0.34 and -7.64°C, respectively) after CWI resulted in ~3% faster completion time in the heat compared to no PreC (Maia-Lima et al., 2017). However, a disadvantage of this method is that it presents considerable practical and logistical challenges (e.g., difficulty in keeping water cold or having access to large volumes of sanitary water), which increases the difficulty in applying this method as a PreC and PerC technique. Furthermore, it is also likely to cool the working muscles, which may be detrimental to performance due to lower muscle force and power production (Racinais et al., 2017, Ross et al., 2013). This is supported by Peiffer et al. (2010) who used whole body CWI (14°C) or resting in 35°C environment in between two 1-km cycling TT protocols in the heat (~35°C, ~40% RH, ~39°C AT). Their findings showed that whole body CWI lowered quadriceps muscle temperature (~-1.3°C), peak PO (~-86 W), and mean PO (~-24 W) in the second TT compared to the first TT (Peiffer et al., 2010).

Another external technique is placing cooled items on the body (e.g., ice vests, cool or frozen towels), prior to exercise (Bongers et al., 2017). Castle et al. (2011) applied ice packs on the anterior, lateral, and posterior areas of the thigh for 20 min prior to a 40 min cycling intermittent sprint protocol which led to reductions in T_{sk} and T_{re} (~-0.2°C) and ~4% increase in peak PO compared to no cooling. However, cooling vests may be considered as a more practical cooling method as it covers a larger body surface area and can be applied easily in the field (Bongers et al., 2017). Cooling vests have been shown to improve endurance performance by 3.4% (ES = 0.19) when applied prior to events (Bongers et al., 2017). This is supported by Bogerd et al. (2010) who concluded the application of a cooling vest significantly decreased T_{sk} (~-2.7°C), leading to ~8 min longer TTE in the heat (~29°C, ~80% RH, ~38°C AT) compared to no cooling. Likewise, 5-km treadmill TT in heat (~32°C, ~50% RH, ~36°C AT) improved by +1.1%, when a cooling vest was applied during a 38 min active warm up prior to exercise (Arngrimsson et al., 2004). Once again, lower T_{sk} and T_{re} were observed when the cooling vest was worn compared to the control group who wore a shirt (~-1.79°C and ~-0.21°C, respectively; Arngrimsson et al., 2004). Improvements in performance in both studies were attributed to a greater heat sink which attenuated the increase in T_{re} and thermal discomfort by increasing the temperature gradient between the skin and environment. This resulted in individuals exercising longer and/or at a higher intensity in the heat when external cooling was applied, compared to no external cooling (Arngrimsson et al., 2004, Bogerd et al., 2010).

Moreover, when cooling vests are used as a PerC technique, improvements in mean PO have been observed in the second half of intermittent cycling exercise when applied during the 15 min half-time break in the heat (~33°C, ~50% RH, ~38°C AT) compared to no cooling (~589 vs. ~561 W; Chaen et al., 2019). This supports previous findings by Cuttell et al. (2016) who observed longer TTE (exercise maintained at 60% peak PO) when a cooling vest was worn (~32 min) compared to a cooling collar (~30 min) or no cooling (~28 min) in the heat (~35°C, ~50% RH, ~41°C AT). Interestingly, no differences in T_c and T_{sk} between conditions were observed (Cuttell et al., 2016). However, Th_s (9-point scale; Hardy & Rejeski, 1989) for the torso region when the cooling vest was used was lower (median Th_s = 3.5 to 6.3) from 5 min of exercise until exhaustion compared to the cooling collar (5.5 to 8.0) or no cooling (6.0 to 8.0). This may explain the longer TTE in the cooling vest trial as lower T_{sk} and thermal perceptions levels have previously been observed to improve performance (Schlader et al., 2011).

2.4.2 Internal Cooling

Internal cooling aims to blunt the rise in T_c by creating a heat sink through direct cooling of the internal viscera via ingestion of cold fluids or crushed-ice to improve exercise performance in heat (Gibson et al., 2020, Rodriguez et al., 2020, Ross et al., 2013). Ice also requires a larger amount of energy to be converted from a solid into a fluid (334 $J \cdot g^{-1}$), and therefore may have a greater cooling capacity when ingested in the form of crushed-ice compared to cold fluid (Ihsan et al., 2010). In addition to thermoregulatory benefits, internal cooling methods have the capacity to provide a source of hydration and nutrition for athletes before or during events (e.g., addition of carbohydrates and electrolytes in fluids or crushed-ice; Gibson et al., 2020).

Ingestion of ~900 mL of cold water (2-4°C) 30 min prior to prolonged endurance exercise in the heat (32-35°C, 60% RH, 38-42°C AT) has been shown to reduce T_c or T_{re} (~0.4°C and ~0.5°C, respectively), and improve Th_s and Th_c. This resulted in ~3% greater distance cycled during a 30 min cycling TT and ~20% longer TTE in the heat (Byrne et al., 2011, Lee et al., 2008). Crushed-ice ingestion has also been shown to induce similar benefits to physiological variables and performance with less quantity (Ihsan et al., 2010, Zimmermann et al., 2018). For example, Ihsan et al. (2010) instructed endurance trained males to ingest 6.8 g·kg body mass (BM)$^{-1}$ of crushed-ice (~476 g for a 70 kg individual) 30 min prior to completing a 40-km cycling TT in heat (30°C, 75% RH, 37°C AT). They observed a ~1.1°C reduction in T_c and resulted in ~6.5% faster completion time compared to no PreC. Researchers concluded improvements in performance were due to the greater energy requirement to convert ice to a liquid, which may have augmented the heat storage capacity of the body and attenuated rising T_c during the initial stages of the cycling TT (Ihsan et al., 2010).

When cold water (4°C) was ingested ad libitum during a 40-km cycling TT in the heat (35°C, 60% RH, 42°C AT), Maunder et al. (2017) observed faster completion times compared to crushed-ice ingestion (~60 vs. ~62 min). It must be noted that no significant differences in physiological variables (e.g., T_{re}, mean T_{sk}, heart rate, and sweat loss) were observed between each intervention however, less crushed-ice was ingested compared to cold water (~0.81 vs. ~1.09 L). In addition to this, participants reported greater ratings of discomfort when crushed-ice was ingested compared to cold-fluid (d = 0.31 – 0.95). As there was no control group included in the study, conclusions on the effects of crushed-ice ingestion during prolonged endurance performance cannot be made. A recent study by Morito et al. (2022) however, observed greater peak PO in the 2nd half of high intensity interval exercise in the heat (29°C, 50% RH, 32°C AT) in rugby union players after 450 g of crushed-ice (-2°C) was ingested during half-time compared to ingesting a 30°C beverage.

To induce beneficial changes to physiological variables and in turn performance, cold fluid ingestion requires larger quantities to be ingested where less is required for crushed-ice ingestion. However, crushed-ice ingestion has been shown to increase levels of discomfort which may negatively affect performance, therefore it is recommended that the intervention be tested during training before it is applied prior to or during competition.

2.4.3 Mixed-method Cooling

Mixed-method cooling proposes a more vigorous approach to cooling by combining external and/or internal cooling methods to cool the body simultaneously (Bongers et al., 2017, Ross et al., 2013). A systematic review by Bongers et al. (2017) showed that mixed-method PreC was a more effective method of improving exercise (endurance and intermittent sprint performance pooled) performance in heat (7.3% improvement, $d = 0.72$) compared to using external (e.g., CWI; 6.5%, $d = 0.49$) or internal (e.g., cold-fluid or crushed-ice ingestion; 6.3%, $d = 0.40$) methods alone. This is supported by Aldous et al. (2019) who investigated the effects of 30 min mixed-method PreC (7.5 $g \cdot kg$ BM^{-1} crushed-ice ingestion and ice packs on the quadriceps and hamstrings) and 15 min half-time cooling on individualised soccer-specific simulation (2 x 45 min halves comprised of three 15 min exercise blocks) on a treadmill in heat (30°C). Their findings showed mixed-method PreC reduced T_{re} (~-0.6°C), T_{sk} (~-1.1°C) and Th_s (~-1.0 units) and remained lower throughout the first half of exercise. In addition, mixed-method PreC resulted in greater total (~+108 m), high speed (~+56 m), and variable (~+15 m) running distances covered during the first half of exercise compared to no-cooling (Aldous et al., 2019). This supports previous findings by Brade et al. (2012) who observed higher total mean PO (~972 vs. ~882 W) and work (~234 vs. ~212 kJ) when mixed-method PreC (7 g·kg BM^{-1} crushed-ice + cooling vest) was used, compared to only crushed-ice ingestion. Similar findings have been observed for endurance performance in heat (46.4 km cycling TT in 32 – 35°C, 50 – 60% RH, 42°C AT) preceded by 30 min mixed-method PreC (14 g·kg BM^{-1} crushed-ice ingestion + iced towel; Ross et al., 2011). Findings from a pilot study conducted prior to the main study (as stated by Ross et al. (2011)) showed large reductions in T_{re} when mixed-method PreC (1.0 L crushed-ice ingestion + iced towels) was used compared to crushed-ice ingestion alone (~-0.72 vs. ~-0.60°C). These findings were then used to investigate the efficacy of mixed-method PreC (14 g·kg BM^{-1} crushed-ice ingestion + iced towel) on 46.4 km cycling TT in the heat. Results showed higher mean PO (~284 W) and faster completion times (~79.10 min) when mixed-method PreC was used compared to 10°C CWI alone (~279 W; ~79.70 min), and no cooling (~276 W; ~80.20 min; Ross et al., 2011). Although the studies discussed above utilised mixed-method PreC, data regarding reductions in T_{re} and T_{sk} were limited or not provided (Aldous et al., 2019, Ross et al., 2011). For example, qualitative conclusions (e.g., likely reductions in T_{re}/T_{sk}) make it difficult to determine the minimum reductions in T_{re} and/or T_{sk} required to have a positive effect on exercise performance in heat. Furthermore, Aldous et al. (2019) and Ross et al. (2011) investigated the effects of mixed-method PreC and/or other cooling methods in one type of environment (hot-dry or hot-humid) only. This indicates that knowledge regarding the environmental conditions where mixed-method PreC has the greatest benefit on endurance performance is lacking.

2.4.4 What's Best? Internal, External, or Mixed-method Cooling?

Current PreC recommendations for endurance performance in heat involve the use of different combinations of internal and external cooling methods to reduce T_c, T_{sk}, and muscle temperature (Taylor et al., 2020). However, systematic and meta-analytical reviews by Bongers et al. (2017) and Ross et al. (2013) have outlined that mixed-method PreC results in greater performance gains (endurance and intermittent sprint performance pooled) compared to using internal or external cooling methods in isolation. Mixed-method PreC may be considered a more efficient approach as it is able to cool athletes externally and internally (T_c or T_{re} reduced by ~0.3 – 0.7°C), creating a larger temperature gradient between the body and environment, leading to improvements in performance (Bongers et al., 2017, Brade et al., 2012, Ross et al., 2013). Nevertheless, knowledge regarding the environmental conditions where mixed-method PreC has the greatest benefit on endurance performance is lacking.

Alternatively, PerC recommendations for endurance performance involve any cooling combinations which: (1) decrease T_{sk}, (2) benefit Th_s/Th_c responses and, (3) attenuates the increase in T_c (Taylor et al., 2020, Tyler et al., 2015). An example may be ingesting cold water every 10 min or wearing an ice-cold hat or neck towels. However, these recommendations are derived from studies analysed in reviews and meta-analyses looking at the effects of PreC and PerC in one thermal condition only. This increases the difficulty in comparing the effects of PreC and PerC across several different environments using the same protocol.

2.5 Chronic Heat Alleviation

Heat acclimation is the process of intentional and frequent exercise (active) or exposure (passive) in artificially hot conditions (e.g., inside a climate chamber). Alternatively, heat acclimatisation follows the same process as heat acclimation but is performed in the natural environment (Sawka et al., 2011). Both methods aim to increase T_c (≥38.5°C) and T_{sk}, skin blood flow and profuse sweating, which has been considered as the primary effectors for adaptations to heat occurring (Périard et al., 2021). Specifically this stimulus leads to the attainment of physiological (e.g., lower exercising HR, T_{sk} and T_c, expansion of blood plasma volume and increased sweat rate) and perceptual (e.g., improved thermal comfort and sensation) adaptations that might improve endurance exercise performance in hot environments (Benjamin et al., 2019, Gibson et al., 2020, Sawka et al., 2011). Confirmation of HA status is commonly achieved by performing a heat stress test (HST), typically involving fixed-intensity exercises (e.g., fixed-speed or percentage of $\dot{V}O_{2max}$; Gibson et al., 2015, Mikkelsen et al., 2019) before, and after a HA protocol to observe any heat adaptations post-acclimation (Daanen et al., 2018, Périard et al., 2021). Self-paced exercises may also be used as an HST, however this method is less common due it's increased difficulty in detecting adaptations, as external (e.g., PO) and internal (e.g., HR) loads may change in similar proportions.

Traditionally HA has been achieved by exposing athletes to ≥30°C environments whilst working constantly at 50 – 60% $\dot{V}O_{2max}$ for approximately

60 to 120 min over ≥15 days (i.e., long-term), 8 – 14 days (i.e., medium-term), or ≤7 days (i.e., short-term) with no more than 2 – 3 days between exposures (Garrett et al., 2011, Périard et al., 2021). Short-term HA has been shown to only partially acclimate athletes to heat (Petersen et al., 2010). Petersen et al. (2010) investigated the effects of 4 days HA (~30-45 min repeat-sprint) in the heat (~30°C, ~61%, ~35°C AT) and found lower HR (~-11 bpm), and lower sweat electrolyte concentrations (Sodium: ~-19%, Potassium: ~-16%, and Chloride: ~-22%) post-acclimation. No difference in T_c and T_{sk} was observed. It is recommended that at least 14 days of HA are required to maximise the adaptations to heat as peak improvements in performance (e.g., exercise capacity) and physiological variables are observed after this time period (Périard et al., 2021). This is supported by Nielsen et al. (1993) who investigated the effects of 9 – 12 days HA exercising for 90 min (60% $\dot{V}O_{2max}$) in the heat (40°C, 10% RH, 38°C AT). Authors found longer cycling TTE (~+32 min), greater sweat rate (~+2.8 $g \cdot min^{-1}$), and lower mean face and chest T_{sk} (~-0.5°C) on the last day of HA compared to the first day (Nielsen et al., 1993). Various methods can be utilised for HA and include: controlled-hyperthermia (isothermic HA), fixed-intensity, self-regulated, intermittent exercise, or passive (Daanen et al., 2018).

2.5.1 Controlled-Hyperthermia (Isothermic Heat Acclimation)

Controlled-hyperthermia or isothermic HA is the achievement and maintenance of an elevated T_c during HA training (Fox et al., 1967). One advantage of this method is that it ensures the stimulus or thermal strain required to induce adaptations is maintained in each HA session (Fox et al., 1967, Périard et al., 2021). Core temperature ≥ 38.5°C has been considered as the primary effector to induce physiological adaptations to heat and may be achieved through a passive or active heat stress phase in a HA protocol, and maintained through periods of rest and exercise including manipulations in external work (Daanen et al., 2018). Likewise, Gibson et al. (2015) acclimated 24 male participants by implementing a 90 min cycling protocol in the heat (~40°C, ~39% RH, ~46°C AT) over 5-10 sessions. A target T_{re} ≥ 38.5°C was achieved by initially cycling at 65% $\dot{V}O_{2max}$, and then maintained by adjusting workload every 5 min for the remainder of the sessions (± 5% $\dot{V}O_{2max}$ or seated rest). A HST performed 48 h after the HA protocol (30 min running at 9 $km \cdot h^{-1}$ at 2% incline), showed a decrease in exercise HR (~-10 bpm), T_c (~-0.2°C), T_{sk} (~-0.51°C) and increased sweat rate (~+0.36 $L \cdot h^{-1}$) compared to baseline (Gibson et al., 2015).

2.5.2 Constant Work Rate or Intensity

Fixed-external-load HA involves exercising at a pre-selected intensity for the whole duration of a HA program (Périard et al., 2021). For instance, Mikkelsen et al. (2019) acclimated their athletes by implementing five weekly 60 min heat training sessions (cycling at 60% $\dot{V}O_{2max}$) over 5½ weeks in the heat (35 – 40°C, 30% RH, 43°C AT). Results from a HST (cycling until volitional exhaustion at 60% $\dot{V}O_{2max}$ in 40°C conditions) on day 1, 14, and 28 showed reduced end-training HR (-~14 bpm), increased plasma volume (+~6.5%), and longer exercise TTE (~+26 min) on day 28 compared to day 1 (Mikkelsen et al., 2019). However, it is important to note that a limitation of fixed-external-load HA protocols is that the stimulus for adaptation diminishes in the early stages of the HA program as athletes acclimate to the environment (Gibson et al., 2020). Therefore, changes in temperature, exercise intensity or duration of exposure to heat is essential to ensure athletes are exposed to similar levels of physiological strain as the HA protocol progresses which induces adaptations (Gibson et al., 2020).

To ensure a maintenance of thermal stimulus without the need to change external variables, Périard et al. (2015) proposed the use of a fixed-internal-load which identifies the HR associated with a specific percentage of $\dot{V}O_{2max}$. An advantage of this method is that absolute work rate required to maintain a given heart rate increases as adaptations occur. Travers et al. (2020) provides support to this approach and acclimated recreationally trained males over 10 days (90 min cycling at HR equivalent to ~65% $\dot{V}O_{2max}$). Findings post-acclimation found partial HA evident by lower T_{sk} (~-0.6°C), increased sweat rate (~+0.19 $L \cdot h^{-1}$), and greater mean PO for the 30 min cycling TT (~+19 W) compared to day 1.

Constant work rate or intensity are effective methods to induce HA in individuals. However, a possible disadvantage of this method is the low relative external- or internal-load prescribed for training. This may pose as a barrier for coaches in situations where high relative or absolute training intensity must be maintained.

2.5.3 Passive Heat Acclimation

Adaptations to the heat may also be achieved whilst resting in heat (i.e., passive HA), as an elevated T_c can still be attained through the use of a heat chamber (>45°C), sauna (70-90°C), or hot-water immersion (40°C; Heathcote et al., 2018, Leppaluoto et al., 1986, Périard et al., 2021). For example, Leppaluoto et al. (1986) observed lower HR (~-8 bpm) and T_{re} (~-0.50°C) after a daily total of 2 hours seated rest in a sauna (80°C) over seven days. Likewise, Zurawlew et al. (2016) investigated whether 6 days hot-water immersion (40 min at 40°C) after exercise in a temperate environment (40 min treadmill run at 65% $\dot{V}O_{2max}$ in 18°C, 40% RH, 18°C AT) induced adaptations to the heat. Their findings showed lower resting T_{re} (~-0.27°C) and 4.9% faster 5-km run TT finish times post-acclimation in the heat (33°C, 40% RH). Passive HA may be used as an alternative method of HA for athletes who reside in cooler conditions or if there is no access to a heat chamber (Gibson et al., 2020, Heathcote et al., 2018). However, direct comparisons between active and passive HA to determine which method may be more beneficial for acclimating individuals are lacking.

2.5.4 Self-regulated Heat Acclimation

Heat acclimation with self-paced exercise is another approach which allows athletes to freely adjust their pacing to attain or maintain intensity around a prescribed prescription during HA sessions. An early investigation by Armstrong et al. (1986) evaluated the effectiveness of 8 days self-regulated HA on male soldiers (9 x 5-10 min treadmill exercise with 2-10 min rest breaks in ~41°C, ~39% RH, ~46°C AT conditions). Results from the HST (100 min variable intensity treadmill running) showed lower final HR (~-26 bpm), T_{re} (~-0.65°C) and T_{sk} (~-1.05°C) on day 8 compared to day 1. Despite this, Armstrong et al. (1986) concluded that self-paced efforts during the training did not protect participants from exceeding safety limits (HR > 180 bpm, T_{re} >

39.5°C) and experiencing symptoms of heat illness. In fact, it was reported that ~79% of the participants in the study experienced more than one symptom of heat illness (i.e., dizziness, vomiting, etc.) during the sessions. However, it must be noted that research on self-regulated HA as a method to acclimate endurance athletes is limited. It is possible that this method is not commonly used as high T_{sk} from the heat has previously been shown to result in lower self-selected exercise intensities, which may not provide sufficient stimulus for HA to occur (Schlader et al., 2011). Although it may seem contradictory to the aims of HA, the application of PreC or PerC during HA training sessions may provide an ergogenic benefit to self-paced performance in heat. This could lead to greater self-selected exercise intensities, providing similar physiological stimuli as those induced by traditional continuous exposure to the heat.

2.6 Combining Cooling and Heat acclimation/acclimatisation

Previous research supports the use of HA or cooling (PreC or PerC) to improve endurance performance in hot-humid conditions (Périard et al., 2021, van de Kerkhof et al., 2023, Zimmermann et al., 2018). Both methods have been shown to reduce the thermal strain experienced by individuals, specifically through inducing heat adaptations (i.e., HA), and increasing heat storage capacity and improving thermal perception (i.e., PreC). Theoretically, combining both methods (i.e., applying cooling during HA training or on heat acclimated individuals) may further decrease T_c and/or T_{sk}, improve perceptual responses and result in improved endurance exercise performance in heat.

The application of cooling techniques during HA training may maintain or improve training quality compared to traditional continuous exposure to the heat by increasing heat storage capacity and improving athletes' perception to heat (Bongers et al., 2017). This idea is supported by previous research investigating the use of regular PreC (30 min ~21°C cold-water immersion) prior to 10 HA training sessions in the heat (60 min cycling at RPE15 in ~35°C, ~53% RH, ~41°C AT; Choo et al., 2020). Findings showed no difference in mean PO between session 1 and 10 when PreC was applied (~166 *vs.* ~173 W) compared to no PreC (~168 *vs.* ~157 W). Heat stress tests (25 min cycling at 60% $\dot{V}O_{2max}$ followed by 20-km cycling TT in same conditions) confirmed partial HA through observation of lower resting T_{re} (~-0.3°C) and exercising HR (~-5 bpm) post-HA compared to pre-HA when PreC was applied. Similarly, Naito et al. (2022) found that using PerC (1.25 and 7.5 g·kg BM^{-1} crushed-ice ingestion during breaks and half-time, respectively) during five HA sessions (80 min intermittent repeated sprint protocol in ~36.5°C, ~50% RH conditions) resulted in lower resting T_{re} (~-0.3°C) post-acclimation, and ~+3.3% greater total work done on day 5 of the HA sessions compared to no PerC. It must be noted however, that both studies by Choo et al. (2020) and Naito et al. (2022) only observed lower resting T_c and T_{re} (~-0.3°C, and ~-0.2°C, respectively) post-acclimation compared to pre-acclimation. No evidence of acclimation was observed for other physiological variables such as HR, T_{sk}, and sweat rate or loss. The absence of evidence of full HA may be attributed to the insufficient magnitude of the heat stimulus. This includes the combination of exercise tasks (duration: 60 to 80 min, mode: repeat sprint vs. self-paced endurance) and environmental conditions (35-37°C and 50-53% RH) during the training period, thus preventing the achievement of HA.

Previous research which applied PreC to heat acclimated individuals prior to intermittent exercise (i.e., intermittent-sprint or repeat sprint efforts) in heat found no further benefits to performance compared to no PreC (Brade et al., 2013a, Castle et al., 2011). Castle et al. (2011) investigated the effects of HA (10 days cycling at 50% $\dot{V}O_{2max}$ for 60 min) with and without PreC (ice pack on thighs for 20 min) prior to intermittent cycling sprint in the heat (~33°C, ~52% RH, ~38°C AT). Findings showed lower resting T_{re} (~-0.4°C), lower exercising HR (~-18 bpm), Th_s (~-1 units), and RPE (~-2 units) on day 10 compared to day 1. Subsequently, 2% higher peak PO was observed during intermittent cycling sprint performance post-acclimation (Castle et al., 2011). However, when PreC was also applied, no additional benefit to cycling sprint performance were observed (Castle et al., 2011). Similarly, Schmit et al. (2017) investigated the effects of PreC (cooling vest) in heat acclimatised triathletes performing a 20-km cycling TT in heat (35°C, 50% RH). Their findings showed that PreC had unclear effects on 20-km cycling TT completion time post-HA (~+4 W). These findings support previous suggestions that PreC may only be of benefit when the thermal stress (combination of endogenous, air temperature and humidity, and exogenous, acclimation status) experienced by the individual is of a sufficient level (Brade et al., 2013a, Castle et al., 2011, Duffield & Marino, 2007, Schmit et al., 2017). The absence of evidence of PreC to benefit performance in heat may be attributed to the insufficient magnitude of the heat stimulus. This includes the combination of exercise tasks (duration:

30 to 60 min, mode: repeat sprint vs. self-paced endurance) and environmental conditions (~33-35°C and ~50-52% RH) during the training period, thus preventing the achievement of HA. This highlights the need for further research to determine the environmental conditions where cooling may provide benefit and ascertain if the application of PreC on heat acclimatised individuals at that environmental temperature will provide further benefits to performance compared to no PreC.

2.7 Conclusion

Acute (i.e., PreC and/or PerC) and chronic heat alleviation (i.e., HA) methods may be used to combat the negative effects of the heat on endurance exercise performance. However, knowledge regarding which environment these provide the greatest benefit to endurance exercise performance is lacking. Furthermore, IHE has not been used to acclimate athletes to the heat, although the benefits to an athlete’s perception can lead to improvements in the quality of training sessions.

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Chapter 3: Study One

Mixed-method pre-cooling enhances self-paced 20-km cycling time-trial performance when apparent temperature is >46°C, but may not be a priority in <46°C.

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3.1 Abstract

Purpose: Precooling (PreC) may only benefit performance when thermal strain experienced by an individual is sufficiently high. We explored the effect of mixed-method PreC on 20-km cycling time-trial (CTT) performance under 3 different apparent temperatures (AT). ***Methods***: On separate days, 12 trained or highly trained male cyclists/triathletes completed six 20-km CTTs in 3 different ATs: hot-dry (35 °C AT), moderately hot-humid (40 °C AT), and hot-humid (46 °C AT). All trials were preceded by 30 minutes of mixed-method PreC or no PreC (control [CON]). ***Results***: Faster 2.5-km-split completion times occurred in PreC compared with CON in 46 °C AT (P = .02), but not in 40 °C AT (P = .62) or 35 °C AT (P = .57). PreC did not affect rectal and body temperature during the 20-km CTT. Skin temperature was lower throughout the CTT in PreC compared with CON in 46 °C AT (P = .01), but not in 40 °C AT (P = 1.00) and 35 °C AT (P = 1.00). Heart rate had a greater rate of increase during the CTT for PreC compared with CON in 46 °C AT (P = .01), but not in 40 °C AT (P = .57) and 35 °C AT (P = 1.00). Ratings of perceived exertion (P < .001) and thermal comfort (P = .04) were lower for PreC compared with CON in 46 °C AT only, while thermal sensation was not different between PreC and CON. ***Conclusion***: Mixed-method PreC should be applied prior to 20-km CTTs conducted in hot-humid conditions (≥46 °C AT). Alternatively, mixed-method PreC may be a priority in moderately hot-humid (~40 °C AT) conditions but should not be in hot-dry (~35 °C AT) conditions for 20-km CTT.

Key Words**:** cooling, heat stress, self-paced exercise

3.2 Introduction

Worldwide, competitive endurance events regularly take place in hot-dry (≥30°C, ≤40% relative humidity [RH]) and hot-humid (≥30°C, >40% RH) conditions. Compared to thermoneutral conditions (20–25°C), competing in hot-dry and hot-humid environmental conditions is often associated with slower finish times, lower sustained power output, and increased risk of exertional heat illness.[1-3]

Pre-cooling (PreC) techniques, including external cooling (e.g., cooling vest), internal cooling (e.g., ice ingestion), or a combination of both (mixed-method), have been used to combat the negative effects of heat, subsequently improving exercise performance.[4] Mixed-method PreC is considered more effective in maintaining or improving exercise performance in the heat (7.3% improvement, effect size=0.72) when compared to external (6.5%, effect size=0.49) or internal (6.3%, effect size=0.40) methods alone.[4] Studies show that improvements in endurance exercise performance (~30–90-min) after PreC are observed in environmental conditions exceeding 27°C and RH above 50%.[5-7] Conversely, no performance improvement is observed in less thermally challenging conditions (≤24°C, ≤68% RH; ~55–60-min).[6,7] Due to high variability in ambient temperatures and RH used in PreC literature, comparisons are difficult when two environmental constructs need considering and overlook how that environment feels.

Apparent temperature (AT), represents temperature in terms of how it is perceived using both RH and ambient temperature (e.g., 40°C, 40% RH feels like 46°C).[8] Therefore, using AT improves our understanding of the thermal conditions required for PreC to improve exercise performance. For instance, a study by Faulkner, et al.,[6] explored the effects of PreC (30-min cooling vest + sleeve) on a ~60-min cycling time-trial (CTT) across varying temperatures. They showed faster CTT completion time under the hottest condition (35°C, 50% RH), while no improvement was observed under the coolest condition (24°C, 50% RH). Using AT unveils ~16°C AT difference between the hottest (41°C AT) and coolest (25°C AT) conditions compared to an absolute 11°C air temperature difference. This outcome is unsurprising, considering that heightened RH, alongside elevated temperatures, can exacerbate thermal strain as the impaired evaporation of sweat limits heat loss due to low water vapor pressure difference between the environment and skin.[9] Consequently, several factors such as cardiovascular function (increased skin blood flow and decreased stroke volume), central neural drive (heightened perception of effort), and skeletal muscle function and metabolism (increased carbohydrate oxidation) are adversely impacted.[3]

Researchers have yet to determine the specific AT conditions necessary for mixed-method PreC to have the largest positive effect on maximal, self-paced endurance cycling performance. Therefore, we aimed to investigate the effect of mixed-method PreC on self-paced endurance performance in non-heat acclimatised individuals exposed to three different AT conditions, and accompanying thermal, cardiovascular, and perceptual responses. We hypothesised that mixed-method PreC would yield greater benefits for 20-km CTT performance under the highest AT.

3.3 Methods

Participants

Twelve male cyclists and/or triathletes (mean±SD: age=35.8±8.4 yrs; body-mass (BM)=80.23±13.27 kg; height=178.19±7.49 cm; sum of eight skinfolds=77.27±26.57 mm; body surface area=1.96±0.14 m^2,[10]) provided written informed consent to participate. All athletes corresponded with an athlete classification 2 or 3 (trained/highly trained).[11] Participants were not involved in a heat acclimation protocol, did not train in a heated environment (>30°C), and had not resided in a summer environment in the previous month (non-heat acclimatised). The study was approved by Curtin University Human Research Ethics Committee (HREC2020-0685). No participant dropout occurred in the study.

Study Overview

Testing occurred during the cooler months in Western Australia (March – October) to minimise exposure to high environmental temperatures and RH, which may induce heat adaptations and potentially influence the effectiveness of the PreC intervention. Participants completed a familiarisation session and six experimental sessions, in random order, separated by 7±2 days. Experimental sessions took place under three thermal conditions: hot-dry (31°C, 49% RH, 35°C AT), moderately hot-humid (34°C, 55% RH, 40°C AT), and hot-humid (36°C, 71% RH, 46°C AT). Experimental sessions consisted of 30 min of mixed-method PreC (crushed-ice ingestion and cooling vest; PreC) or a no cooling control (CON) in a thermoneutral environment (22°C, 57% RH, 23°C AT), followed by a maximal, self-paced 20-km cycling TT in a heat chamber. Participants avoided vigorous exercise, alcohol intake (24-h) and caffeine consumption (6-h) prior to testing. They were also instructed to replicate a similar diet from 24-h prior to their first experimental session using a food diary for nutritional consistency. To ensure adequate hydration, participants were instructed to drink 6 $mL\cdot kg^{-1}$ BM of water every 2.5-h on the day before experimental sessions.

Familiarisation Session

Anthropometric measures were obtained during the familiarisation session. Standing height (Holtain Limited, Crymych, United Kingdom) and BM (Seca 360° Wireless, Hamburg, Germany) were recorded. Skinfold thickness was assessed using International Society for the Advancement of Kinanthropometry (ISAK) standardised methodology at eight sites (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, and medial calf). Calibrated Harpenden skinfold calipers (Baty International, West Sussex, UK) were used by an ISAK accredited Level 1 Anthropometrist (intra-technical error measurement = 2.9%). Subsequently, participants underwent 30 min mixed-method PreC in a thermoneutral environment followed by a 10 min warm-up in a heat chamber (34°C, 55% RH, 40°C AT). After the warm-up, participants performed a 20-km cycling TT in the heat chamber on a cycling ergometer (BikeErg, Concept 2, Queensland, Australia) to familiarise themselves with the exercise protocol.

Experimental Session

Upon arrival at the laboratory, participants' hydration status was determined using urine specific gravity (USG; Portable Refractometer, RS PRO, RS Components, New South Wales, Australia). Participants with a USG reading

>1.021 ingested 600-mL of water and then re-tested after 5-min. This process was repeated until USG readings were ≤1.021. Nude BM was measured pre- and post-experimental session on a set of calibrated electronic scales (Seca 360° Wireless, Seca, Birmingham, United Kingdom) to calculate sweat loss (pre – post nude BM + fluid ingested). A rectal thermistor (Mon-a-Therm™ General Purpose Probe, 400TM, Medtronic, Minneapolis, USA) was then self-inserted 10-cm past the anal sphincter for measurement of rectal temperature (T_{re}; SQ2010 data logger, Grant Instruments, Cambridgeshire, UK). Surface skin temperature (T_{sk}) was measured by placing dermal temperature sensors (DS1922L; iButton™, Maxim Integrated Products, California, USA) on the mesosternale, mid-forearm, mid-quadriceps and medial calf to calculate mean T_{sk}.[12] A heart rate (HR) monitor (A300 Polar Electro, Kempele, Finland) was placed on the participants' chest to measure HR. During the PreC sessions, participants sat in a thermoneutral environment while wearing an activated cooling vest (as per manufacturer's instructions, Arctic Heat, Queensland, Australia). At the same time, crushed-ice (made with an ice shaver, Avalanche Model IS6800, Sunbeam, New South Wales, Australia) was ingested at 2.3 $g \cdot kg^{-1}$ BM every 10-min over a 30-min period (total of 7 $g \cdot kg^{-1}$ BM) to ensure consistent ingestion rates between sessions.[13] Throughout the CON sessions, participants sat in a thermoneutral environment for 30-min wearing a non-activated cooling vest.

Following 30-min PreC or CON period, participants entered the heat chamber and completed a 10-min cycling warm-up at a perceptually regulated exercise intensity (rating of perceived exertion [RPE] 0-10 Borg scale of 5, 7, and 8 for

5, 3, and 2-min, respectively)[14]. This was followed by a self-paced 20-km CTT on a cycle ergometer at the participants' highest sustainable intensity. Participants only received feedback on the distance completed throughout the CTT and were allowed to drink heat chamber room temperature water *ad libitum*. During the PreC period, HR, T_{re}, and T_{sk} data were collected every 5-min. During the warm-up, these measurements were taken every 2-min, and every 2.5-km distance completed during the 20-km CTT. Thermal comfort (0 = very comfortable, 20 = very uncomfortable; Th_c) and sensation (0 = very cold, 20 = very hot; Th_s) were recorded every 5-min during the PreC period, pre- and post-warm up, and every 2.5-km distance completed during the 20-km CTT [15]. Similarly, RPE (6–20 scale)[16] was obtained every 2.5-km distance completed during the 20-km CTT. Exercise performance was assessed by measuring split-times every 2.5-km of distance completed and completion time for the 20-km CTT.

3.4 Data and Statistical Analyses

Body temperature (T_b) was calculated using the equation: $0.65 \times T_{re} + 0.35 \times T_{sk}$.[17] Intraclass correlation coefficients (ICC) were calculated for all variables to determine the percentage of variance explained by individual differences between participants. When ICC values exceed .5, analysts are advised to utilise statistical models that account for non-independence in the data.[18] Data sampled repeatedly was not independent and ICCs in 7 of 20 outcomes were >.5 (see supplementary material) thus multilevel modelling was used to analyse data. Multilevel modelling analysed the relationship between T_{re}, mean T_{sk}, T_b, HR, RPE, Th_c, Th_s and total and split completion times, with estimates reported as unstandardized regression coefficients (β), which indicate the amount of change (in the unit of measurement e.g., BPM, ˚C) between different levels of categorical independent variables (i.e., AT condition, PreC intervention) or per second increase in time.[19] Additionally, linear mixed-effects regression model was used to determine differences in hydration, total fluid ingested, sweat loss, and completion time. Where significant two- or three-way interaction effects were identified, post-hoc simple slopes analyses with adjustments for multiple comparisons were conducted to compare the relationship between distance/time, intervention and condition pairings reported as β coefficients, t-value, and degrees of freedom. Key findings are summarised in text and full results and 95% confidence intervals (CI) are provided as supplementary material. All analyses were conducted using R (R Foundation for Statistical Computing, Austria) with significance accepted at p<.05.

3.5 Results

Hydration and Sweat Loss

Urine specific gravity was greater in CON compared to PreC in 35°C AT (1.012±0.007 *vs* 1.010±0.007, β=-.001, p<.001) and 46°C AT (1.010±0.006 *vs* 1.008±0.006, β=-.002, p<.001). Alternatively, lower USG was observed in CON compared PreC in 40°C AT (1.009±0.008 *vs* 1.010±0.007, β=.001, p<.001).

Greater total fluid ingestion (i.e., total ice + water ingested during PreC and total water ingested during CON) was observed for PreC compared to CON in

35°C AT (1.08 *vs* 0.85 L, β=.241, p<.001), 40°C AT (1.31 *vs* 1.14 L, β=.189, p<.001), and 46°C AT (1.45 *vs* 1.23 L, β=.231, p<.001).

Lower sweat loss was observed for PreC compared to CON in 35°C AT (1.11 *vs* 1.25 L, β=-0.151, p<.001) and 40°C AT (1.31 *vs* 1.53 L, β=-0.172, p<.001), while higher sweat loss was noted for PreC compared to CON in 46°C AT (1.53 *vs* 1.48 L, β=.061, p<.001).

Performance Data

Total completion time is summarised in Table 3.1. There was a significant three-way interaction between condition, distance, and intervention for split completion times in 40 (β=-1.115, p=.024) and 46°C AT (β=-1.717, p<.001). Post-hoc analysis (Figure 3.1) indicated faster split completion times over the course of the cycling TT in PreC compared to CON in 46°C AT (β=1.146, t(595)=3.23, p=.016), but not in 40 (β=.544, t(595)=-1.64, p=.623) or 35°C AT (β=-.571, t(595)=1.56, p=.571). Comparisons between PreC conditions showed split completion times increased over the course of the cycling TT in 46°C AT, which was significantly different to the pattern of decreasing split times across the cycling TT in 40°C AT (β=-1.304, t(595)=-3.72, p=.003), but was not significantly different from 35°C AT (β=-.929, t(595)=-2.648, p=.088). Comparisons between CON conditions showed split completion times increased over the course of the cycling TT in 46°C AT compared to both 40 (β=-1.906, t(595)=-5.42, p<.001) and 35°C AT (β=-2.646, t(595)=-7.53, p<.001).

Table 3.1. 20-km cycling time-trial (CTT) completion time in 35, 40, and 46°C apparent temperature (AT) conditions after control (CON) or pre-cooling (PreC) interventions.

	20-km cycling TT Completion Time (s)	
Thermal Conditions	CON	PreC
35°C AT	2434 ± 182[b]	2502 ± 231*
40°C AT	2474 ± 172[a]	2470 ± 188
46°C AT	2652 ± 237[a,b]	2557 ± 256[*,b]

*Significantly different from control, [a]Significantly different from 35°C AT, [b]Significantly different from 40°C AT.

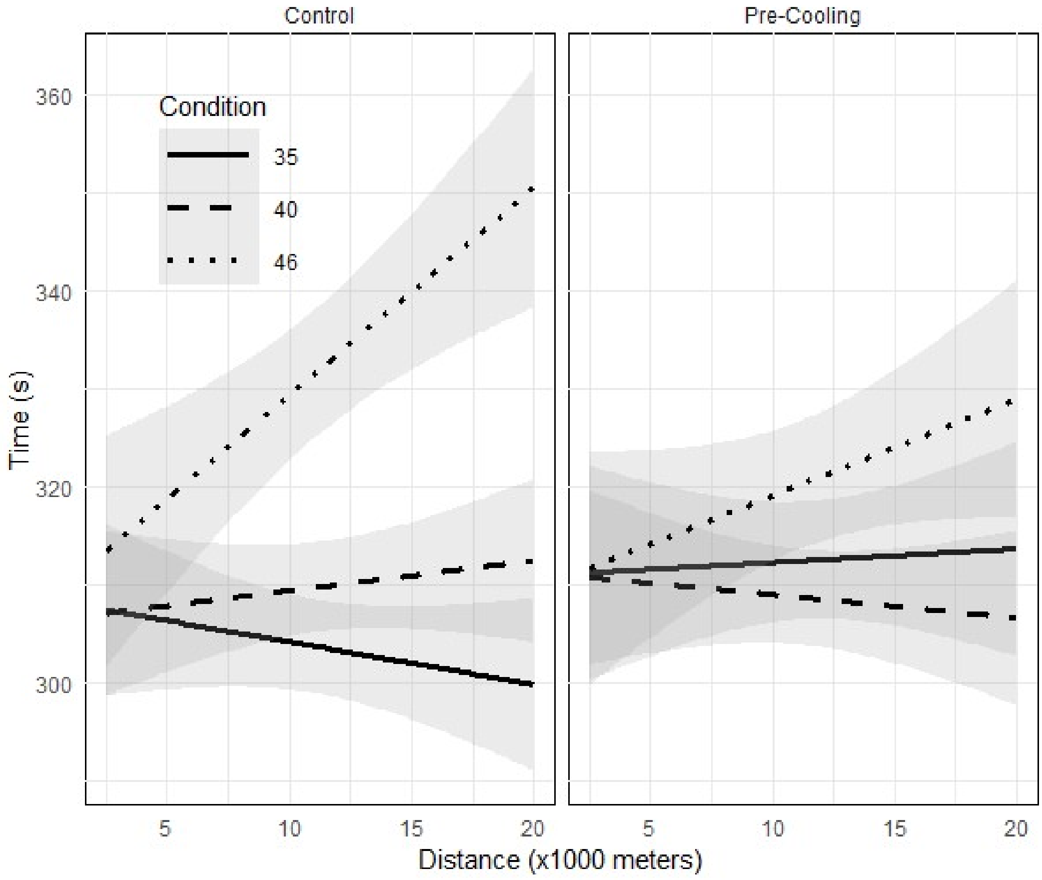

Figure 3.1. Split completion times as a function of distance cycled, intervention (control or pre-cooling), and 35, 40, and 46°C apparent temperature conditions during the 20-km cycling time-trial. *Note: Shaded areas indicate 95% confidence intervals.*

Physiological Responses

PreC Period

There was a significant two-way interaction between time and intervention for T_{re} (β=-.012, p=.004; Figure 3.2A), T_{sk} (β=-.012, p=.004; Figure 3.2A), and T_b (β=-.012, p<.001). Post-hoc analysis indicated faster decline over time in PreC compared to CON for T_{re} (β=.016, t(418)=9.03, p<.001), mean T_{sk} (β=.016, t(437)=4.02, p<.001), and T_b (β=.016, t(412)=7.59, p<.001). No other significant main effects or interactions were observed.

There was a significant main effect for time during the PreC period for HR when no PreC was applied in 35°C AT (β=-154, p=.009). This indicated a significant decrease in HR from baseline (0 min) over the course of the PreC period. Similarly, a significant decrease in HR from baseline was observed when PreC was applied in 35 (β=-.024, p<.001), 40 (β=-.294, p<.001), and 46°C AT (β=-.286, p<.001).

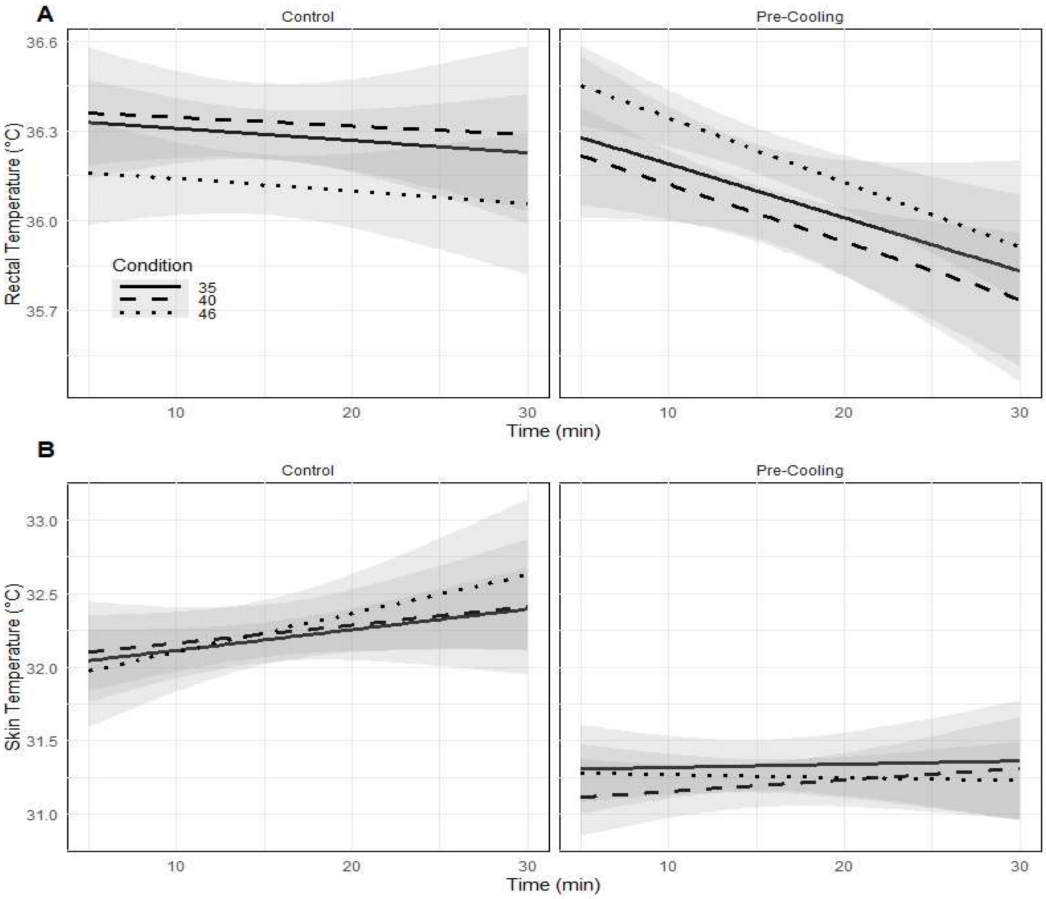

Figure 3.2. (A) Rectal and (B) skin temperature as a function of time, and 35, 40, and 46°C apparent temperature conditions during the pre-cooling period. *Note: Shaded areas indicate 95% confidence intervals.*

Cycling Time-Trial

There was a significant two-way interaction between condition and distance in 46°C AT for T_{re} (β=.028, p<.001; Figure 3.3A) and T_b (β=.041, p<.001), as well as 40°C AT for T_b (β=.015, p=.005; Figure 3.3B). Post-hoc analysis indicated faster increase in T_{re} and T_b over the course of the cycling TT in 46°C AT compared to 35 (β=-.030, t(536)=-5.66, p<.001, and β=-.050, t(524)=-10.39, p<.001, respectively) and 40°C AT (β=-0.016, t(536)=-3.00, p=.008, and β=-.024, t(524)=-4.78, p<.001, respectively). Similarly, faster increases in T_{re} and

T_b over the course of the cycling TT were observed in 40°C AT compared to 35°C AT (β=-.014, t(536)=-2.45, p=.039, and β=-.026, t(524)=-5.09, p<.001, respectively).

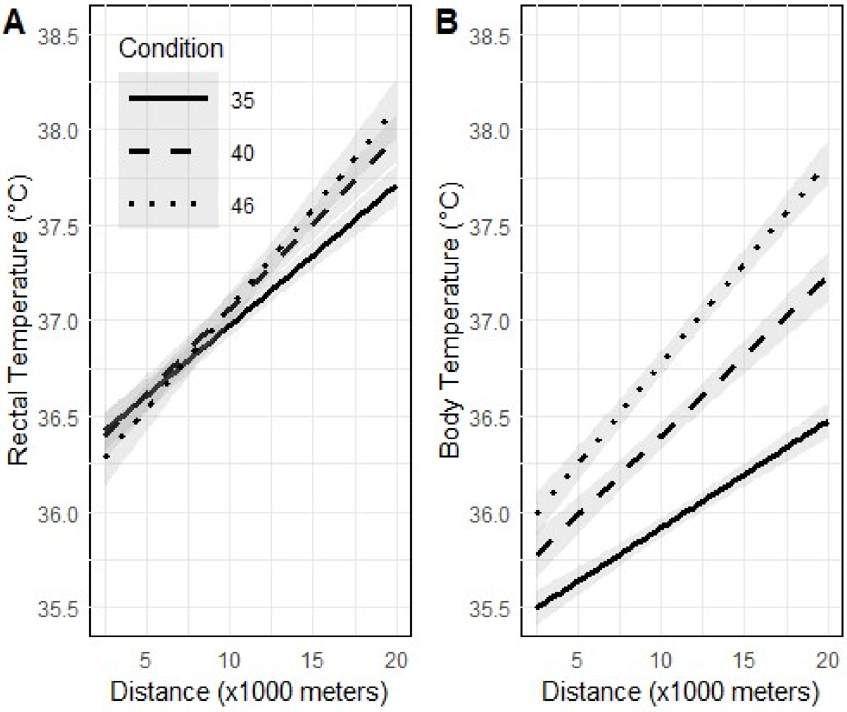

Figure 3.3. (A) Rectal and (B) body temperature as a function of distance cycled, and 35, 40, and 46°C apparent temperature conditions during the 20-km cycling time-trial. *Note: Shaded areas indicate 95% confidence intervals. Intervention effects are excluded from these graphs due to no difference between interventions. Data is therefore averaged over pre-cooling and control.*

There was a significant three-way interaction between distance, condition, and intervention for HR (β=.589, p=.010; Figure 3.4A) and T_{sk} (β=.037, p=.001; Figure 3.4B). Post-hoc analysis indicated greater rate of increase in T_{sk} during the cycling TT in PreC compared to CON in 46°C AT (β=-.038, t(582)=-3.54, p=.006), but not in 40 (β=.003, p=.999) and 35°C AT (β=-.002, p=.999). Comparisons between PreC conditions showed greater T_{sk} over during the cycling TT in 46°C AT than 40 (β=-.062, t(582)=-5.84, p<.001) and 35°C AT (β=-.103, t(582)=-9.62, p<.001), and 40°C AT compared to 35°C AT (β=-.041, t(582)=-3.87, p=.002). Comparisons between CON conditions showed greater T_{sk} over the course of the cycling TT in 46°C AT compared to 35°C AT (β=-.067, t(582)=-6.34, p<.001), and 40°C AT compared to 35°C AT (β=-.046, t(582)=-4.28, p<.001) but not between 46°C AT and 40°C AT (β=-.021, p=.359).

Post-hoc analysis for HR indicated greater rate of increase in HR over the course of the cycling TT for PreC compared to CON in 46°C AT (β=-.669, t(559)=-3.48, p=.007), but not in 40 (β=-.310, p=.570) or 35°C AT (β=-.032, p=1.000).

No other significant main effects or interactions were observed.

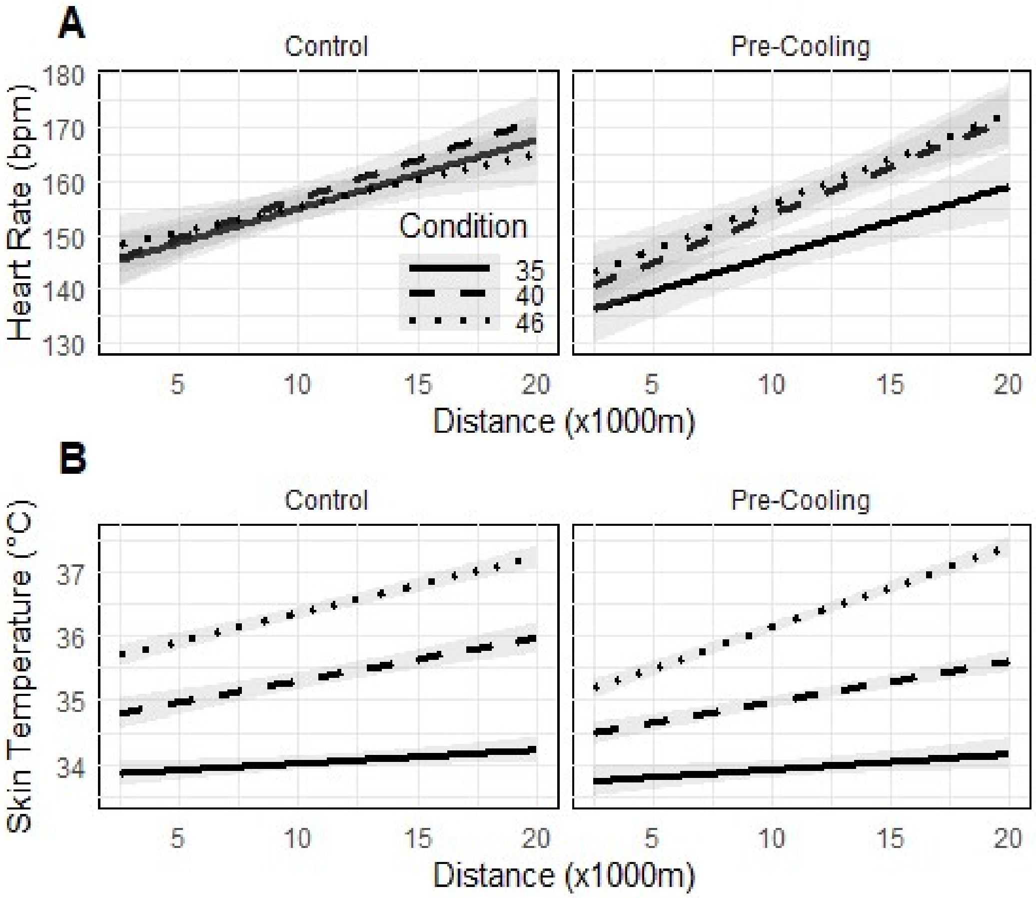

Figure 3.4. (A) Heart rate and (B) mean skin temperature as a function of distance cycled, intervention (control or pre-cooling), and 35, 40, and 46 apparent temperature conditions during the 20-km cycling time-trial. *Note: Shaded areas indicate 95% confidence intervals.*

Perceptual Data

PreC Period

There was a significant two-way interaction between time and intervention for Th_c and Th_s in 35°C AT (β=.146, p<.001, and β=-.134, p<.001, respectively). Post-hoc analysis indicated greater Th_c and lower Th_s over time in PreC compared to CON (β=-.107, t(443)=-3.99, p<.001, and β=.113, t(443)=7.49, p<.001, respectively). No other significant main effects were observed.

Cycling Time-Trial

There was a significant two-way interaction between condition and intervention for RPE in 46°C AT (β=-1.032, p=.005). Post-hoc analysis indicated lower RPE for PreC compared to CON in 46°C AT (β=.740, t(595)=6.31, p<.001). Comparisons between PreC conditions showed lower RPE in 35°C AT than 40 (β=-.423, t(595)=-3.65, p=.004) and 46°C AT (β=-.663, t(595)=-5.69, p<.001). Comparisons between CON conditions showed greater RPE in 46°C AT compared to 35 (β=-1.202, t(595)=-10.28, p<.001) and 40°C AT (β=-1.019, t(595)=-8.72, p<.001).

There was a significant three-way interaction between distance, condition, and intervention for Th_c (Figure 3.5A) and Th_s (Figure 3.5B) in 46°C AT (β=.123, p=.002, and β=.070, p=.036, respectively). Post-hoc analysis indicated a greater rate of increase in Th_c and Th_s over the course of the cycling TT for PreC compared to CON in 46°C AT (β=-.102, t(595)=-3.67, p=.004, and β=-.076, t(595)=-3.21, p=.017, respectively), and Th_s in 40°C AT (β=-.072, t(595)=-3.10, p=.025). Comparisons between PreC conditions showed lower Th_c and Th_s in 35°C AT compared to 40 (β=-.106, t(595)=-3.89, p=.002, and β=-.088, t(595)=-3.79, p=.002, respectively) and 46°C AT (β=-.094, t(595)=-3.40, p=.009, and β=-.098, t(596)=-4.17, p=.001, respectively). Comparisons between CON conditions showed lower Th_c in 40°C AT compared to 46°C AT (β=-.086, t(596)=3.131, p=.022). No other significant main effects or interactions were observed.

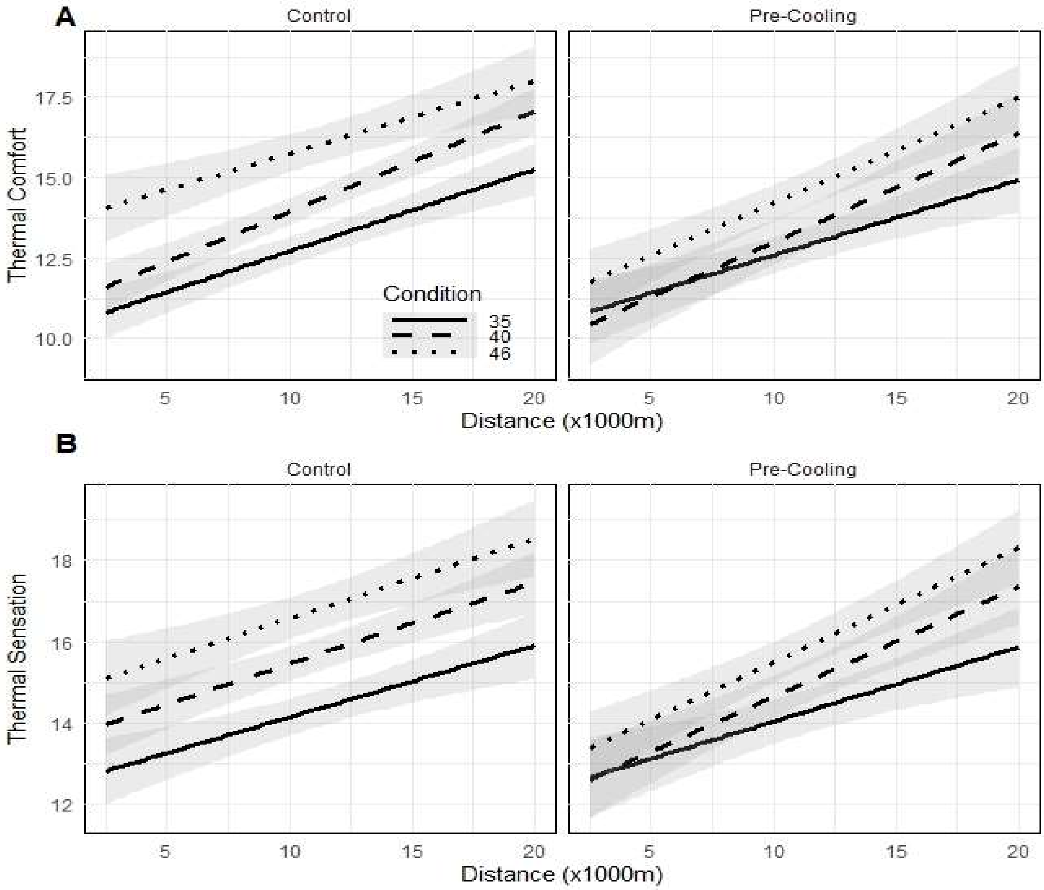

Figure 3.5. (A) Thermal comfort and (B) thermal sensation as a function of distance cycled, intervention (control or pre-cooling), and 35, 40, and 46 apparent temperature conditions during the 20-km cycling time-trial. *Note: Shaded areas indicate 95% confidence intervals.*

3.6 Discussion

This study is the first examining the effect of mixed-method PreC on self-paced cycling endurance performance in different ATs. Mixed-method PreC improved 20-km CTT completion time by ~95 s in 46°C AT compared to CON, yet no performance benefits in 40°C AT and was detrimental to 20-km CTT completion time in 35°C AT. Lower T_{sk} and HR, and improved RPE, Th_s, and

Th_c in the 46°C AT condition may help explain the observed performance improvements.

Faster total completion time observed after mixed-method PreC in 46°C AT, as well as slower or no difference in 35°C AT and 40°C AT (~ +68 s and -4 s, respectively), provides support for emerging claims that PreC may only enhance performance in environments with higher thermal strain (>27°C, <50% RH).[5,20-22] Previous findings by Ross, et al.,[23] showed ~66 s faster completion time when 15-min mixed-method PreC (14 $g{\cdot}kg^{-1}$ BM crushed-ice ingestion + cooling vest) was utilised compared to no cooling prior to 46.4-km CTT in the heat (~35°C, ~60% RH, ~42°C AT). Faster CTT completion times observed by Ross, et al.,[23] in PreC compared to CON in ~42°C AT condition, may be due to the longer CTT compared to our study (46.4 *vs* 20-km), higher RH (55 *vs* 60% RH), and greater change in T_{re} from baseline to the end of the CTT (~2.5 *vs* ~1.3°C). Which together, may have elicited greater thermal strain. Bright, et al.,[9] provides support to this claim with their investigation on the effects of different skin-to-air vapor pressure gradients on 30-km CTT in hot-humid conditions (≥28°C, ≥72% RH, ≥33°C AT) compared to cooler conditions (≤20°C, ≤70% RH, ≤21°C AT). Greater thermal strain was experienced by participants, evident through greater T_{re} (~39.1–39.6°C *vs* 38.7–38.8°C), HR (159–163 bpm *vs* 153–159 bpm), and RPE (15–17 *vs* 15) in hot-humid compared to cooler conditions. This corresponded to lower power output during the 30-km CTT in hot-humid conditions (~228–262 W) compared to cooler conditions (~272–275 W).

Although our study observed no difference in 2.5-km split completion times between PreC and CON in ≤40°C AT, 20-km CTT completion time was slower or not different in PreC compared to CON. This contrasts with previous findings where PreC (6.8 $g \cdot kg^{-1}$ BM crushed-ice ingestion) prior to 1200-kJ CTT (~40-km CTT) in heat (30°C, 75% RH, 37°C AT) resulted in ~348 s faster completion time compared to no PreC.[13] This disparity in findings may be due to a greater heat sink in T_c at the end of the PreC period in the previous study compared to our study (~0.46 *vs* ~1.10°C).[13] Additionally, longer exercise and exposure to heat resulted in higher final T_c compared to our study (~38.5-39.0 *vs* ~37.5-38.0°C), eliciting greater thermal strain and therefore greater PreC benefits leading to faster mean split completion times compared to no PreC. Possibly the duration of the exercise task and environmental conditions in our study did not elicit sufficient thermal strain, and therefore mixed-method PreC did not benefit performance in <40°C AT. In fact, Faulkner, et al.,[6] observed ~95 s faster 60-min CTT completion time after applying external PreC (30-min) in less thermally stressful conditions compared to our study (~27°C, ~50%RH, ~29°C AT). This suggests that PreC may only benefit self-paced endurance performance in ≤40°C AT when exercise duration is >60-min in duration.

Rectal temperature was not significantly different over the course of the 20-km CTT when PreC was applied compared to CON. However, mixed-method PreC resulted in lower mean T_{sk} in 46°C AT compared to CON, which was accompanied by faster 20-km CTT completion time. This supports previous suggestions that exercise intensity at the commencement of exercise is more closely associated with T_{sk} compared to T_c.[24] Schlader, et al.,[24] observed greater mean power output (~258 *vs* ~251 W) from the commencement of exercise during a 60-min CTT when T_{sk} (manipulated by a perfused-suit) changed from cold (~6.6°C) to hot (~61.4°C) compared to hot-to-cold, despite no difference between conditions in T_c. Lower initial work rates in the hot-to-cold condition were attributed to greater Th_s and Th_c when T_{sk} was high, leading to an anticipatory response resulting in a lower self-selected intensity to ensure exercise task completion.[25] Our findings support this, as we also observed lower in T_{sk}, improved Th_c and RPE during the initial stages of the 20-km CTT in 46°C AT. It is possible that mixed-method PreC blunts the anticipatory response by lowering T_{sk} at the commencement of exercise, resulting in higher initial work outputs.

Sweat loss was lower when PreC was applied compared to CON in ≤40°C AT but higher in 46°C AT. This supports previous research, which found lower sweat loss when PreC is applied prior to self-paced endurance cycling in <41°C AT conditions.[6] The difference in sweat rate in the 46°C AT condition may be due to a higher mean T_{sk} than in ≤40°C AT, resulting in greater blood flow to the skin for sweating to occur.[3,26] It must be noted that all participants were classified as hydrated prior to commencement and no indications of dehydration were noted physiologically or perceptually during any condition.

Ecological validity is impacted by the methodology of this study because mixed-method PreC was applied in a thermoneutral environment prior to the 20-km CTT, which may not always be achievable in a real-world setting and a fan was not utilised during the 20-km CTT. These methodological limitations may overestimate the effect of the mixed-method PreC intervention. Future research should investigate the effects of different combinations of temperature and RH to achieve the same ATs and the effect on self-paced endurance performance.

3.7 Practical Applications

Mixed-method PreC should be applied prior to 20-km cycling TT conducted in hot-humid conditions (≥46°C AT). Contrastingly, mixed-method PreC may not be a priority or is not recommended in moderately hot-humid (~40°C AT) or hot-dry (~35°C AT) conditions, respectively.

3.8 Conclusion

Mixed-method PreC yielded the greatest benefit to 20-km cycling TT performance in hot-humid environmental conditions (≥46°C AT). Detrimental or no benefit to 20-km cycling TT performance was observed when applied in moderately hot-humid (~40°C AT) or hot-dry (~35°C AT) conditions, respectively. Improved performance may result from lower T_{sk} and HR, and perceptual responses when mixed-method PreC was utilised compared to no cooling.

3.9 References

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3.10 Supplementary Materials

Table S3.1. Intraclass correlation coefficients for hydration and sweat loss, performance, physiological, and perceptual data during the pre-cooling (PreC) period and 20-km cycling time-trial (CTT).

Variable	**PreC period**	**20-km cycling TT**
Urine specific gravity	61%	
Total fluid ingestion	65%	
Sweat loss	67%	
Total completion time	78%	
Mean split completion time	66%	
Rectal temperature	50%	20%
Mean skin temperature	32%	6%
Body temperature	37%	12%
Core-to-skin temperature gradient	39%	18%
Heart rate	42%	48%
Thermal comfort	43%	67%
Thermal sensation	31%	54%
Rating of perceived exertion	-	46%

Table S3.2. Mean ± SD for physiological and perceptual data at the start and finish of the pre-cooling period for control (CON) and pre-cooling (PreC) interventions.

		CON		PreC	
		Start	Finish	Start	Finish
Rectal temperature (°C)	35°C AT	36.34 ± 0.35	36.21 ± 0.34	36.24 ± 0.67	35.90 ± 0.33
	40°C AT	36.39 ± 0.44	36.27 ± 0.52	36.22 ± 0.36	35.74 ± 0.37
	46°C AT	36.16 ± 0.38	36.07 ± 0.39	36.47 ± 0.30	35.90 ± 0.30

Skin temperature (°C)	35°C AT	30.84 ± 0.81	32.28 ± 0.42	31.06 ± 0.76	31.13 ± 0.62
	40°C AT	30.86 ± 0.77	32.33 ± 0.71	30.70 ± 0.77	31.09 ± 0.52
	46°C AT	30.66 ± 1.26	32.42 ± 0.86	30.94 ± 0.76	31.10 ± 0.34
Body temperature (°C)	35°C AT	34.42 ± 0.32	34.83 ± 0.23	34.43 ± 0.57	34.25 ± 0.30
	40°C AT	34.41 ± 0.45	34.87 ± 0.55	34.29 ± 0.36	34.11 ± 0.32
	46°C AT	34.24 ± 0.64	34.80 ± 0.50	34.54 ± 0.37	34.22 ± 0.28
Core-to-skin temperature gradient (°C)	35°C AT	5.50 ± 0.96	3.93 ± 0.60	5.18 ± 0.89	4.72 ± 0.76
	40°C AT	5.67 ± 0.41	3.99 ± 0.46	5.51 ± 0.85	4.65 ± 0.59
	46°C AT	5.51 ± 1.10	3.63 ± 0.73	5.49 ± 0.78	4.79 ± 0.30
Heart Rate (bpm)	35°C AT	64 ± 8	69 ± 11	73 ± 10	64 ± 6
	40°C AT	65 ± 8	67 ± 7	71 ± 8	67 ± 14
	46°C AT	73 ± 17	67 ± 9	70 ± 10	61 ± 6
Thermal Comfort	35°C AT	6 ± 4	6 ± 4	7 ± 4	11 ± 5
	40°C AT	6 ± 4	7 ± 4	8 ± 4	11 ±5
	46°C AT	5 ± 4	6 ± 4	9 ± 4	12 ± 5
Thermal Sensation	35°C AT	8 ± 2	8 ± 2	6 ± 3	3 ± 3
	40°C AT	8 ± 2	8 ± 2	7 ± 2	4 ± 3
	46°C AT	8 ± 2	8 ± 2	7 ± 3	3 ± 2

Table S3.3. Mean ± SD for performance, physiological and perceptual data during the 20-km cycling time-trial for control (CON) and pre-cooling (PreC) interventions.

	CON	PreC

2.5-km Split completion time (s)		
35°C AT	304 ± 25	312 ± 31
40°C AT	310 ± 24	309 ± 25
46°C AT	332 ± 35	320 ± 34
2.5-km Split mean power output (W)		
35°C AT	201 ± 47	188 ± 51
40°C AT	189 ± 43	192 ± 45
46°C AT	159 ± 45	175 ± 48
Rectal temperature (°C)		
35°C AT	37.25 ± 0.52	36.87 ± 0.58
40°C AT	37.27 ± 0.64	37.00 ± 0.69
46°C AT	37.13 ± 0.91	37.23 ± 0.75
Skin temperature (°C)		
35°C AT	34.05 ± 0.56	33.95 ± 0.68
40°C AT	35.39 ± 0.75	35.05 ± 0.60
46°C AT	36.45 ± 0.69	36.27 ± 0.83
Body temperature (°C)		
35°C AT	36.13 ± 0.43	35.82 ± 0.47
40°C AT	36.63 ± 0.64	36.31 ± 0.63
46°C AT	36.89 ± 0.75	36.89 ± 0.75
Core-to-skin Temperature Gradient (°C)		
35°C AT	3.21 ± 0.68	2.96 ± 0.87
40°C AT	1.89 ± 0.64	1.97 ± 0.52
46°C AT	0.66 ± 0.78	0.94 ± 0.46
Heart Rate (bpm)		
35°C AT	151 ± 22	148 ± 19
40°C AT	153 ± 23	156 ± 18
46°C AT	151 ± 22	158 ± 18
Thermal Comfort		
35°C AT	13 ± 3	13 ± 3
40°C AT	14 ± 3	13 ± 3
46°C AT	16 ± 3	15 ± 3
Thermal Sensation		
35°C AT	14 ± 3	14 ± 3
40°C AT	16 ± 2	15 ± 3
46°C AT	17 ± 3	16 ± 3
Ratings of Perceived Exertion		
35°C AT	16 ± 2	15 ± 2

40°C AT	16 ± 2	16 ± 2
46°C AT	17 ± 2	16 ± 2

Table S3.4. Summary of fixed effects for the linear mixed-effects model for urine specific gravity (USG), total fluid ingestion, and sweat loss variables. Intercept denotes USG levels, total fluid ingestion and sweat loss at the reference category: 35°C apparent temperature (AT) condition with no pre-cooling. Fixed effects describe changes in variables from the intercept at different levels of the independent variables.

	Estimate	Standard Error	Degrees of Freedom	T-value	P-value	95% Confidence Interval
USG						
Intercept	1.011	.002	12.06	688.101	<.001	[1.008, 1.014]
40°C AT	-.003	.0001	16,360	-24.468	<.001	[-.003, -.003]
46°C AT	-.001	.0001	16,360	-9.088	<.001	[-.001, -.0001]
Intervention (pre-cooling)	-.001	.0001	16,360	-11.185	<.001	[-.002, -.001]
40°C AT (pre-cooling)	.002	.0002	16,360	15.324	<.001	[.002, .003]
46°C AT (pre-cooling)	-.001	.0002	16,360	-5.438	<.001	[-.001, -.001]
Total Fluid Ingestion (L)						
Intercept	.866	.141	12.030	6.150	<.001	[.580, .310]
40°C AT	.273	.008	16,360	32.850	<.001	[.257, .289]
46°C AT	.350	.008	16,360	42.104	<.001	[.334, .366]
Intervention (pre-cooling)	.241	.008	16,360	28.964	<.001	[.225, .257]
40°C AT (pre-cooling)	-.052	.011	16,360	-4.384	<.001	[-.075, -.029]
46°C AT (pre-cooling)	-.010	.012	16,360	-.851	.395	[-.033, .013]
Sweat Loss (L)						
Intercept	1.252	.108	12.03	11.552	<.001	[1.032, 1.473]

40°C AT	.238	.006	16,360	38.297	<.001	[.226, .250]
46°C AT	.216	.006	16,360	34.826	<.001	[.204, .228]
Intervention (pre-cooling)	-.151	.006	16,360	-24.292	<.001	[-.163, -.139]
40°C AT (pre-cooling)	-.022	.009	16,360	-2.454	.014	[-.039, -.004]
46°C AT (pre-cooling)	.212	.009	16,360	24.100	<.001	[.194, .229]

Table S3.5. Summary of fixed effects for the linear mixed-effects model for physiological and perceptual data during the pre-cooling period. Intercept denotes values of physiological and perceptual data at the reference category: 35°C apparent temperature (AT) condition with no pre-cooling at zero seconds (i.e., baseline). Fixed effects describe changes in the variables from the intercept at different levels of categorical independent variables (i.e., AT condition; pre-cooling intervention) or for each one unit increase in continuous independent variables (i.e., a one second increase in time).

	Estimate	Standard Error	Degrees of Freedom	T-value	P-value	95% Confidence Interval
Heart rate (bpm)						
Intercept	2.251	1.764	376.000	1.276	.203	[-1.16, 5.659]
Time	-.154	.058	376.000	-2.637	.009	[-.267, -.041]
40°C AT	-2.375	1.369	376.000	-1.735	.084	[-5.020, .269]
46°C AT	-1.594	1.369	376.000	-1.164	.245	[-4.237, 1.050]
Intervention (pre-cooling)	1.347	1.375	376.000	.980	.328	[-1.309, 4.002]
40°C AT x time	.159	.083	376.000	1.921	.056	[-.001, .318]

46°C AT x time	.106	.083	376.000	1.287	.199	[-.053, .266]
Intervention x time	-.089	.083	376.000	-1.070	.285	[.248, .071]
40°C AT x intervention	3.128	1.941	376.000	1.611	.108	[-.622, 6.878]
46°C AT x intervention	2.233	1.940	376.000	1.151	.250	[-1.514, 5.980]
40°C AT x intervention x time	-.210	.117	376.000	-1.798	.073	[-.436, .016]
46°C AT x intervention x time	-.150	.117	376.000	-1.284	.200	[-.376, .076]
Rectal Temperature (°C)						
Intercept	-.210	.423	372.000	-.497	.619	[.075, .086]
Time	.014	.003	372.000	4.909	.000	[-.563, .875]
40°C AT	.027	.068	372.000	.391	.696	[-.007, -.001]
46°C AT	-.182	.067	372.000	-2.723	.007	[-.084, .049]
Intervention (pre-cooling)	.177	.068	372.000	2.604	.010	[-.066, .067]
40°C AT x time	1.000	.013	372.000	76.576	<.001	[.140, .273]
46°C AT x time	-.002	.004	372.000	-.432	.666	[.978, 1.017]
Intervention x time	.012	.004	372.000	3.010	.003	[-.003, .005]
40°C AT x intervention	-.012	.004	372.000	-2.919	.004	[-.004, .004]
46°C AT x intervention	-.111	.096	372.000	-1.159	.247	[-.018, -.010]
40°C AT x intervention x time	.246	.095	372.000	2.600	.010	[-.057, .130]
46°C AT x intervention x time	.007	.006	372.000	1.282	.201	[-.078, .112]
Mean Skin Temperature (°C)						
Intercept	-.210	.423	372.000	-.497	.619	[.149, .172]
Time	.014	.003	372.000	4.909	.000	[-1.027, .607]
40°C AT	.027	.068	372.000	.391	.696	[.009, .020]
46°C AT	-.182	.067	372.000	-2.723	.007	[-.105, .159]

Intervention (pre-cooling)	.177	.068	372.000	2.604	.010	[-.312, -.053]
40°C AT x time	1.000	.013	372.000	76.576	<.001	[.046, .308]
46°C AT x time	-.002	.004	372.000	-.432	.666	[0.975, 1.025]
Intervention x time	.012	.004	372.000	3.010	.003	[-.01, .006]
40°C AT x intervention	-.012	.004	372.000	-2.919	.004	[.004, .02]
46°C AT x intervention	-.111	.096	372.000	-1.159	.247	[-.02, -.004]
40°C AT x intervention x time	.246	.095	372.000	2.600	.010	[-.296, .074]
46°C AT x intervention x time	.007	.006	372.000	1.282	.201	[.063, .429]
Body Temperature (°C)						
Intercept	.016	.350	355.000	.044	.965	[.071, .082]
Time	.002	.001	355.000	1.676	.095	[-.660, .691]
40°C AT	-.007	.033	355.000	-.204	.838	[-.0003, .005]
46°C AT	-.068	.033	355.000	-2.107	.036	[-.071, .057]
Intervention (pre-cooling)	.185	.033	355.000	5.632	.000	[-.131, -.006]
40°C AT x time	.999	.010	355.000	99.713	<.001	[.121, .248]
46°C AT x time	.001	.002	355.000	.226	.821	[.979, 1.018]
Intervention x time	.005	.002	355.000	2.326	.021	[-.003, .004]
40°C AT x intervention	-.012	.002	355.000	-6.298	<.001	[.001, .008]
46°C AT x intervention	.001	.047	355.000	.024	.981	[-.016, -.009]
40°C AT x intervention x time	.113	.046	355.000	2.443	.015	[-.089, .091]
46°C AT x intervention x time	.000	.003	355.000	-.030	.976	[.024, .203]
Thermal Comfort						
Intercept	.023	.353	377.000	.065	.948	[-.658, .704]
Time	-.002	.021	377.000	-.075	.940	[-.041, .038]

40°C AT	-.023	.480	377.000	-.048	.962	[-.951, .905]
46°C AT	-.415	.480	377.000	-.865	.388	[-1.343, .512]
Intervention (pre-cooling)	-2.192	.484	377.000	-4.535	<.001	[-3.126, -1.258]
40°C AT x time	.002	.029	377.000	.053	.958	[-.054, .058]
46°C AT x time	.028	.029	377.000	.956	.340	[-.028, .084]
Intervention x time	.146	.029	377.000	5.047	<.001	[.090, .202]
40°C AT x intervention	.531	.679	377.000	.781	.435	[-.781, 1.843]
46°C AT x intervention	.531	.679	377.000	.781	.435	[-.781, 1.843]
40°C AT x intervention x time	-.035	.041	377.000	-.864	.388	[-.115, .044]
46°C AT x intervention x time	-.035	.041	377.000	-.864	.388	[-.115, .044]
Thermal Sensation						
Intercept	-.254	.285	377.000	-.891	.374	[-.804, .297]
Time	.017	.014	377.000	1.217	.224	[-.010, .044]
40°C AT	.554	.326	377.000	1.699	.090	[-.076, 1.184]
46°C AT	.139	.326	377.000	.425	.671	[-.491, .768]
Intervention (pre-cooling)	2.008	.335	377.000	5.999	<.001	[1.361, 2.654]
40°C AT x time	-.037	.020	377.000	-1.878	.061	[-.075, .001]
46°C AT x time	-.009	.020	377.000	-.470	.639	[-.047, .029]
Intervention x time	-.134	.020	377.000	-6.808	<.001	[-.172, -.096]
40°C AT x intervention	-.462	.462	377.000	-1.000	.318	[-1.35, .430]
46°C AT x intervention	-.415	.461	377.000	-.901	.368	[-1.306, .475]
40°C AT x intervention x time	.031	.028	377.000	1.107	.269	[-.023, .085]
46°C AT x intervention x time	.028	.028	377.000	.996	.320	[-.026, .081]

Table S3.6. Summary of fixed effects for the linear mixed-effects model for performance, physiological and perceptual data during the 20-km cycling time-trial. Intercept denotes values of performance, physiological and perceptual data at the reference category: 35°C apparent temperature (AT) condition with no pre-cooling at zero seconds (i.e., baseline). Fixed effects describe changes in the variables from the intercept at different levels of categorical independent variables (i.e., AT condition; pre-cooling intervention) or for each one unit increase in continuous independent variables (i.e., a one second increase in time).

	Estimate	Standard Error	Degrees of Freedom	T-value	P-value	95% Confidence Interval
2.5-km split completion time (s)						
Intercept	308.544	7.716	16.847	39.990	<.001	[293.113, 323.975]
Time	-.433	.246	595.001	-1.761	.079	[-.911, .045]
40°C AT	-2.190	4.390	595.001	-.499	.618	[-10.73, 6.351]
46°C AT	-.962	4.404	595.003	-.219	.827	[-9.528, 7.603]
Intervention (pre-cooling)	2.374	4.390	595.001	.541	.589	[-6.167, 10.914]
40°C AT x time	.740	.348	595.001	2.128	.034	[.063, 1.416]
46°C AT x time	2.646	.352	595.012	7.528	<.001	[1.962, 3.33]
Intervention x time	.571	.348	595.001	1.642	.101	[-.105, 1.248]
40°C AT x intervention	2.665	6.209	595.001	.429	.668	[-9.413, 14.742]
46°C AT x intervention	-1.287	6.226	595.001	-.207	.836	[-13.399, 10.824]
40°C AT x intervention x time	-1.115	.492	595.001	-2.266	.024	[-2.071, -.158]
46°C AT x intervention x time	-1.717	.497	595.001	-3.458	.001	[-2.683, -.751]

Heart rate (bpm)						
Intercept	-14.427	3.421	606.000	-4.217	<.001	[7.857, 17.557]
Time	1.262	.113	606.000	11.205	<.001	[13.25, 14.731]
40°C AT	-2.125	2.012	606.000	-1.057	.291	[119.906, 135.79]
46°C AT	2.736	2.017	606.000	1.357	.175	[1.889, 2.673]
Intervention (pre-cooling)	-.350	2.019	606.000	-.173	.862	[-5.854, 7.355]
40°C AT x time	.189	.159	606.000	1.184	.237	[-3.001, 10.232]
46°C AT x time	-.226	.161	606.000	-1.405	.161	[-13.972, -.763]
Intervention x time	.032	.159	606.000	.202	.840	[-.423, .687]
40°C AT x intervention	-3.129	2.847	606.000	-1.099	.272	[-.852, .270]
46°C AT x intervention	-6.587	2.857	606.000	-2.305	.021	[-.647, .463]
40°C AT x intervention x time	.277	.225	606.000	1.231	.219	[-7.997, 10.684]
46°C AT x intervention x time	.589	.227	606.000	2.589	.010	[-9.979, 8.734]
Rectal Temperature (°C)						
Intercept	1.692	.983	56.694	1.721	.091	[-.196, 3.582]
Time	.068	.004	534.096	16.051	<.001	[.060, .076]
40°C AT	.030	.077	535.820	.385	.701	[-.120, .181]
46°C AT	-.301	.075	536.093	-4.035	<.001	[-.446, -.156]
Intervention (pre-cooling)	-.128	.077	541.774	-1.669	.096	[-.277, .021]
40°C AT x time	.001	.006	537.868	.213	.832	[.883, .985]
46°C AT x time	.028	.006	535.119	4.674	<.001	[-.011, .013]
Intervention x time	.010	.006	534.355	1.613	.107	[.016, .039]
40°C AT x intervention	-.130	.109	535.009	-1.193	.234	[-.002, .021]
46°C AT x intervention	-.008	.106	540.334	-.075	.941	[-.343, .081]

40°C AT x intervention x time	.011	.009	535.181	1.270	.205	[-.215, .198]
46°C AT x intervention x time	.003	.008	534.285	.325	.746	[-.006, .028]
Mean Skin Temperature (°C)						
Intercept	.166	.953	594.000	.174	.862	[-1.684, 2.017]
Time	.021	.005	594.000	3.913	<.001	[.010, 0.031]
40°C AT	-.500	.103	594.000	-4.828	<.001	[-.701, -.299]
46°C AT	-.678	.116	594.000	-5.856	<.001	[-.903, -.453]
Intervention (pre-cooling)	-.019	.095	594.000	-.205	.838	[-.204, .165]
40°C AT x time	.046	.008	594.000	5.993	<.001	[.934, 1.042]
46°C AT x time	.064	.008	594.000	8.481	<.001	[.031, .061]
Intervention x time	.002	.008	594.000	.269	.788	[.049, .079]
40°C AT x intervention	.044	.135	594.000	.322	.747	[-.013, .017]
46°C AT x intervention	-.424	.134	594.000	-3.158	.002	[-.219, .307]
40°C AT x intervention x time	-.005	.011	594.000	-.422	.673	[-.685, -.163]
46°C AT x intervention x time	.037	.011	594.000	3.483	.001	[-.025, .016]
Body Temperature (°C)						
Intercept	2.700	.986	51.239	2.739	.008	[.807, 4.600]
Time	.051	.004	522.428	14.035	<.001	[.044, .058]
40°C AT	-.085	.068	530.985	-1.236	.217	[-.217, .049]
46°C AT	-.376	.067	516.912	-5.625	<.001	[-.506, -.247]
Intervention (pre-cooling)	-.084	.066	530.075	-1.277	.202	[-.212, .044]
40°C AT x time	.015	.006	525.820	2.794	.005	[.857, .962]
46°C AT x time	.041	.005	523.351	8.124	<.001	[.005, .026]
Intervention x time	.006	.005	523.627	1.242	.215	[.031, .051]

40°C AT x intervention	-.103	.095	523.660	-1.084	.279	[-.004, .017]
46°C AT x intervention	-.163	.091	526.640	-1.793	.074	[-.287, .081]
40°C AT x intervention x time	.007	.008	523.581	.868	.386	[-.339, .013]
46°C AT x intervention x time	.015	.007	523.098	2.126	.034	[-.008, .022]
Thermal Comfort						
Intercept	-2.860	.339	606.000	-8.437	.000	[-3.519, -2.202]
Time	.255	.019	606.000	13.175	<.001	[.217, .292]
40°C AT	-.647	.346	606.000	-1.870	.062	[-1.318, .025]
46°C AT	.368	.350	606.000	1.052	.293	[-.312, 1.048]
Intervention (pre-cooling)	.231	.345	606.000	.669	.504	[-.439, .901]
40°C AT x time	.058	.027	606.000	2.105	.036	[.965, 1.035]
46°C AT x time	-.029	.028	606.000	-1.048	.295	[.004, .111]
Intervention x time	-.021	.027	606.000	-.751	.453	[-.083, .025]
40°C AT x intervention	-.548	.488	606.000	-1.123	.262	[-.074, .033]
46°C AT x intervention	-1.374	.490	606.000	-2.805	.005	[-1.496, .400]
40°C AT x intervention x time	.049	.039	606.000	1.261	.208	[-2.325, -.423]
46°C AT x intervention x time	.123	.039	606.000	3.144	.002	[-.026, .124]
Thermal Sensation						
Intercept	-2.009	.321	606.000	-6.266	<.001	[-2.632, -1.386]
Time	.177	.016	606.000	10.733	<.001	[.145, .209]
40°C AT	-.257	.295	606.000	-.871	.384	[-.831, .316]
46°C AT	-.282	.298	606.000	-.945	.345	[-.861, .297]
Intervention (pre-cooling)	-.074	.294	606.000	-.252	.801	[-.646, .498]
40°C AT x time	.023	.023	606.000	.974	.330	[.968, 1.034]

46°C AT x time	.028	.024	606.000	1.196	.232	[-.023, .068]
Intervention x time	.007	.023	606.000	.283	.777	[-.018, .074]
40°C AT x intervention	-.737	.416	606.000	-1.770	.077	[-.039, .052]
46°C AT x intervention	-.781	.418	606.000	-1.870	.062	[-1.545, .072]
40°C AT x intervention x time	.066	.033	606.000	1.989	.047	[-1.593, .030]
46°C AT x intervention x time	.070	.033	606.000	2.098	.036	[.002, .130]
Rating of perceived exertion						
Intercept	13.740	.565	14.770	24.298	<.001	[12.599, 14.873]
Time	.170	.014	595.000	11.847	<.001	[.142, .197]
40°C AT	.179	.256	595.000	.699	.485	[-.319, .676]
46°C AT	1.216	.256	595.000	4.743	<.001	[.717, 1.714]
Intervention (pre-cooling)	-.173	.256	595.000	-.677	.499	[-.670, .324]
40°C AT x time	.0004	.020	595.000	.018	.986	[-.039, .040]
46°C AT x time	-.001	.020	595.000	-.061	.952	[-.041, .039]
Intervention x time	-.003	.020	595.000	-.127	.899	[-.042, .037]
40°C AT x intervention	.278	.361	595.000	.768	.443	[-.426, .981]
46°C AT x intervention	-1.032	.363	595.000	-2.846	.005	[-1.737, -.327]
40°C AT x intervention x time	-.003	.029	595.000	-.115	.908	[-.059, .052]
46°C AT x intervention x time	.044	.029	595.000	1.524	.128	[-.012, 0.100]

Chapter 4: Study Two

Single session intermittent heat exposure with more frequent and shorter cooling breaks facilitates greater training intensity and elicits comparable physiological responses to continuous heat exposure

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4.1 Abstract

Purpose: To investigate the influence of shorter, more frequent rest breaks with per-cooling (PerC) as an alternative heat acclimation session on physiological, perceptual, and self-paced maximal cycling performance, compared to continuous heat exposure. ***Methods:*** Thirteen participants completed one continuous and three intermittent heat exposure (IHE) maximal self-paced cycling protocols in a random order in heat (36°C, 80% relative humidity): 1 x 60-min exercise (CON), 3 x 20-min exercise with 7.5-min rest between sets (IHE-20), 4 x 15-min exercise with 5-min rest between sets (IHE-15), 6 x 10-min exercise with 3-min rest between sets (IHE-10). Mixed-method PerC (crushed-ice ingestion and cooling vest) was applied during rest periods of all IHE protocols. ***Results:*** Total distance completed was greater in IHE-10, IHE-15, and IHE-20 compared to CON (+11%, +9%, and +8%, respectively), with no difference observed between IHE protocols. Total time spent above 38.5°C core temperature (T_c) was longer in CON compared to IHE-15 and IHE-20 (+62% and +78%, respectively), but similar to IHE-10 (+5%). Furthermore, a longer time above 38.5°C T_c occurred in IHE-10 versus IHE-15 and IHE-20 (+54% and +69%, respectively). Sweat loss did not differ between conditions. ***Conclusion:*** Intermittent heat exposure with PerC may be a viable alternative heat acclimation protocol in situations where training quality takes precedence over thermal stimulus, or when both factors hold equal priority.

Key Words: Cooling, heat acclimation, self-paced, cycling, intermittent

4.2 Introduction

Heat acclimation (HA) is critical to mitigate the adverse effects of heat on exercise performance.[1] Traditional HA protocols involve exercise bouts in hot environments (>30°C) lasting 60 to 120-min over 10 to 14 days.[2,3] To elicit heat adaptations, including reductions in exercising and resting heart rate (HR), skin (T_{sk}) and core temperature (T_c), expansion of plasma volume, increased sweat rate, and improvements in thermal comfort (Th_c) and sensation (Th_s), various methodologies have been used.[2] These methodologies encompass controlled hyperthermia (e.g., maintaining T_c at 38.5°C throughout exercise), constant work rate (e.g., exercising at 60% maximal aerobic capacity), controlled intensity (e.g., exercising at 65% HR_{max}), and/or passive heating (e.g., water immersion or sauna bathing). While these methods yield physiological adaptations to heat, they also result in greater internal training load.[4] This can potentially compromise training quality and lead to overreaching. Therefore, there is a need for HA protocols that address situations where training quality is crucial, yet the heat stimulus is necessary.

Another method of HA that may maintain training quality without compromising heat stimulus is self-paced exercise. This approach allows athletes to freely adjust their pacing to attain or maintain intensity around a prescribed perception.[1,2,5] Furthermore, acute cooling such as pre-cooling and per-cooling (PerC) may assist in preserving exercise quality during HA sessions, despite appearing contradictory to the aims of HA training (i.e., increase T_c and T_{sk}, and induce sweating).[1] However, application of cooling can blunt increases in T_c and T_{sk}, improve Th_c, Th_s, and effort, thus improving/maintaining training and exercise performance.[6] Choo, et al.,[7] investigated the use of pre-cooling (30-min of ~22°C cold-water immersion) prior to 10 HA sessions (60-min cycling at a rating of perceived exertion [RPE] of 15,[8]) in heat (~35°C;~53% relative humidity [RH]). Their findings showed maintenance of a greater mean power output over the 10-day period with pre-cooling (+2.9%) compared to no pre-cooling (-2.6%). Similarly, Naito, et al.,[9] found that using PerC (via crushed-ice ingestion) during five HA sessions (80-min intermittent repeated sprint protocol in 36.5°C, 50% RH) resulted in greater total work observed on day 1 (~+4%) and day 5 (~+3%) of the HA sessions compared to no PerC. This may be attributed to lower end-exercise rectal temperature and T_{sk} in the PerC condition compared to no PerC on day 1 (~-0.4, and ~-1.0°C, respectively) and day 5 (~-0.4, and ~-0.4°C, respectively). These findings suggest that incorporating cooling during HA training sessions facilitates greater training intensity, presenting a viable training alternative for a HA program.

Another potential method of reducing the internal training load and mitigating athlete fatigue during HA involves using an intermittent heat exposure (IHE) protocol. This approach exposes athletes to heat during exercise intervals, followed by passive rest in thermoneutral conditions. To our knowledge, the use of IHE as a means of HA has not been previously explored. Potentially IHE could maintain and/or improve training quality by implementing cooling strategies (i.e., PerC) during rest breaks, thus benefitting from the ergogenic effects of PerC compared to no PerC.[10] However, before implementing IHE with PerC into a full HA protocol (chronic effects), we first need to better understand the performance, physiological, and perceptual responses to different rest breaks with PerC at the level of a single session (acute effects).

Therefore, the aim of this study was to investigate the acute physiological, perceptual and performance responses to shorter, more frequent rest breaks with PerC as an alternative HA session compared to a traditional continuous HA session without cooling in non-heat acclimatised endurance athletes. To understand the effect of different IHE set structures (i.e., short, frequent breaks *vs.* longer, less frequent breaks) on exercise responses, we compared three structures matched for total exercise and rest time. We hypothesised that training intensity and thermal perception would improve in the IHE sessions with shorter, more frequent cooling breaks while producing acute physiological responses comparable to those observed in a traditional continuous heat exposure.

4.3 Methods

Participants

Thirteen male cyclists and/or triathletes (mean±SD: age=34.0±11.1 y; body mass [BM]=78.08±9.37 kg; height=177.79±6.30 cm; sum of eight skinfolds=77.27±26.57 mm; body surface area=1.96±0.14 m^2[11]; training distance=241±120 km per week; training frequency=5±2 sessions per week), corresponding with an athlete classification of 2 or 3 (trained or highly trained[12]) provided written informed consent. Participants had not undertaken a HA protocol, trained in environments exceeding 30°C, or resided in a summer (warm) climate in the last month (i.e., non-heat acclimatised). The study was approved by Curtin University Human Research Ethics Committee (HRE2021-0398).

Study Overview

Testing occurred during the cooler months in Western Australia (March – October) to control for exposure to high environmental temperatures and RH, which may induce heat adaptations and influence the cooling intervention. Participants attended a familiarisation session followed by four experimental sessions, separated by 7±2 days. These sessions were completed in a randomised order (http://www.randomization.com/, January 2022). All experimental sessions comprised self-paced cycling exercise in a heat chamber (35.8°C, 77.7% RH; ~48°C apparent temperature [AT], no fan and radiant heat[13]) for a total of 60-min with 15-min rest in a thermoneutral environment. During rest, mixed method (crushed ice plus cooling vest) PerC was applied. The four experimental sessions had different set structures: 1 x 60-min exercise (CON), 3 x 20-min exercise with 7.5-min rest between sets (IHE-20), 4 x 15-min exercise with 5-min rest (IHE-15), and 6 x 10-min exercise with 3-min rest (IHE-10). Participants refrained from vigorous exercise, alcohol (within 24-h), and caffeine (within 6-h) prior to each experimental session. Additionally, participants were instructed to keep a food diary and replicate their dietary intake for the 24 hours prior to each experimental session to ensure nutritional consistency. To ensure adequate hydration prior to each experimental session, participants were instructed to drink 6 $mL \cdot kg^{-1}$ BM of water every 2.5-h on the preceding day.

Familiarisation Session

During the familiarisation session, participants' anthropometric measures (height, BM and sum of eight skinfolds) were obtained. Standing height and BM were recorded using a calibrated stadiometer (Holtain Limited, United Kingdom) and electronic scales (Seca 360° Wireless, Seca, United Kingdom), respectively. Skinfold thickness was assessed using International Society for Advancement of Kinanthropometry (ISAK) standardised methodology at eight sites (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, and medial calf) using calibrated Harpenden skinfold calipers (Baty International, United Kingdom) by an ISAK accredited Level 1 anthropometrist (intra-technical error measurement = 1.1%). Subsequently, participants completed the entire IHE-15 protocol on a cycling ergometer (BikeErg, Concept 2, Queensland, Australia) to become familiar with the demands of experimental sessions.

Experimental Session

Eight hours prior to each experimental session, participants ingested a core body temperature pill (E-Celsius© Performance, Bodycap Medical, Caen, France) to measure T_c. Upon arrival at the laboratory, hydration status was determined by urine specific gravity (USG; Portable Refractometer, RS PRO, RS Components, New South Wales, Australia). Participants with a USG reading of >1.021 µg were instructed to ingest water and re-tested every 10-min until USG readings were ≤1.021 µg. Nude BM was measured pre- and post-experimental session on a set of calibrated electronic scales (Model CW11, OHAUS Corporation, New Jersey, USA) to calculate sweat loss (pre – post nude BM + fluid ingested). Surface T_{sk} was measured by placing dermal temperature sensors (DS1922L; iButton™, Maxim Integrated Products, California, USA) on the mesosternale, mid-forearm, mid-quadriceps and medial calf to calculate mean T_{sk}.[14] A HR monitor (A300 Polar, Electro, Kempele, Finland) was also placed on the participants' chest to measure HR.

Participants entered the heat chamber and completed a 10-min cycling warm-up at a perceptually regulated exercise intensity (RPE of 13, 15 and 17 on the 6-20 Borg scale for 5, 3 and 2-min, respectively[8]) on the same cycling ergometer as the familiarisation session. This was followed by completing the first exercise interval at the participant's highest sustainable intensity. Participants were blinded to their completed distance but received continuous feedback on time remaining. After each exercise interval, participants exited the heat chamber and moved to a thermoneutral environment (23.7°C, 53.9% RH; ~25°C AT) to passively rest and receive the PerC intervention. During the rest period, participants sat wearing an activated cooling vest (as per manufacturer's instructions, Arctic Heat, Queensland, Australia). At the same time, crushed-ice (using an ice shaver, Avalanche Model IS6800, Sunbeam, New South Wales, Australia) was ingested to achieve a session total of 3.5 $g \cdot kg^{-1}$ BM, which represents 15-min of previously utilised ingestion rate (IHE-10: 0.7 $g \cdot kg^{-1}$ BM per rest break, IHE-15: 1.17 $g \cdot kg^{-1}$ BM, and IHE-20: 1.75 $g \cdot kg^{-1}$ BM).[15] No cooling occurred during or after the CON trial. Upon completion of the rest interval, participants re-entered the heat chamber and repeated this process for all exercise/rest intervals. Participants had access to *ad libitum* water consumption throughout all sessions. Water was retrieved from a water fountain (~16°C), held in a non-insulated water bottle which entered the chamber when participants commenced the warm-up and remained in the heat chamber until a refill was necessary. After refilling, the water bottle was returned and remained in the heat chamber. Heart rate, T_c, and T_{sk} data were collected every 2-min during the warm-up, and every 5-min during exercise intervals, and pre- and post-rest intervals. Core temperature was monitored continuously (15 s sample rate) throughout the session to calculate time spent above 38.5°C T_c. Thermal comfort and Th_s were recorded (0-20 scales ranging from 'very cold' to 'very hot' and from 'very comfortable' to 'very uncomfortable'[16]) pre- and post-warm up, and every 5-min during exercise and pre- and post-rest intervals. Rating of perceived exertion (6–20 scale[8]) was obtained every 5-min during the exercise protocol. Exercise performance was assessed by measuring split-distance every 5-min as well as total distance completed.

4.4 Data and Statistical Analyses

Body temperature (T_b) was calculated using the equation: $0.65 \times T_c + 0.35 \times T_{sk}$.[17] Intraclass correlation coefficients (ICC) were calculated for all variables to determine the percentage of variance explained by individual differences between participants. When ICC values exceed 0.5, analysts are advised to utilise statistical models that account for non-independence in the data.[18] Given data sampled repeatedly from participants was not independent and ICCs in 9 of 20 outcomes were >0.5 (see supplementary material) multilevel modelling was used to analyse the data. Multilevel modelling analysed the relationship between T_c, mean T_{sk}, T_b, HR, RPE, Th_c, Th_s and total and split distance completed for each condition, with estimates reported as unstandardised regression coefficients (β). This indicates the amount of change (in the unit of measurement e.g., BPM, °C) between different levels of categorical independent variables (i.e., condition) or per second increase in time.[19] Additionally, linear mixed effects regression model was used to determine differences in hydration, total fluid ingested, sweat loss and time spent above 38.5°C T_c during exercise intervals. Where significant interaction effects were identified, post-hoc simple slopes analyses, corrected for multiple comparisons, were conducted to compare the relationship between distance and condition pairings. Key findings are summarised in text and full results are provided as supplementary material. Linear mixed-effects regression and post-hoc analyses were conducted using the “lme4” and “emmeans” packages, respectively, in R (R Foundation for Statistical Computing, Austria) with significance accepted at p<.05.[20,21]

4.5 Results

Hydration and Sweat Loss

Urine specific gravity was significantly lower in CON (1.011±.010 µg) compared to IHE-10 (1.016±.010 µg, β=-.01, p<.01) and IHE-15 (1.015±.010 µg, β=-.004, p<.01), but not in IHE-20 (1.012±.010 µg, β=-.001, p=.32).

Total fluid ingestion was greater in CON (1.85±.57 L) compared to IHE-10 (1.49±.67 L, β=.36, p<.01), IHE-15 (1.36±.67 L, β=.50, p<.01), and IHE-20 (1.53±.82 L, β=.32, p<.01).

No significant difference in sweat loss was observed between CON (2.12±.53 L) and IHE-10 (2.07±.65 L, β=-.05, p=.39), IHE-15 (2.07±.56 L, β=-.05, p=.30), and IHE-20 (2.02±.60 L, β=-.10, p=.06).

Cycling Performance

Total distance completed was 11%, 9%, and 8% greater in IHE-10 (26,765±1,300 m, β=-2203.88, p<.01), IHE-15 (26,406±1,300 m, β=-1928.64, p<.01), and IHE-20 (26,167±1330 m, β=-1,689.95, p<.01) than CON (24,232±1,858 m), respectively. No significant difference was observed between IHE protocols. There was a significant (p<.01) two-way interaction between condition and time in IHE-10 (β=4.11), IHE-15 (β=4.79), and IHE-20 (β=4.73) for split distance completed. Post-hoc comparisons (Figure 4.1) indicated lower split distance completed over time in CON than all IHE protocols. No differences in split completion distance were identified between IHE protocols.

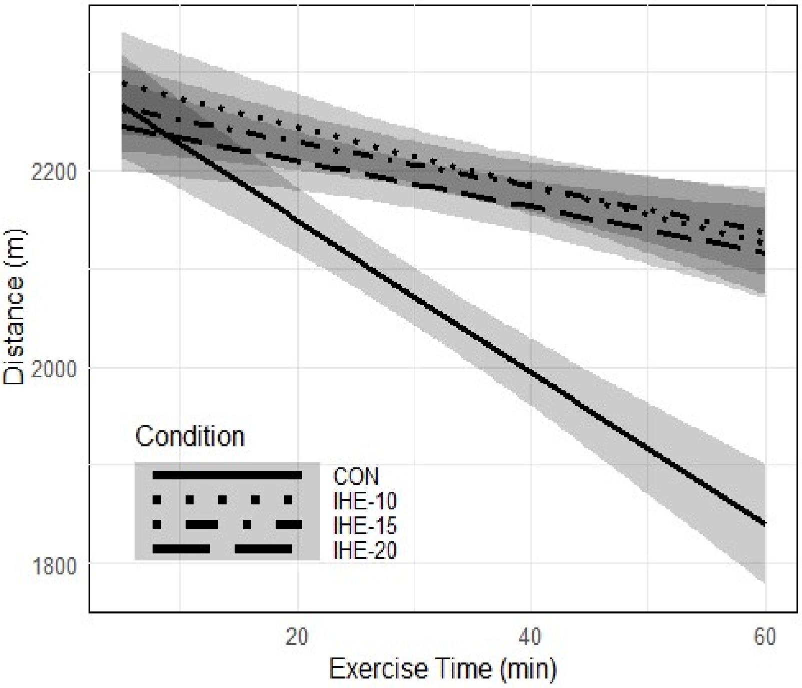

Figure 4.1. Split distance completed as a function of total-time cycled in control (CON), 6 x 10 min, (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

Thermal Response

Exercise

Accumulated time (and % of total session) above 38.5°C T_c was longer in CON (1,815±1,109 s, ~50%) compared to IHE-15 (1,124±1,394 s, ~31%, β=-500.28, p=.03) and IHE-20 (1,019±1,618 s, ~28%, β=-893.23, p<.01). No difference was observed between IHE-10 and CON (1,726±1,471 s, ~48%, β=32.19, p=.89). Longer time above 38.5°C T_c was observed in IHE-10 compared to IHE-15 (β=-532.47, p=.02) and IHE-20 (β=-925.43, p<.01). There was a significant two-way interaction (condition x time) in all IHE protocols for T_c (IHE-10: β=-.02, p<.01, IHE-15: β=-.02, p<.01, IHE-20: β=-.03, p<.01), mean T_{sk} (IHE-10: β=-.02, p<.01, IHE-15: β=-.02, p<.01, IHE-20: β=-.02, p<.01), and T_b (IHE-10: β=-.02, p<.01, IHE-15: β=-.02, p<.01, IHE-20: β=-.03, p<.01) during exercise intervals. Post-hoc comparisons indicated a greater increase in T_c (Figure 4.2A) over time in CON than all IHE protocols (IHE-10: β=.02, t[509]=7.14, p<.01, IHE-15: β=.02, t[509]=6.38, p<.01, IHE-20: β=.03, t[509]=9.31, p<.01), and greater increase over time in IHE-15 compared to IHE-20 (β=.01, t[509]= 3.55, p<.01). Similarly, post-hoc comparisons indicated greater mean T_{sk} (Figure 4.2B) over time in CON than all IHE protocols (IHE-10: β=.02, t[584]=4.68, p<.01, IHE-15: β=.02, t[584]=5.07, p<.01, IHE-20: β=.02, t[584]=4.69, p<.01). No significant difference between IHE protocols was observed. Post-hoc comparisons for T_b (Figure 4.3) indicated greater T_b over time in CON than all IHE protocols (IHE-10: β=.02, t[509]=7.23, p<.01, IHE-15: β=.003, t[509]=7.06, p<.01, IHE-20: β=.03, t[509]=9.10, p<.01), and greater over time in IHE15 compared to IHE20 (β=.01, t[509]= 2.60, p<.01).

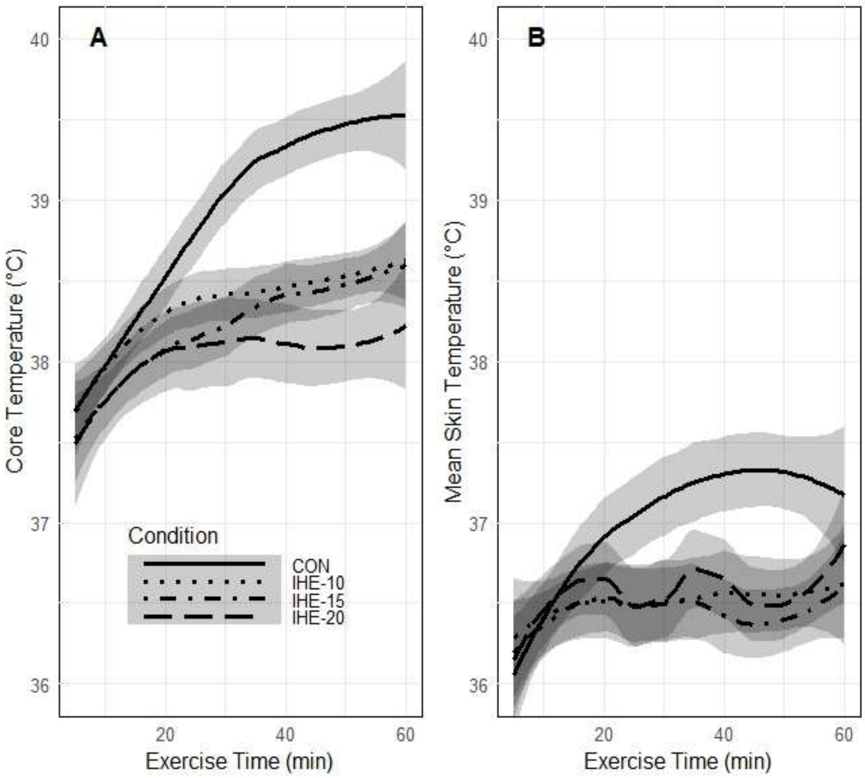

Figure 4.2. (A) Core and (B) mean skin temperature as a function of total-time cycled in control (CON), 6 x 10 min, (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

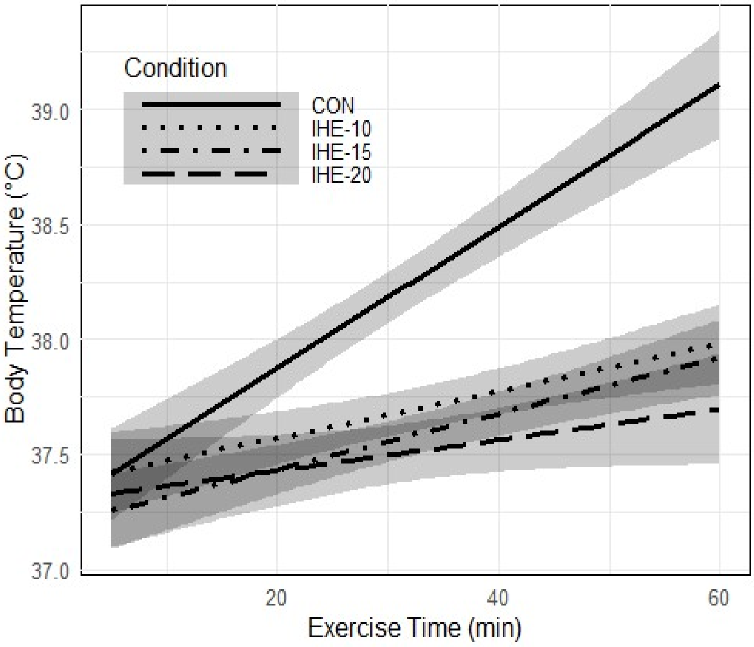

Figure 4.3. Body temperature as a function of total-time cycled in control (CON), 6 x 10 min, (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

There was a significant two-way interaction (condition x time) for HR in IHE-20 (β=-.24, p<.01). Post-hoc comparisons (Figure 4.4) indicated greater HR over time in CON than IHE-20 (β=.24, t[580]=2.89, p=.02), and no difference compared to IHE-15 (β=.14, t[580]=1.75, p=.30) and IHE-10 (β=.18, t[580]=2.15, p=.14). No significant difference between IHE protocols was observed.

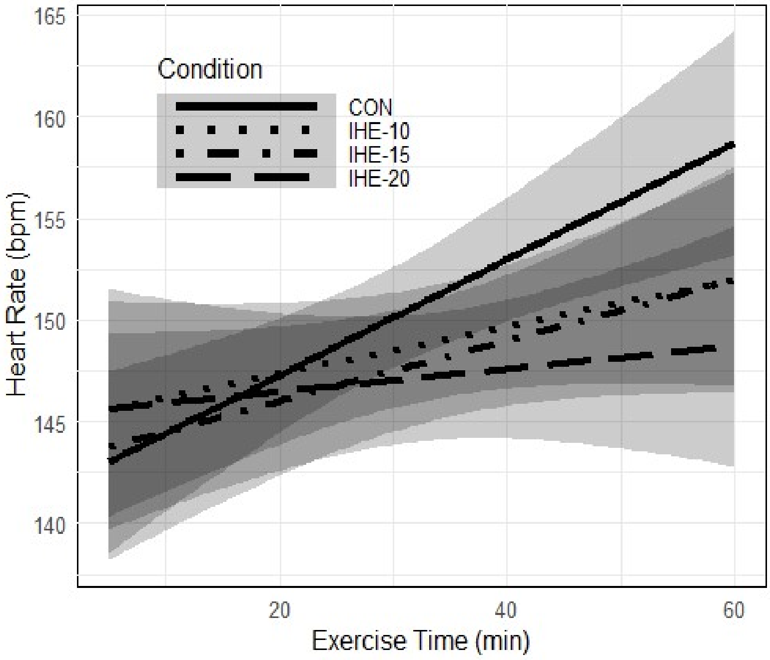

Figure 4.4. Heart rate as a function of total-time cycled in control (CON), 6 x 10 min, (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

Rest

There was a significant two-way interaction (condition x time) in IHE-20 for T_c (β=-.06, p<.01), mean T_{sk} (β=-.13, p<.01), T_b (β =-.10, p<.01), and HR (β=-2.65, p<.01) during the rest periods. Post-hoc comparisons indicated lower T_c (Figure 4.5; β=.06, t[204]=3.81, p<.01), mean T_{sk} (β=.14, t[242]=4.07, p<.01), T_b (β=.10, t[204]=5.34, p<.01), and HR (β=2.65, t[220]=3.28, p<.01) over time in IHE-20 compared to IHE-10 during rest periods. Similarly, lower T_c (β=.05, t[204]=2.97, p=.01), and T_b (β=.05, t[204]=2.80, p=.02) over time was observed in IHE-20 compared to IHE-15, and lower T_b in IHE-15 compared to IHE-10 (β=.04, t[204]=2.70, p=.02) during rest periods.

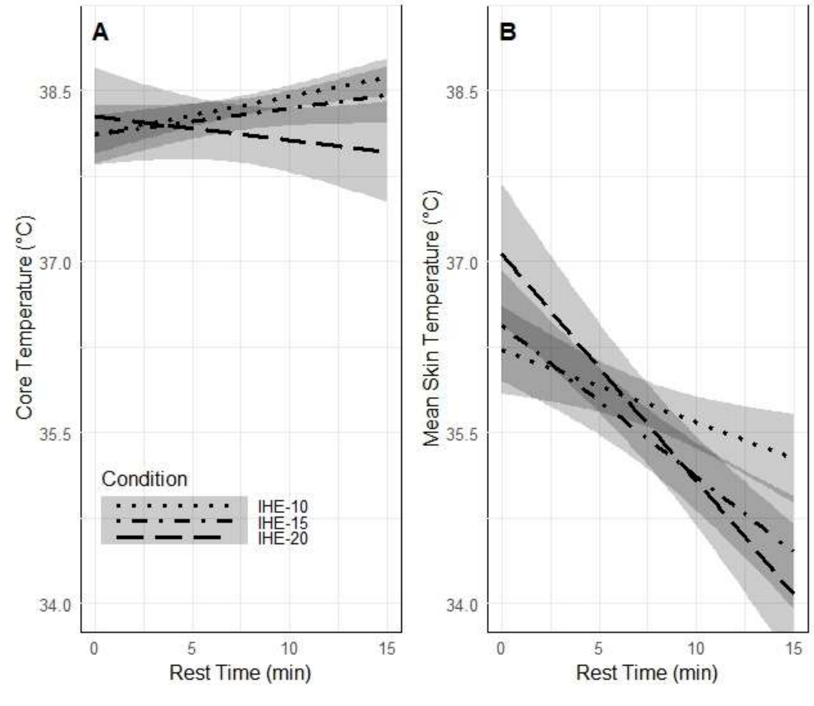

Figure 4.5. (A) Core, and (B) skin temperature as a function of total rest time in 6 x 10 min (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

Perceptual Response

Exercise

There was a significant two-way interaction (condition x time) for Th_c (β=-.07, p<.01), Th_s (β=-.06, p<.01), and RPE (β=-.02, p<.01) for IHE-20. Post-hoc comparisons indicated improved Th_c (IHE-10: β=.09, t[584]=7.72, p<.01, IHE-15: β=.09, t[584]=7.63, p<.01, IHE-20: β=.07, t[584]=6.15, p<.01), Th_s (IHE-10: β=.07, t[584]=6.89, p<.01, IHE-15: β=.06, t[584]=6.48, p<.01, IHE-20: β=.06, t[584]=5.86, p<.01), and RPE (IHE-10: β=.03, t[584]=3.44, p<.01, IHE-15: β=.03, t[584]=3.18, p<.01, IHE-20: β=.02, t[584]=2.78, p<.01) over time in all IHE protocols compared to CON. No difference between IHE protocols were observed.

Rest

There was a significant two-way interaction (condition x time) for Th_c (β=-.18, p<.01) and Th_s (β=-.18, p<.01) in IHE-20. Post-hoc comparisons indicated lower Th_c and Th_s (Figure 4.6) over time in IHE-20 compared to IHE-10 (β=.18, t[242=3.71, p<.01, and β=.18, t(241)=3.15, p=.01, respectively).

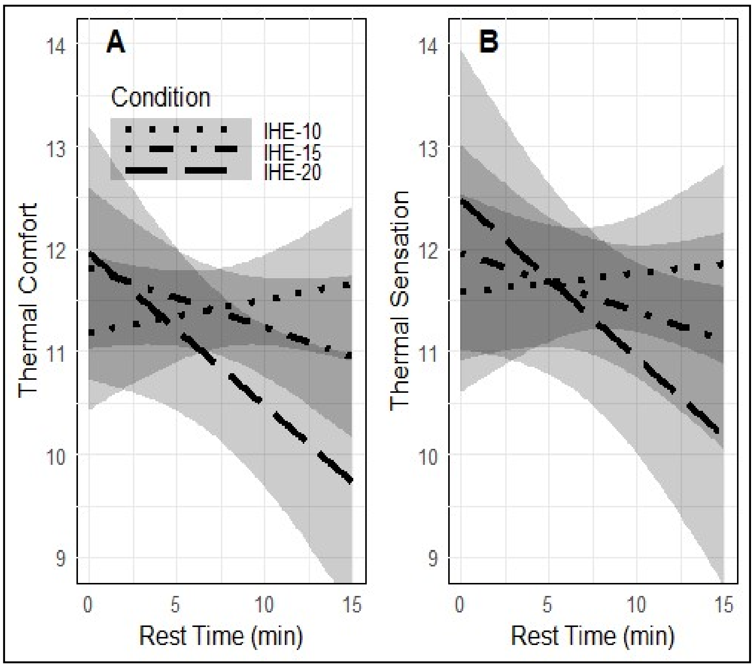

Figure 4.6. (A) Thermal comfort and (B) thermal sensation as a function of total rest time in 6 x 10 min (IHE-10), 4 x 15 min (IHE-15), and 3 x 20 min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

4.6 Discussion

To the best of our knowledge, this is the first study examining the effect of shorter, more frequent rest breaks with PerC as an alternative HA session compared to traditional continuous heat exposure with no cooling. One key finding was that 5-min split distances (total and mean) were greater in all IHE protocols compared to CON, with no discernible difference between IHE protocols. Additionally, time spent above 38.5°C T_c was not significantly different between CON and IHE-10, but was longer in CON compared to IHE-15 and IHE-20. Thermal comfort, Th_s, and RPE were lower in all IHE protocols compared to CON, and no difference between conditions was observed for sweat loss. These results confirm our hypothesis that IHE sessions with shorter exercise durations and more frequent breaks can result in improved self-paced maximal cycling performance and thermal perception compared to continuous heat exposure. Importantly, IHE-10 with shorter exercise bout durations and more frequent breaks achieved these performance and perceptual benefits without compromising necessary acute physiological stimuli required for HA (i.e., time spent above 38.5°C T_c and sweat loss).

Enhanced performance in IHE protocols supports previous findings by Naito, et al.,[9] who observed significantly greater total work following PerC compared to control on Day 1 (~206 *vs.* ~198-kJ) of HA training. Performance improvements were attributed to lower T_c and T_{sk} resulting from PerC. Our study also observed lower T_b in all IHE protocols compared to CON, which may explain improved performance in heat.

Core temperature increased over time for all conditions, but consistently remained lower during all IHE protocols compared to CON. This result aligns with previous research suggesting that PerC attenuates increases in T_c during exercise compared to scenarios without PerC, and added breaks may further contribute to blunting T_c increases.[6,22,23] Additionally, a lower rate of increase was evident in IHE-20 compared to IHE-15 due to the longer rest break. However, no difference was observed in IHE-10 compared to IHE-15 and IHE-20. This indicates that IHE protocol selection may depend on the specific requirements of the training session, as different IHE set structures have varying effects on T_c.

To induce HA, it is commonly proposed that maintaining T_c of ≥38.5°C is a key factor for adaptation.[24,25] Despite cooler T_b evident in all IHE protocols, IHE-10 spent ~48% of the total exercise time with T_c ≥38.5°C, IHE-15 spent ~31%, and IHE-20 spent ~28%. Notably, no difference was observed between CON and IHE-10 for the percentage of total exercise time with T_c ≥38.5°C (~50% *vs.* ~48%). This may be due to greater total and mean 5-min split distance completed in IHE-10 compared to CON, resulting in greater intensity driven metabolic heat production equalling the continuous heat exposure thermal strain. Thus, an IHE protocol with shorter and more frequent breaks (e.g., IHE-10) may enhance performance whilst promoting maintenance of high T_c closer to that of continuous exposure despite cooling rest periods.

Additionally, there was consistently lower mean T_{sk} observed over time during all IHE protocols compared to CON. This aligns with previous literature, as PerC is known to delay increases in T_{sk}, ultimately resulting in improved performance.[6,22,23] Schlader, et al.,[26] suggested that high T_{sk} (>35°C[1,27]) initiates an anticipatory response, leading to lower initial work rates during exercise in heat. Alternatively, lower T_{sk} corresponds with improved levels of Th_s and Th_c, resulting in improved distance completed during a 60-min cycling time-trial. Our findings support this, as the lower T_{sk} observed in all IHE protocols corresponded with improved Th_c, Th_s, and RPE, along with greater total and 5-min split-distance completed.

Sweat loss was not different between CON and IHE protocols, despite the intermittent nature of the exercise and the application of PerC, suggesting sweat rates are comparable between IHE and continuous heat exposure. Our study observed greater sweat loss in single IHE sessions (~2.05 L) compared to what Choo, et al.,[7] and Naito, et al.,[9] reported in their pre-acclimation sessions (1.15 and 1.32 L, respectively). This difference in sweat loss may be due to different levels of RH observed in both studies (~50% RH) compared to our study (~78% RH). This may have exacerbated the thermal strain experienced by participants in our study, as impaired sweat evaporation can limit heat loss due to low water vapour pressure differences between the environment and skin.[28]

Although findings of this study support the notion that IHE with PerC may be an alternative method of HA, it must be noted that our study only provides an acute assessment of this protocol. Thus, further research is required to determine the chronic effects of repeated exposures (i.e., a HA program). Another limitation of the study lies in its execution on non-heat acclimatised individuals, and indoor conditions. Therefore, it cannot be excluded that a greater/lesser magnitude of change in performance, physiological, and perceptual responses is observed with heat-acclimated individuals, or when performed outdoors with the addition of environmental factors (e.g., radiant heat or wind). Consequently, the outcomes of this study may not be directly applicable to partially or fully heat-acclimated individuals. Additionally, athletes in this study were classified as level 2-3 (trained or highly trained), and it remains uncertain whether athletes at level 4-5 (elite or world class), individuals at level 0 (sedentary), or those who are not healthy would respond similarly. These results also do not provide insight into the effect of IHE alone, and it is possible that the intermittent rest breaks may account for the differences on physiological, perceptual and performance variables, irrespective of the PerC intervention.

4.7 Practical Applications

Traditional continuous exercise heat exposure training is recommended when the overall training load permits prioritising a maximal HA stimulus over training quality. Conversely, in situations where training quality takes precedence over HA stimulus or when training load needs to be limited, IHE-20 and IHE-15 protocols may be preferred. Finally, IHE-10 may be the preferred choice when both HA stimulus and training quality hold equal priority.

4.8 Conclusion

Intermittent heat exposure with PerC resulted in increased training intensity compared to traditional continuous heat exposure. Despite the shorter exercise duration and more frequent breaks in IHE-10, the necessary stimulus (e.g., time above 38.5°C T_c) and physiological responses (e.g., sweat loss) required for effective HA were not compromised. This can be attributed to lower T_c and T_{sk} as well as improved perceptual responses when PerC was applied.

4.9 References

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4.10 Supplementary Material

Table S4.1. Intraclass correlation coefficients for hydration and sweat loss, performance, physiological, and perceptual data during the exercise and rest periods of the continuous and IHE protocols.

Variable	Pre-exercise	
Urine specific gravity	25%	
Total fluid ingestion	60%	
Sweat loss	80%	
Total distance completed	54%	
5 min split distance completed	33%	
	Exercise Period	**Rest Period**
Core temperature	34%	45%
Mean skin temperature	54%	21%
Body temperature	45%	40%
Heart rate	60%	19%
Thermal comfort	48%	51%
Thermal sensation	58%	63%
Rating of perceived exertion	51%	-

Table S4.2. Mean ± SD for physiological data at the start and finish of the rest per-cooling period for 6x10 min, (IHE-10), 4x15 min (IHE-15), and 3x20 min (IHE-20) intermittent heat exposure conditions.

	Start	Finish
Core Temperature (°C)		
IHE-10	38.40 ± 0.44	38.32 ± 0.45
IHE-15	38.40 ± 0.56	38.17 ± 0.54
IHE-20	38.30 ± 0.88	37.95 ± 0.72
Skin Temperature (°C)		
IHE-10	36.62 ± 0.76	34.86 ± 0.80
IHE-15	36.53 ± 0.76	34.37 ± 0.80
IHE-20	37.00 ± 0.82	34.15 ± 0.85

Heart Rate (bpm)		
IHE-10	160 ± 19	115 ± 15
IHE-15	156 ± 17	108 ± 16
IHE-20	158 ± 20	106 ± 19

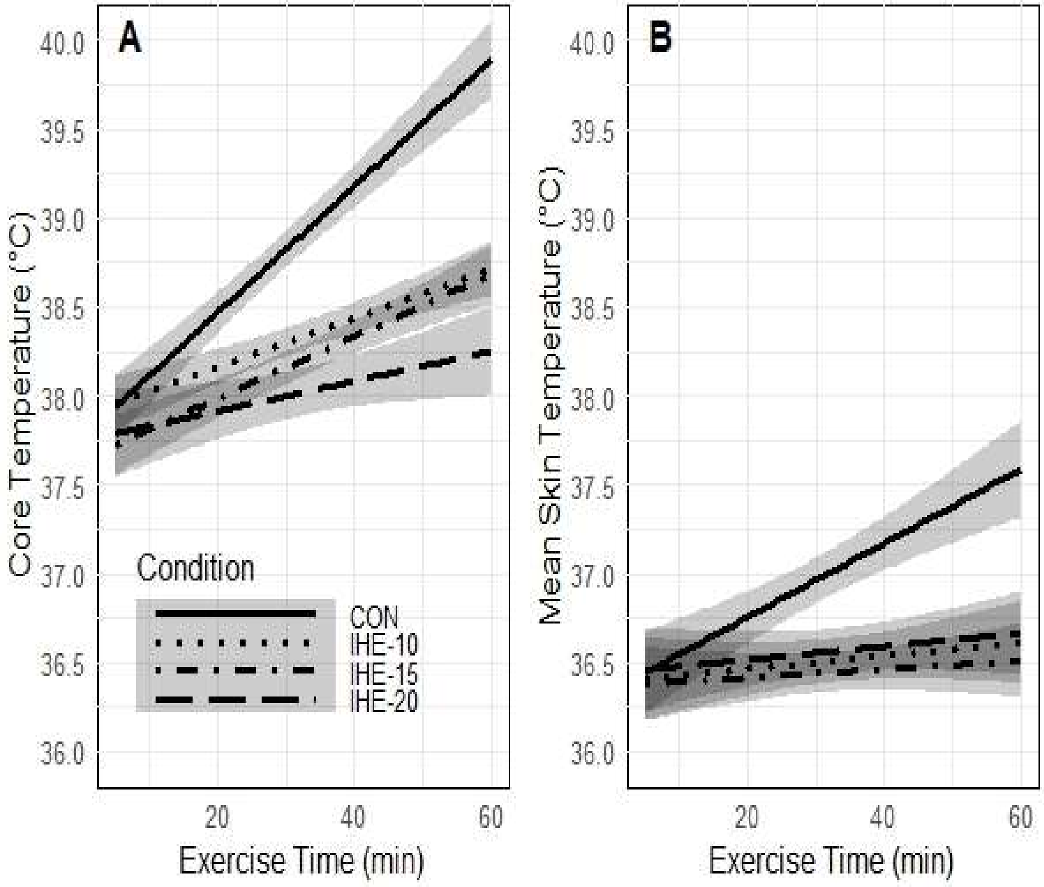

Figure S4.7. (A) Core and (B) skin temperature as a function of total-time cycled in control (CON), 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions. *Note: Shaded areas indicate 95% confidence intervals.*

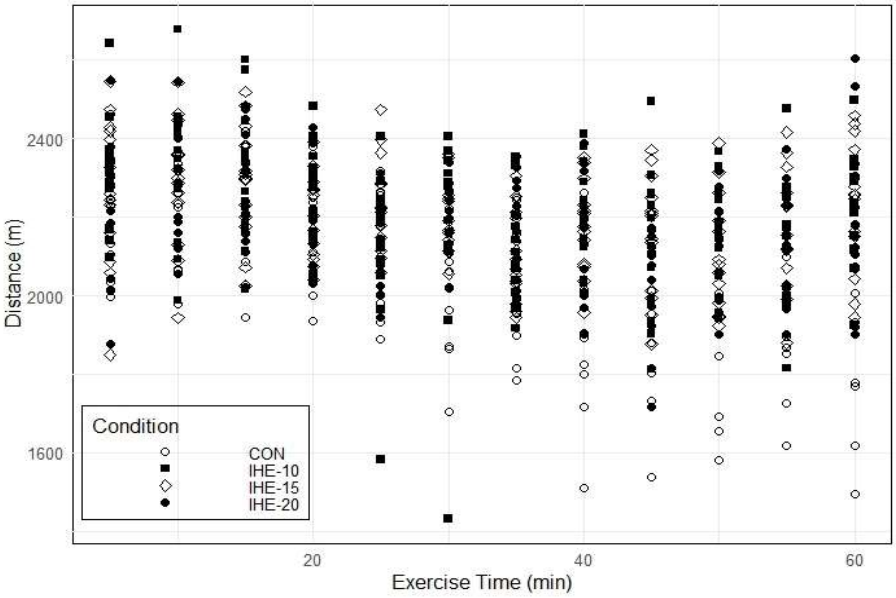

Figure S4.8. Individual split distance completed over total-time cycled in in control (CON), 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

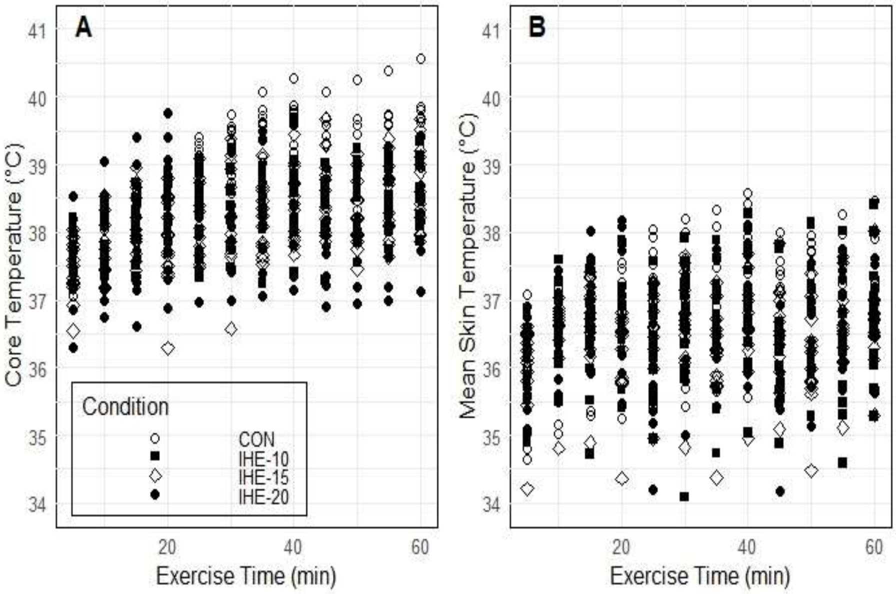

Figure S4.9. Individual (A) Core and (B) skin temperature over total-time cycled in in control (CON), 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

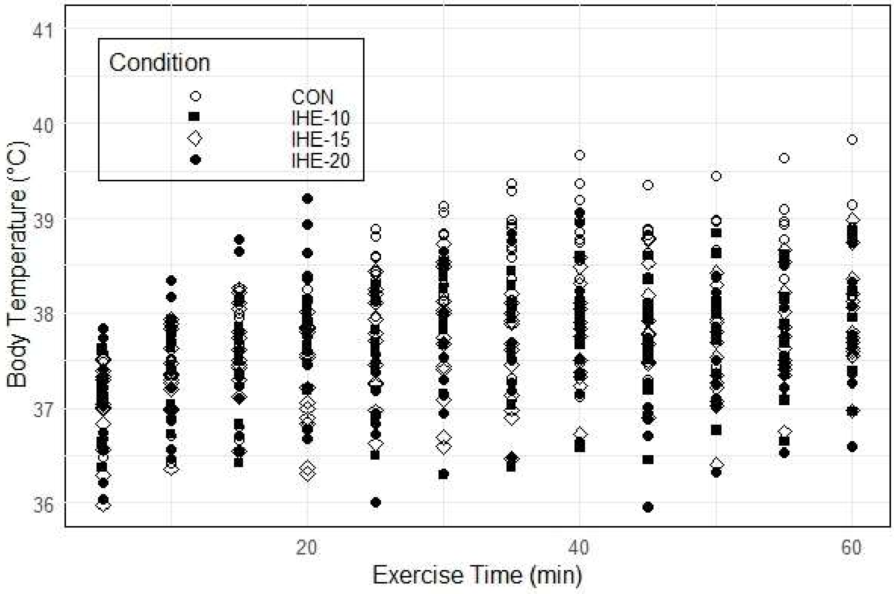

Figure S4.10. Individual body temperature over total-time cycled in in control (CON), 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

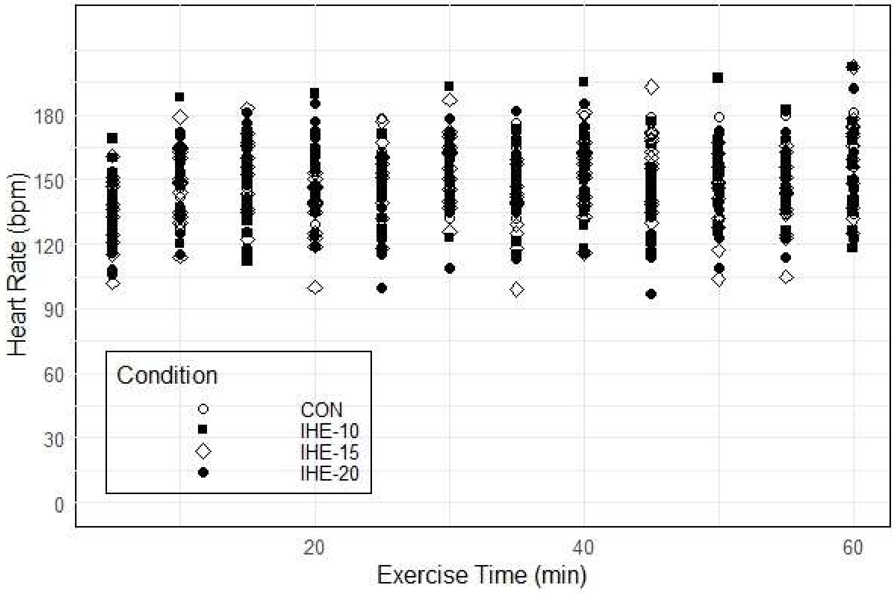

Figure S4.11. Individual heart rate over total-time cycled in in control (CON), 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

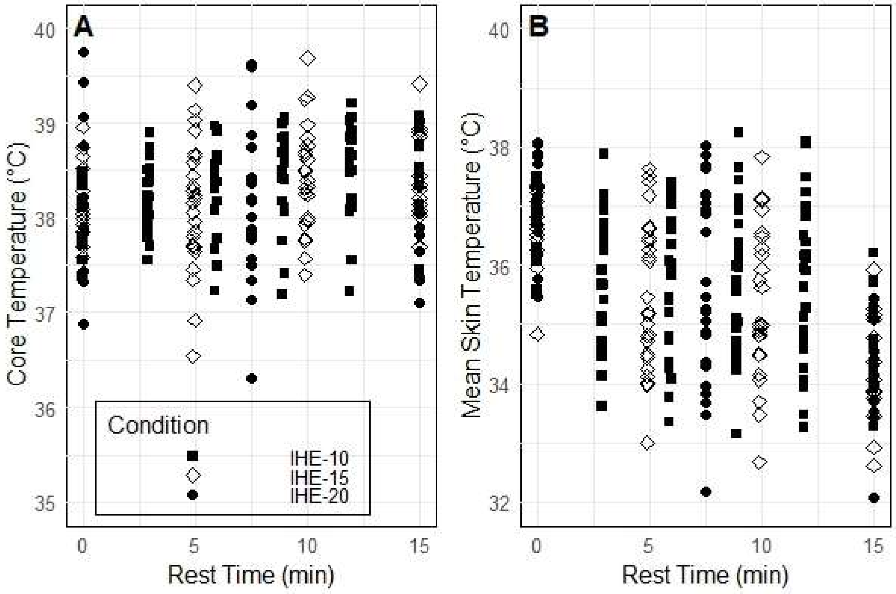

Figure S4.12. Individual (A) Core and (B) skin temperature over total rest time in 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

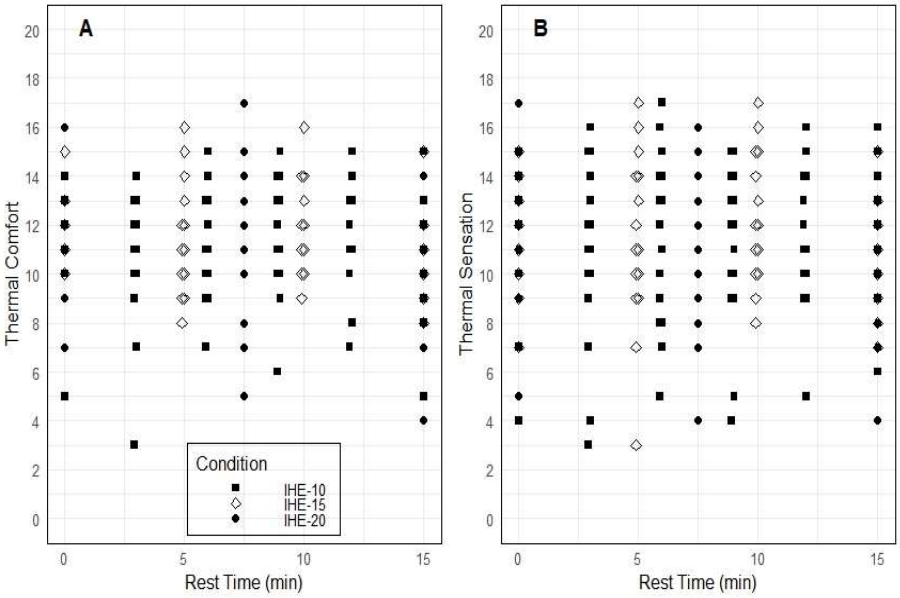

Figure S4.13. Individual thermal (A) comfort and (B) sensation over total rest time in 6 x 10-min, (IHE-10), 4 x 15-min (IHE-15), and 3 x 20-min (IHE-20) intermittent heat exposure conditions.

Chapter 5: Study Three

Pre-cooling alters pacing profiles resulting in no additional benefit to 20-km self-paced maximal cycling time-trial performance in heat acclimated endurance athletes

Julian A. P. Ramos[a], Kagan J. Ducker[a], Hugh Riddell[a], Olivier Girard[b], Grant Landers[b], Carly J. Brade[a]

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5.1 Abstract

Objective: To examine the effect of pre-cooling (PreC) on 20-km cycling time-trials (CTT) in hot conditions, both before and after heat acclimation (HA) sessions in endurance trained athletes.

Design: Randomised crossover.

Method: Ten trained or highly trained male (Tier 3 or 4) cyclists and/or triathletes completed two 20-km CTT before (PreHA) HA training sessions (10 x 60 min intermittent-heat exposure protocol in 36°C, 50-80% relative humidity), and another two after (PostHA; i.e., four total). Mixed-method PreC (30 min via crushed-ice ingestion plus cooling vest) or no cooling (CON) was applied prior to PreHA and PostHA.

Results: No meaningful direct relations were observed for 20-km CTT completion time between PreHA-CON (2682±228 s) and PostHA-CON (2671±370 s; b=-44.43[-110.68,22.02]) or PreHA+PreC (2671±242; b=-50.78[-166.15, 64.44]). Finally, no difference was observed between PostHA-CON and PostHA+PreC (2663±307 s; b=37.81[-109.98,170.56]). Greater sweat loss (intervention pooled) was observed in PostHA compared to PreHA (2.24±0.54 vs. 1.98±0.71 L; b=0.333[0.002,0.675]). During the CTT, core temperature was lower over the course of PostHA-CON compared to PostHA+PreC (b=-0.02[-0.04,-0.01]). Mean skin temperature was also lower during the CTT (condition pooled) with PreC compared to CON (b=-0.16[-0.27,-0.05]).

Conclusions: Insufficient evidence exists to support a meaningful performance improvement in 20-km CTT in hot-humid environmental conditions when mixed-method PreC was applied to heat-acclimated individuals. This can be attributed to HA-induced adaptions (e.g., greater sweat loss), which diminished the ergogenic benefit of PreC in heat by reducing mean skin temperature. Additionally, sub-optimal pacing strategies resulting from the application of PreC on heat-acclimated individuals may explain the lack of additional benefit to performance.

Key Words: cooling, heat acclimation, self-paced exercise, endurance, cycling,

5.2 Introduction

To mitigate the negative impact of hot-humid environments (i.e., >30°C and >60% relative humidity; RH) on exercise performance, implementing preventive countermeasures such as heat acclimation (HA) and pre-cooling (PreC) are recommended (Periard et al., 2021, Bongers et al., 2017). In isolation, both methods demonstrate reduced thermal strain by inducing heat adaptations, increasing heat storage capacity (via a decrease in core temperature; T_c), and improving pre-event thermal perception (Alhadad et al., 2019, van de Kerkhof et al., 2023). Theoretically, combining these methods by applying PreC to heat-acclimated/acclimatised ($Heat_{Acc}$) individuals may further decrease T_c and/or skin (T_{sk}) temperature, and improve thermal comfort (Th_c) and sensation (Th_s), potentially improving endurance exercise performance in heat.

To date, only five studies have investigated the application of PreC on $Heat_{Acc}$ individuals and the effect on exercise performance in hot conditions (Brade et al., 2013a, 2013b, Castle et al., 2011, James et al., 2018, Schmit et al., 2017). Among them, three examined the effect of 20–30 min PreC prior to 40–80 min intermittent-sprint or repeat sprint efforts (~27–35°C, ~39–60% RH; Brade et al., 2013a, 2013b, Castle et al., 2011). These studies showed no further benefits when PreC was applied compared to no PreC post HA (~-1–3% mean difference). Two studies explored the effect of 10–20 min PreC prior to running or cycling time-trials (i.e., ~20–32 min continuous efforts; CTT) in heat (32–35°C, 50–60% RH; James et al., 2018, Schmit et al., 2017). Similarly, both studies revealed no statistically significant additional performance benefits compared to HA alone (~-4-0.4% mean difference).

The absence of conclusive evidence indicating performance benefits of PreC in Heat_{Acc} individuals may be attributed to insufficient thermal strain resulting from external (e.g., air temperature and humidity) or internal (e.g., acclimation status and pacing profile) factors. For instance, in intermittent exercise studies, HA alone reduced thermal strain, potentially blunting the ergogenic effects of PreC in enhancing performance in Heat_{Acc} individuals (Brade et al., 2013a, 2013b, Castle et al., 2011). Despite longer total working durations conducted during the Brade et al. (2013a, 2013b) and Castle et al. (2011) studies, compared to endurance exercise studies (James et al., 2018, Schmit et al., 2017),, end-exercise T_c was lower (~38.6 vs. ~39.4°C) for similar environmental conditions. Even with greater thermal strain (i.e., greater T_c) observed in the endurance exercise studies, PreC did not yield additional performance benefits compared to HA alone in Heat_{Acc} individuals. Both studies proposed that PreC could influence pacing profiles, leading to higher initial exercise intensities, potentially exacerbating heat gain during exercise and ultimately limiting performance (James et al., 2018, Schmit et al., 2017). Additionally, it is possible that the environmental conditions (e.g., <35°C, <60% RH) did not induce sufficient thermal stress for PreC to exert an ergogenic effect on performance. This suggests that PreC may benefit endurance efforts, in hot-humid conditions (e.g., >35°C, >60% RH) in Heat_{Acc} individuals.

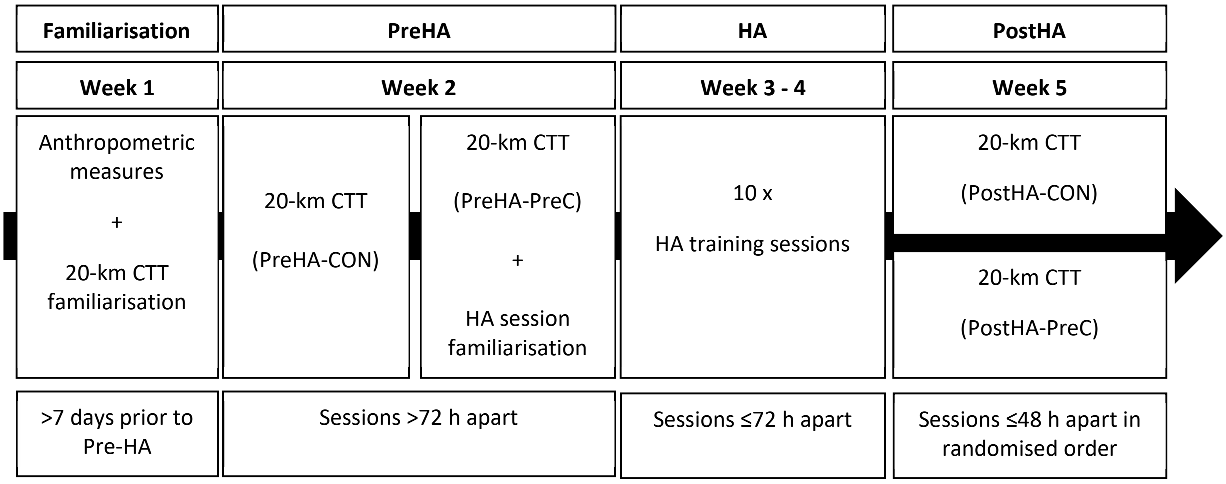
Limited evidence supports the notion that high environmental thermal stress (i.e., >35°C, >60% RH) results in an ergogenic benefit from PreC to enhance self-paced maximal endurance cycling performance in Heat_{Acc} individuals. Therefore, our aim was to determine the effect of PreC on self-paced maximal cycling performance in hot conditions in Heat_{Acc} endurance athletes. We hypothesised that PreC would lead to further decreases in T_c and T_{sk}, and improve Th_c and Th_s in already Heat_{Acc} athletes compared to no cooling, consequently resulting in improved performance.

5.3 Methods

Ten male cyclists and/or triathletes (mean±SD: age=35.2±8.2 yrs; body-mass (BM)=83.72±16.40 kg; height=177.04±4.78 cm; body surface area=2.01±0.19 m^2,Du Bois & Du Bois, 1916; total training distance=390±201 km per week; training frequency=5±2 sessions per week) provided written informed consent. All participants were classified as 'trained/highly trained' (Tier 2 or 3; McKay et al., 2022). None were undergoing HA, trained in heat (>30°C), or had resided in a summer environment in the previous month (i.e., non-heat acclimatised). One participant withdrew for personal reasons, resulting in a final sample size of nine participants. Data collected prior to withdrawal was retained for analysis. Females were excluded due to best practice methodological procedures for conducting research in women being beyond the feasibility of this study (Elliott-Sale et al., 2021). The study was approved by <<BLINDED FOR REVIEW>> Human Research Ethics Committee (<<BLINDED FOR REVIEW>>) meeting the requirements described in the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research.

This study followed a randomised crossover study design. Participants completed a familiarisation session, four experimental sessions, and 10 HA sessions over five-weeks (Figure 5.1). Participants underwent two pre- and post-HA experimental sessions, one including 30 min of mixed-method PreC (crushed-ice ingestion plus cooling vest), and the other serving as a no-cooling control (CON) in a thermoneutral environment (22.2±1.5°C, 54.2±7.3% RH) followed by a maximal self-paced 20-km CTT in a heat chamber (35.8±0.4°C, 78.8±3.9% RH). Ten HA training sessions were conducted between the pre- and post-HA sessions. To minimise acclimation before HA training sessions, the PreHA-CON trial was completed first, followed by PreHA-PreC, while PostHA-CON and PostHA-PreC trials were randomised (http://www.randomization.com, January 2023). Testing occurred during the cooler months in Western Australia (March – November) to control for exposure to high environmental temperatures which may induce adaptations to heat, and therefore affect the cooling capacity of the PreC intervention. Participants avoided vigorous exercise, alcohol intake (24-h), and caffeine consumption (6-h) prior to testing. They were also instructed to replicate a consistent diet from 24-h prior to their first experimental session, recorded using a food diary for nutritional consistency. To ensure adequate hydration, participants were instructed to drink 6 mL·kg^{-1} BM of water every 2.5-h on the day before experimental sessions.

Anthropometric measures, including standing height (Holtain Limited, Crymych, United Kingdom, ±0.01 cm) and BM (Seca 360° Wireless, Hamburg, Germany, ±0.01 kg), were obtained during the familiarisation session. Subsequently, participants underwent 15-min mixed-method PreC in a thermoneutral environment to familiarise themselves with the cooling intervention. This was followed by a 10 min warm-up in a heat chamber (36°C, 80% RH). After the warm-up, participants performed a 20-km CTT in the heat chamber on a cycling ergometer (BikeErg, Concept 2, Queensland, Australia) to familiarise themselves with the exercise protocol.



Familiarisation	PreHA		HA	PostHA
Week 1	Week 2		Week 3 - 4	Week 5
Anthropometric measures + 20-km CTT familiarisation	20-km CTT (PreHA-CON)	20-km CTT (PreHA-PreC) + HA session familiarisation	10 x HA training sessions	20-km CTT (PostHA-CON) 20-km CTT (PostHA-PreC)
>7 days prior to Pre-HA	Sessions >72 h apart		Sessions ≤72 h apart	Sessions ≤48 h apart in randomised order

Figure 5.1. Protocol overview. HA = Heat acclimation, CTT = cycling time-trial, PreC = pre-cooling.

Eight hours prior to experimental sessions, participants ingested a pill (E-Celsius© Performance, Bodycap Medical, Caen, France, ±0.01°C) to measure T_c. Upon arrival at the laboratory, participants' hydration status was determined using urine specific gravity (USG; Portable Refractometer, RS PRO, RS Components, New South Wales, Australia). Participants with a USG reading >1.021 ingested 600 mL of water and then re-tested after 5 min. This process was repeated until readings were ≤1.021. Nude BM was measured pre- and post-experimental session on a set of calibrated electronic scales (Seca 360° Wireless, Seca, Birmingham, United Kingdom, ±0.01 kg) to calculate sweat loss (pre – post nude BM + fluid ingested). Surface T_{sk} was measured by placing dermal temperature sensors (DS1922L; iButton™, Maxim Integrated Products, California, USA, ±0.01°C) on the mesosternale, mid-forearm, mid-quadriceps and medial calf to calculate mean T_{sk} (Ramanathan, 1964). A HR monitor (A300 Polar Electro, Kempele, Finland) was placed on the participants' chest to measure HR. During the PreC experimental session, participants sat in a thermoneutral environment while wearing an activated cooling vest (as per manufacturer's instructions, Arctic Heat, Queensland, Australia). Concurrently, crushed-ice (made with an ice shaver, Avalanche Model IS6800, Sunbeam, New South Wales, Australia) was ingested at 2.3 $g \cdot kg^{-1}$ BM every 10 min over a 30 min period (total of 7 $g \cdot kg^{-1}$ BM) to ensure consistent ingestion rates between sessions (Ihsan et al., 2010). Throughout the CON experimental sessions, participants sat in the same thermoneutral environment for 30 min wearing a non-activated cooling vest and drank room temperature water (retrieved from a water fountain supplying 16°C water) of similar rate and volume as performed in the PreC experimental sessions.

Participants then entered the heat chamber and completed a 10 min cycling warm-up at a perceptually regulated exercise intensity (ratings of perceived exertion [RPE] of 13, 15 and 17 for 5, 3 and 2 min, respectively, 6–20 scale; Borg, 1970). This was followed by a self-paced 20-km CTT on a cycle ergometer at the participants' highest sustainable intensity. Participants received feedback only on the distance completed throughout the CTT. Participants had access to water (retrieved from a fountain supplying ~16°C water), kept in non-insulated water bottles in the heat chamber, and were allowed to drink *ad libitum*. During the PreC period, HR, T_c, and T_{sk} data were collected every 5 min. During the warm-up, these measurements were taken every 2 min, and every 2.5-km distance completed during the 20-km CTT. Thermal comfort (0 = very comfortable, 20 = very uncomfortable; Th_c) and sensation (0 = very cold, 20 = very hot; Th_s) were recorded every 5 min during the PreC period, pre- and post-warm up, and every 2.5-km distance completed during the 20-km CTT (Gaoua et al., 2012). Similarly, RPE (6–20 scale; Borg, 1970) was obtained every 2.5-km distance completed during the 20-km CTT. Exercise performance was assessed by measuring split-times every 2.5-km of distance completed and 20-km CTT total completion time.

Prior to starting the HA protocol (>72 h after the HA_{pre}-PreC trial), hydration status was assessed using USG. Participants with USG >1.021 underwent the same re-hydration process employed prior to the experimental sessions to ensure adequate hydration. Capillary blood samples were collected from the fingertip in 75-mm heparinised sterile tubes (Haematokrit-Kapillaren, Hirschmann Laborgerate, Eberstadt, Germany) before being centrifuged for 6 min at 3,500 rpm to obtain haematocrit (Hct; as per manufacturer's instructions, E8 Centrifuge, LW Scientific, Georgia, USA). Haemoglobin (Hb) was also obtained (Hb-801 Analyzer, HemoCue, Angelholm, Sweden). Both variables were again collected prior to the commencement of the final HA session to determine changes in plasma volume, which was calculated as: 100 x (pre-Hb/post-Hb) x (1 –post-Hct/1 – pre-Hct) – 100 (Strauss et al., 1951). Following blood sample collection, participants entered the heat chamber and completed a 10 min cycling warm-up at a perceptually regulated exercise intensity (RPE of 13, 15 and 17 for 5, 3 and 2 min, respectively, 6-20 scale, Borg, 1982) on the same cycle ergometer as the experimental sessions. This was followed by 6 x 10 min of self-paced cycling protocol in the heat chamber, which has been previously shown to elicit comparable physiological responses as continuous HA (Ramos et al., 2024). After each work interval, participants exited the heat chamber and moved to a thermoneutral environment (24±1.2°C, 49.6±8.5% RH) to perform 3 min of per-cooling (PerC). Training sessions were conducted in a 36°C air temperature environment and were periodised by increasing RH between sessions (session 1-2 = ~50% RH, session 3-5 = ~65% RH, session 6-9 = ~80% RH and session 10 = ~50% RH). The environmental conditions for the first and final HA training sessions were matched to enable comparisons between performance, physiological, and perceptual changes resulting from the HA program (i.e., heat stress test). Within 48 h after the final HA session, participants performed the first of two PostHA experimental sessions, conducted the same as the PreHA experimental sessions and within 48 h of each other.

5.4 Statistical Analyses

Body temperature (T_b) was calculated using the equation: 0.65 x T_c + 0.35 x T_{sk} (Burton, 1935). Core-to-skin temperature gradient (T_g) was calculated using the equation: T_c - T_{sk} (Ely et al., 2009). Data sampled repeatedly was not independent and intraclass correlation coefficients in 6 of 18 outcomes were >0.5 (see supplementary material) thus multilevel modelling was used to analyse data to account for non-independence (Bryk & Raudenbush, 1992).

Bayesian multilevel modelling with random slopes and intercepts was utilised to analyse the relationship between T_c, mean T_{sk}, T_b, HR, RPE, Th_c, Th_s, and 2.5-km split completion times, with estimates reported as unstandardised regression coefficients (b), with 95% credible intervals. Additionally, Bayesian multilevel regression modelling was used to determine differences in hydration, total fluid ingested, sweat loss, and total completion time. This analytic approach is particularly advantageous for small sample sizes as it enables the researcher to integrate prior expectations (priors) about typical effect sizes, thus increasing the precision of estimated effects (Zondervan-Zwijnenburg et al., 2017). We derived priors for the current study from meta-analyses and previous studies that used a similar PreC protocol and self-paced endurance exercise (i.e., time-trial).

Posterior distributions derived from the Bayesian estimation process describe the relative probabilities of different values for a parameter, which can be used to make statistical inferences. Credibility intervals provided by Bayesian analysis are interpreted similarly to that of frequentist credibility intervals: if an interval does not contain zero, the true value of the parameter is – with 95% certainty – non-zero. We defined statistically meaningful or “significant” effects as coefficients with credibility intervals that do not include zero. These intervals are reported in brackets alongside unstandardised regression coefficients (b). Where significant coefficients for interaction effects were identified, post-hoc simple slopes analyses were conducted to compare the relationship between distance/time, intervention and condition pairings reported as b coefficients. Key findings are summarised in text and full results as well as prior values are provided as supplementary material.

Sensitivity analysis was conducted for all models with non-informative priors (priors that assume little to no pre-existing knowledge about typical effects; see supplementary material, Table 5) to assess the stability of our results (Depaoli & van de Schoot, 2017). We controlled for the influence of PreC and HA on physiological, perceptual and performance variables. Priors are preferred when sample sizes are small but the sensitivity analysis indicates that some degree of caution is required when interpreting results (Mcneish, 2016). Post-hoc analyses were conducted using the “emtrends” package, and Bayesian multilevel models were implemented using “brms” package (Burkner, 2017, Searle et al., 2012) in R (R Foundation for Statistical Computing, Austria).

5.5 Results

No meaningful direct relations were observed between the first and final HA session for starting exercise HR (84±19 vs. 89±11 bpm, b=4.742[-6.824,14.899]), T_c (36.99±0.36 vs. 37.00±0.33°C, b=-0.025[-0.290,0.219]), and sweat loss (2.23±0.75 vs. 2.42±0.78 L; b=0.113[-0.220,0.446]). Similarly, capillary blood samples from six participants indicated no meaningful direct relations between the first and final HA session for change in plasma volume (-1.63±7.97%; b=2.106[-5.522,13.245]).

No meaningful direct relations were observed for USG. We found a main effect for intervention for total fluid ingested (total ice + water ingested) during the 20-km CTT, indicating lower fluid intake when PreC was applied compared to CON (1.47±0.83 vs. 1.67±0.74 L, b=-0.210[-0.268,-0.149]). There was a main effect for condition for sweat loss, indicating greater sweat loss in HA_{post} compared to HA_{pre} (2.24±0.54 vs. 1.98±0.71 L; b=0.333[0.002,0.675].

During the PreC period, we found a two-way interaction between time and intervention for T_c (b=-0.014[-0.018,-0.010]), mean T_{sk} (b=-0.029[-0.047,-0.009]), and T_b (b=-0.019[-0.028,-0.010]). Post-hoc analysis indicated T_c (b=0.013[0.010,0.016]) remained relatively stable in CON compared to PreC which decreased across the PreC period. Post-hoc analysis for mean T_{sk} (b=0.034[0.016,0.048]) and T_b (b=0.019[0.013,0.025]) indicated greater mean T_{sk} and T_b in CON compared to PreC across the PreC period. There was a three-way interaction for Th_c (b=0.127[0.032,0.222]) and Th_s (b=-0.055[-0.108,-0.004]) during the PreC period. Post-hoc analysis indicated lower Th_c (i.e., participants were more comfortable) over the course of HA_{post}-CON compared to HA_{post}-PreC (b=-0.082[-0.151,-0.017]). Comparisons between PreC interventions indicated lower Th_c over the course of HA_{pre}-PreC compared to HA_{post}-PreC (b=-0.135[-0.203,-0.017]). Post-hoc analysis for Th_s indicated greater ratings (i.e., hotter sensation) over the course of the HA_{post}-CON compared to HA_{post}-PreC (b=0.133[0.098,0.170]). Comparisons between PreC interventions indicated greater Th_s over the course of HA_{pre}-PreC compared to HA_{post}-PreC (b=0.048[0.011,0.083]).

No meaningful direct relations were observed between HA_{post}-CON and HA_{post}-PreC (2671±370 vs. 2663±307 s; b=-37.050[-156.495,70.605]) 20-km CTT completion time. No meaningful direct relations were observed between and HA_{post}-CON and HA_{pre}-CON (2682±228 s; b=-44.712[-119.319,29.350]), and between HA_{pre}-PreC (2671±242 s) and HA_{post}-PreC (b=-40.102[-108.476,24.263]). However, we found a three-way interaction between condition, time, and intervention for 2.5-km split completion time (b=1.501[0.348,2.684]). Post-hoc analysis (Figure 5.2) indicated that the rate at which split times changed across the CTT differed between interventions and conditions. Comparisons between interventions showed that split times decreased (i.e., faster) across HA_{post}-CON compared to HA_{post}-PreC, which increased (i.e., slower) across the CTT (b=-1.224[-2.196,-0.157]). Comparisons between conditions showed faster split times across HA_{pre}-PreC compared to HA_{post}-PreC which increased (i.e., slower) across the CTT (b=-1.632[-2.610,-0.657]).

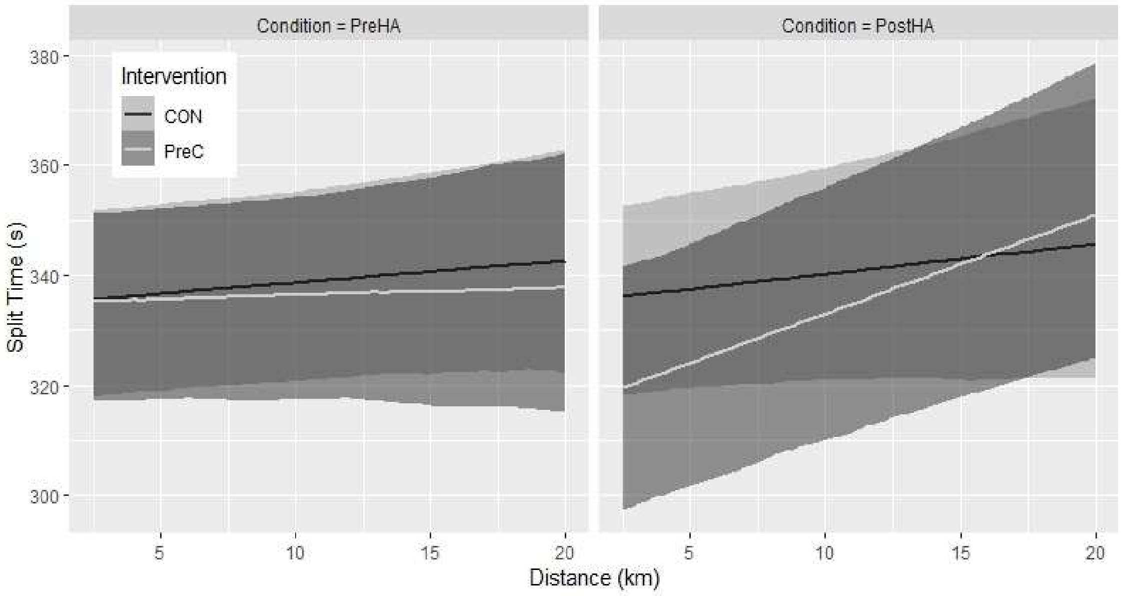


Figure 5.2. 2.5-km split completion time during the 20-km cycling time-trial before (PreHA) and after (PostHA) heat acclimation with pre-cooling (PreC) or no cooling (CON). *Note: Shaded areas indicate 95% credible intervals.*

There was a three-way interaction between condition, time, and intervention for T_c (b=0.023[0.010,0.036]) and T_g (b=0.061[0.026, 0.008]) during the CTT. Post-hoc analysis for T_c (Figure 5.3) indicated that the rate at which T_c changed across the CTT differed between interventions and conditions. Comparisons between interventions showed that T_c was lower over the course of HA_{post}-CON compared to HA_{post}-PreC, which was higher across the CTT (b=-0.023[-0.033,-0.015]). Additionally, greater change in T_c was observed in HA_{post}-PreC compared to HA_{post}-CON (+2.57 vs. +1.92°C; see Table 4 in supplementary material). Comparisons between conditions showed lower T_c over the course of HA_{pre}-PreC compared to HA_{post}-PreC, which was greater over the course of the CTT (b=-0.013[-0.033,-0.004]). Post-hoc analysis for T_g indicated no meaningful direct relations were observed between HA_{post}-PreC and HA_{post}-CON (b=-0.034[-0.068, 0.001]).

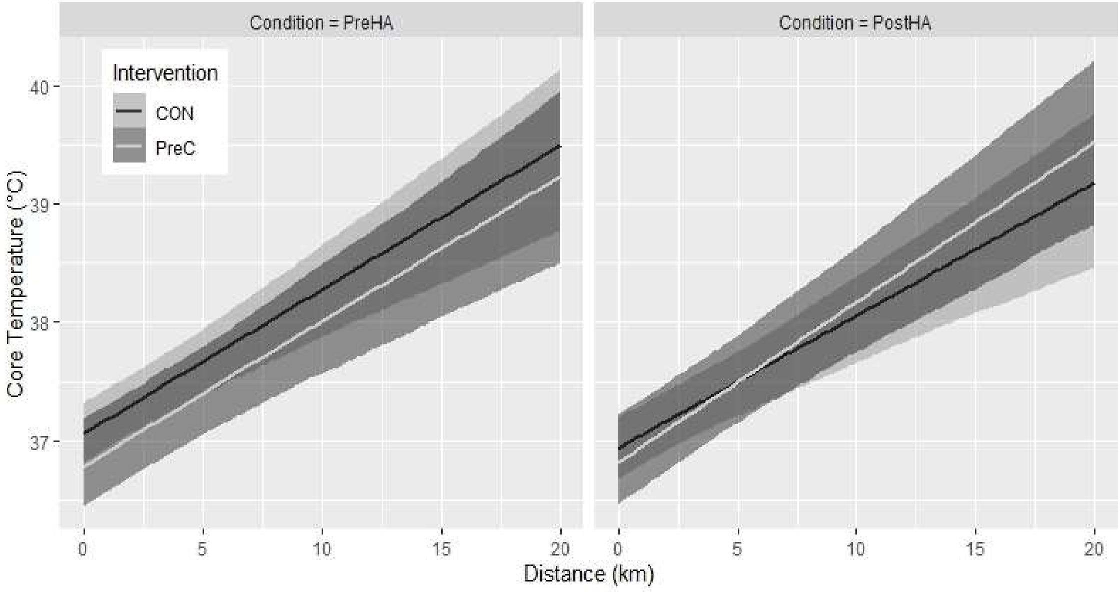


Figure 5.3. Core temperature during the 20-km cycling time-trial before (PreHA) and after (PostHA) heat acclimation training with pre-cooling (PreC) or no cooling (CON). *Note: Shaded areas indicate 95% credible intervals.*

There was a main effect for intervention for mean T_{sk} and T_b during the CTT which indicated lower mean T_{sk} and T_b during the CTT when PreC was applied compared to CON (b=-0.160[-0.270,-0.047], and b=-0.486[-0.561, -0.412], respectively). We found a main effect of time for HR during the CTT which indicated increased HR (b=0.020[0.018,0.022]) over the course of the CTT. No main effect for condition was observed for HR (b=-2.308[-5.781, 1.243]). We found a main effect of time for Th_c during the CTT, indicating increased Th_c (i.e., more uncomfortable; b=0.280[0.068,0.110]) over the course of the CTT. We also found a two-way interaction between intervention and time for Th_s (b=0.075[0.014,0.137]) during the CTT. Post-hoc analysis indicated lower ratings (i.e., cooler) over the course the CTT in CON compared to PreC (b=-0.047[-0.091,-0.003]), which had higher ratings over the course the CTT. We found a three-way interaction between time, condition, and intervention for RPE during the CTT (b=-0.097[-0.185,-0.005]). Post-hoc analysis indicated lower perceived effort over the course of HA_{pre}-CON compared to HA_{pre}-PreC (b=-0.071[-0.138,-0.010]). No meaningful direct relations were observed for RPE between HA_{post}-CON and HA_{post}-PreC (b=0.025[-0.044,0.087]).

Sensitivity analysis indicated substantive changes (i.e., posterior distribution became meaningfully different) in the interpretation of the two-way interactions during the PreC period for HR, and Th_s. During the 20-km CTT, substantive changes (i.e., posterior distribution became meaningfully different) were observed in the interpretation of main effect of intervention (i.e., total fluid ingestion), two-way interactions (i.e., Th_s and T_b), and three-way interaction (i.e., RPE and 2.5-km split completion time).

5.6 Discussion

This is the first study to examine the effect of mixed-method PreC on self-paced maximal 20-km CTT performance in hot (36°C, 79% RH) conditions in $Heat_{Acc}$ individuals. Contrary to our hypothesis, although not detrimental, mixed-method PreC did not improve 20-km CTT completion time beyond the improvements achieved through HA. This challenges the notion that reduced bodily temperatures resulting from mixed-method PreC in $Heat_{Acc}$ individuals would improve maximal self-paced endurance performance in hot conditions.

We observed a shift in pacing profile when PreC was applied in HA_{post} compared to HA_{pre} (from positive [slower intensity over time], to flat [consistent intensity over time] pacing). Similarly, Schmit et al. (2017) investigated the effects of PreC following heat acclimatisation (eight-day training camp in ~30°C, ~74% RH conditions) on 20-km CTT performance in heat (35°C, 50% RH). They observed a positive pacing profile evident through greater initial power output at the start of the 20-km CTT and decreased over the course of the CTT. The observation by Schmit et al. (2017) and our study is potentially linked to cooler T_{sk} at the commencement of the CTT when PreC was applied compared to CON (Table 4 supplementary material). This mechanism is supported by Schlader et al. (2011), who investigated the effect of manipulating T_{sk} (via a liquid-perfused suit) on self-selected exercise intensity during a 60-min CTT and found greater mean power output throughout the CTT when exercise commenced with cooler compared to hotter T_{sk} (~258 vs. ~251 W). However, in our study, this effect only lasted until ~15 km. The higher initial intensity in our study might have increased metabolic heat production, evidenced by a ~0.3°C higher T_c at the end of the CTT, resulting in lower exercise intensity to complete the task and/or facilitate heat loss. This indicates that pacing during the initial stages of exercise in heat may be affected by PreC because of cooler T_{sk}. Therefore, familiarisation with the PreC intervention prior to exercise in heat may be necessary to understand its impact on planned pacing strategies and potential performance enhancement before its application in competition.

Lack of meaningful change in total completion time between HA_{post}-PreC and HA_{post}-CON is in line with findings by the aforementioned research study by Schmit et al. (2017). They suggested that HA-induced adaptations (e.g., +16.60% plasma volume and +0.28 L sweat loss) might diminish the ergogenic benefits of PreC on endurance performance in heat. For example, lower sweating thresholds and greater sweat quantities from HA could led to a greater T_g (i.e., lower T_{sk} compared to T_c), which could have reduced the effectiveness of PreC (Cuddy et al., 2014, Shido et al., 1999). Despite observing similar sweat loss to that reported by Schmit et al. (2017) for HA_{post} compared to HA_{pre} (~+0.26 L greater), regardless of PreC or no cooling, we did not observe any meaningful interactions for T_g. Therefore, greater sweat loss due to HA may account for the diminished effectiveness of PreC in $Heat_{Acc}$ individuals.

Despite observing cooler mean T_{sk} during the CTT when PreC was applied compared to CON, this did not translate into improved Th_c, Th_s, and RPE during HA_{post}. This supports previous findings by James et al. (2018) who investigated the effects of 20 min PreC (via iced towels and 9°C cold-water immersion) on $Heat_{Acc}$ individuals. Participants underwent five sessions consisting of 90 min cycling bouts at ~38.5°C T_c in ~37°C, ~60% RH conditions, followed by a 5-km running time-trial in heat (32°C, 60% RH). Although they observed ~0.5°C lower T_{sk} during HA_{post}-PreC compared HA_{post}-CON, no differences in mean Th_s and RPE were observed. This lack of difference may have contributed to the absence of a meaningful change in completion time compared to HA alone (~1,373 vs. ~1,378 s). Unfortunately, the results observed for Th_s and RPE were not discussed by James et al. (2018), therefore, we can only speculate that the absence of additional benefit on performance in heat when PreC is applied in $Heat_{Acc}$ individuals is due to its failure to improve perception. Indeed, previous research with a similar PreC intervention (i.e., cooling vest plus crushed-ice ingestion), mode of exercise (i.e., cycling time-trial), and environment (i.e., 30°C, 75% RH) in non-$Heat_{Acc}$ individuals have observed cooler ratings of Th_s and ~6.5% faster completion times (Ihsan et al., 2010). This may indicate that improved endurance performance in heat among $Heat_{Acc}$ individuals could be observed if their perception (i.e., Th_s) to heat is also enhanced.

5.7 Conclusion

Insufficient evidence exists to support a meaningful performance improvement in 20-km CTT in hot environmental conditions when mixed-method PreC was applied to $Heat_{Acc}$ individuals. This can be attributed to HA-induced adaptions (e.g., greater sweat loss), which potentially diminished the ergogenic benefit of PreC in heat by reducing T_{sk}. Additionally, sub-optimal pacing strategies resulting from the application of PreC on $Heat_{Acc}$ individuals may explain the lack of additional benefit to performance.

5.8 Practical Applications

- Prior to utilisation in competition, familiarisation with PreC (via crushed-ice ingestion plus cooling vest) is recommended as it can lead to sub-optimal pacing strategies in the initial stages of a 20-km CTT.

- Pre-cooling may not be a priority for Heat$_{Acc}$ individuals performing a 20-km CTT in hot-humid conditions, as greater sweat losses reduce the ergogenic benefit of PreC.
- Pre-cooling (via crushed-ice ingestion plus cooling vest) should not be employed if the goal is to improve Th$_{s}$ in Heat$_{Acc}$ individuals who are about to complete a 20-km CTT in hot-humid conditions.

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5.10 Supplementary Material

Table S5.2. Intraclass correlation coefficients for hydration and sweat loss, performance, physiological, and perceptual data during the exercise and rest-intervals.

Variable	PreC period	20-km CTT
Urine specific gravity	32%	
Total fluid ingestion	83%	
Sweat loss	64%	
Total completion time	67%	
Mean split completion time	65%	
Core temperature	39%	2%
Mean skin temperature	27%	5%
Body temperature	36%	3%
Heart rate	73%	5%
Thermal comfort	17%	25%
Thermal sensation	12%	37%
Rating of perceived exertion	-	57%

Table S5.3. Mean ± SD for physiological and perceptual data during the pre-cooling (PreC) period pre- (Pre) and post-heat acclimation (Post) training after PreC or no cooling control (CON) interventions.

		CON		PreC	
		Start	Finish	Start	Finish
Heart Rate (bpm)	Pre	70 ± 10	68 ± 12	70 ± 11	71 ± 15
	Post	76 ± 14	66 ± 12	69 ± 8	65 ± 9
Core Temperature (°C)	Pre	36.79 ± 0.26	36.64 ± 0.36	36.82 ± 0.40	36.29 ± 0.50
	Post	37.04 ± 0.36	36.75 ± 0.27	36.92 ± 0.39	36.34 ± 0.55
Mean Skin Temperature (°C)	Pre	29.81 ± 0.97	32.17 ± 0.91	30.03 ± 1.29	31.18 ± 0.71
	Post	29.75 ± 1.81	32.40 ± 0.87	29.73 ± 1.34	31.10 ± 0.63
	Pre	34.36 ± 0.43	35.15 ± 0.42	34.47 ± 0.66	34.54 ± 0.39

Body Temperature (°C)	Post	34.54 ± 0.82	35.24 ± 0.44	34.40 ± 0.59	34.51 ± 0.52
Thermal Comfort*	Pre	4 ± 4	6 ± 4	10 ± 2	10 ± 4
	Post	6 ± 4	8 ± 3	8 ± 3	12 ± 3
Thermal Sensation†	Pre	8 ± 2	8 ± 2	7 ± 3	4 ± 2
	Post	9 ± 1	9 ± 2	8 ± 1	4 ± 2

*(0 = very comfortable, 20 = very uncomfortable, †(0 = very cold, 20 = very hot).

Table S5.4. Mean ± SD for performance, physiological and perceptual data at the start and finish of the 20-km cycling time-trial pre- (Pre) and post-heat acclimation (Post) training after pre-cooling (PreC) or no-cooling control (CON) interventions.

		CON		PreC	
		Start	Finish	Start	Finish
Heart Rate (bpm)	Pre	88 ± 14	168 ± 14	81 ± 10	175 ± 12
	Post	87 ± 10	177 ± 8	82 ± 16	175 ± 12
Core Temperature (°C)	Pre	37.09 ± 0.26	39.40 ± 0.14	36.70 ± 0.44	39.03 ± 0.33
	Post	37.08 ± 0.25	39.0 ± 0.64	36.66 ± 0.54	39.23 ± 0.70
Mean Skin Temperature (°C)	Pre	34.22 ± 0.87	37.51 ± 0.58	33.58 ± 0.63	37.27 ± 0.33
	Post	33.81 ± 0.68	37.47 ± 0.75	33.55 ± 0.59	37.33 ± 0.72
Body Temperature (°C)	Pre	36.07 ± 0.25	38.85 ± 0.25	35.63 ± 0.44	38.43 ± 0.26
	Post	35.92 ± 0.37	38.42 ± 0.66	35.57 ± 0.41	38.56 ± 0.64
Thermal Comfort*	Pre	10 ± 1	17 ± 4	10 ± 3	17 ± 3
	Post	9 ± 2	17 ± 2	9 ± 2	18 ± 2
Thermal Sensation†	Pre	12 ± 2	17 ± 4	10 ± 4	17 ± 2
	Post	11 ± 1	18 ± 2	10 ± 3	18 ± 2

*(0 = very comfortable, 20 = very uncomfortable, †(0 = very cold, 20 = very hot).

Table S5.5. Informative and default priors for Bayesian analysis.

	Estimates [95 % credible intervals]	Time Coefficient (Mean ± SD)	Intervention Coefficient (Mean ± SD)	Condition Coefficient (Mean ± SD)
Between-person				
Urine specific gravity	1.012 [1.006, 1.017]	-	0.003 ± 0.01 (Schmit et al., 2017),	0.001 ± 0.01 (Schmit et al., 2017)
Total fluid ingested	1.63 [1.14, 2.15]	-	-0.21 ± 0.03 (Maia-Lima et al., 2017),	0.54 ± 0.61 (Sekiguchi et al., 2021)
Sweat Loss	1.89 [1.43, 2.40]	-	0.43 ± 0.3 (Maia-Lima et al., 2017),	0.24 ± 0.3 (Zimmermann et al., 2018)
Total Time	2721.39 [2559.74, 2883.28]	-	-25 ± 90 (Schmit et al., 2017)	-45.6 ± 36.79 (Schmit et al., 2017)
Pre-cooling period				
Heart rate	68.35 [60.51, 76.90]	0.003 ± 0.100 (Zimmermann et al., 2017)	-	-6 ± 18.6 (Brown et al., 2023)
Core temperature	36.81 [36.51, 37.12]	-0.0003 ± 0.3 (Zimmermann et al., 2017)	-0.48 ± 0.23 (Alhadad et al., 2019)	-
Mean skin temperature	31.00 [30.31, 31.69]	-	-0.24 ± 0.24 (Choo et al., 2017)	-0.73 ± 0.46 (Best et al., 2014)
Body temperature	34.83 [34.38, 35.34]	-0.0003 ± 0.1 (Bogerd et al., 2010)	-0.7 ± 0.1 (Bogerd et al., 2010)	-
Thermal comfort	6.39 [4.60, 8.30]	-	2.3 ± 0.8 (Byrne et al., 2011)	-
Thermal sensation	7.62 [6.05, 9.14]	-0.001 ± 0.5	1.9 ± 0.7	-

		(Zimmermann et al., 2017)	(Zimmermann et al., 2017)	
20-km cycling time-trial				
Heart rate	119.57 [109.49, 132.75]	0.02 ± 0.001 (Byrne et al., 2011)	0.26 ± 0.19 (Choo et al., 2017)	-3 ± 2 (Schmit et al., 2017)
2.5-km split completion time	320.29 [222.12, 372.73]	-	-0.01 ± 0.15 (Schmit et al., 2017)	-0.05 ± 0.15 (Schmit et al., 2017)
Core temperature	37.06 [36.79, 37.32]	0.001 ± 0.1 (Alhadad et al., 2019)	0.03 ± 0.44 (Alhadad et al., 2019)	-0.19 ± 0.26 (Alhadad et al., 2019)
Mean skin temperature	35.11 [34.75, 35.53]	0.10 ± 0.10 (Byrne et al., 2011)	-0.13 ± 0.06 (Byrne et al., 2011)	-1.2 ± 0.7 (Schmit et al., 2017)
Body temperature	36.38 [36.15, 36.62]	0.001 ± 0.1 (Quod et al., 2008)	-0.5 ± 0.4 (Quod et al., 2008)	-
Thermal comfort	10.41 [9.24, 11.52]	0.001 ± 0.1 (Byrne et al., 2011)	-0.5 ± 0.68 (Byrne et al., 2011)	-
Thermal sensation	12.65 [11.17, 14.30]	0.001 ± 0.1 (Duffield et al., 2010)	-0.62 ± 0.71 (Duffield et al., 2010)	-0.8 ± 1.0 (Choo et al., 2020)
Rating of perceived exertion	13.92 [12.65, 15.15]	0.003 ± 0.1 (Byrne et al., 2011)	0.03 ± 2.5 (Byrne et al., 2011)	0.6 ± 1.6 (Choo et al., 2020)

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Chapter 6: Study Four

Intermittent heat exposure with per-cooling as a heat acclimation program does not attain physiological adaptations but maintains exercise performance

This paper has been submitted to the Scandinavian Journal of Medicine and Science in Sports.

Ramos, J. A. P., Ducker, K. J., Riddell, H., Girad, O., Landers, G. J., and Brade, C. J. Intermittent heat exposure with per-cooling as a heat acclimation training protocol does not induce heat adaptations. Submitted to Scandinavian Journal of Medicine and Science in Sports, *in review.*

Presented here in journal submission format.

6.1 Abstract

Objective: Investigate the effects of intermittent-heat exposure (IHE) with PerC as an alternate method of heat acclimation (HA) for non-heat acclimatised endurance athletes. **Method:** Ten participants completed a cross-sectional study comprised of ten IHE HA sessions. Each session consisted of 6 x 10 min maximal self-paced cycling in heat (~36°C, ~50-80% RH), followed by 3 min passive rest with mixed method PerC in thermoneutral conditions (24°C, 50% RH). The first (HA1) and final (HA10) HA sessions were used for performance, physiological, and perceptual comparisons. **Results:** No meaningful direct relations were observed between HA1 and HA10 for sweat loss (b=0.11[-0.24,0.45]) and plasma volume (b=-1.15[-8.51,6.88]). Mean 5-min split distance completed was greater during HA10 compared to HA1 (b=1.29[0.32,2.25]). During the exercise intervals, core-to-skin temperature gradient was higher in HA10 compared to HA1 (b=0.68[0.18,1.19]). Core temperature increased (b=0.0078[0.0004,0.0142]) throughout the exercise protocol and no meaningful direct relations were observed for mean skin temperature (b=-0.05[-0.38,0.26]). Similarly, thermal comfort (b=0.04[0.02,0.07]) and rating of perceived exertion (b=0.022[0.003,0.042]) increased during the exercise intervals. **Conclusions:** Intermittent-heat exposure with PerC did not result in attainment of hallmark adaptations to heat (i.e., increased plasma volume, and sweat loss). However, improved within-session performance post-HA (i.e., distance) which may be attributed to the greater T_g in HA10 created by PerC.

Key Words: cooling, self-paced, cycling, thermoregulation, endurance

6.2 Introduction

Self-paced exercise performance in hot-humid environments (i.e., >30°C and >60% relative humidity [RH]) in most cases, is negatively affected, therefore implementing preventive counter measures such as heat acclimation (HA) and cooling (e.g., per-cooling; PerC) is recommended (Bongers et al., 2017, Périard et al., 2021). Typically, HA protocols involve regular continuous exercise in hot environments (>30°C) for approximately 60-120 min over 10-14 days, with no more than 2-3 days between exposures. The aim of HA protocols is to create a heat stimulus (e.g., core temperature [T_c] ≥38.5°C, and increased sweating) to induce adaptations to heat (Daanen et al., 2018, Périard et al., 2015). These adaptations include lower resting and exercising heart rate (HR), T_c and skin temperature (T_{sk}), expansion of blood plasma volume (PV), increased sweat rate, and improved thermal comfort (Th_c) and sensation (Th_s) and result in maintained or improved exercise performance in heat (Périard et al., 2021). However, this can pose competing challenges for athletes. While HA protocols yield physiological adaptations to heat, the additional external training load (resulting from exercise in heat) within a short timespan can lead to greater fatigue in athletes, potentially compromising training quality and performance due to HA proximity to competitive performances.

One method to assist in maintaining training quality is the application of cooling (e.g., pre-cooling [PreC] or PerC) during HA training. Choo et al. (2020) utilised PreC (30 min, ~21°C cold water-immersion) prior to 10 HA training sessions (60 min cycling at a rating of perceived effort [RPE] of 15 in ~35°C, ~53% RH) and found no difference in mean power output between session 1 (HA1) and

10 (HA10) when PreC was applied (HA1 ~166 vs. HA10 ~173 W) compared to no PreC (HA1 ~168 vs. *HA10* ~157 W). Similarly, Naito et al. (2022) found that using PerC (1.25 and 7.5 $g \cdot kg^{-1}$ body mass [BM] crushed-ice ingestion during breaks and half-time, respectively) during five HA sessions (80 min intermittent repeated sprint protocol in ~36.5°C, ~50% RH conditions) resulted in +3.3% greater total work done on day 5 of the HA sessions compared to no PerC. It must be noted however, that both studies by Choo et al. (2020) and Naito et al. (2022) only observed lower resting T_c and rectal temperature (~-0.3°C, and ~-0.2°C, respectively) post-acclimation compared to pre-acclimation. No further evidence of physiological acclimation adaptations was noted for sweat loss, exercising HR or mean T_{sk} (note plasma volume not measured). Despite seemingly adequate heat stimuli (400-600 min total exercising in 35-37°C, 50-53% RH), it may be that the heat stimulus was not sufficient to achieve hallmark physiological adaptation typically noted post HA. Intermittent-heat exposure (IHE), whereby athletes complete exercise intervals in heat, followed by passive rest plus mixed method PerC in thermoneutral environments has been shown to maintain training quality in single sessions without compromising the physiological stimuli for HA (Ramos et al., 2024). Additionally, Ramos et al. (2024) observed greater distance completed over 60 min total of exercise in three different IHE protocols (3 x 20 min exercise intervals with 7.5 min rest in between [IHE-20], 4 x 15 min intervals with 5 min rest [IHE-15], and 3: 6 x 10 min intervals with 3 min rest [IHE-10]) compared to a traditional continuous heat exposure bout (1 x 60 min exercise interval). Further, time spent ≥38.5°C T_c, which has been previously suggested as an important stimulus for HA (Fox et al., 1967, Tyler et al., 2016),

was shorter in IHE-20 and IHE-15 (~28% and ~31% of total session duration, respectively), but was not different between traditional continuous heat exposure and IHE-10 (~50% and ~48% of total session duration, respectively). Improvements in performance were attributed to lower T_c and T_{sk}, which corresponded with improved thermal comfort (Th_c), sensation (Th_s), and rating of perceived exertion (RPE). These findings were for a single HA session, and we don't yet know if this protocol used for a full HA program (~ 10 sessions) would provide the stimulus needed to induce physiological heat adaptations and exercise performance improvements. Therefore, the aim of this study was to investigate whether non-heat acclimatised endurance athletes could be acclimated via a novel HA program using IHE with mixed-method PerC. We hypothesised that IHE training would induce physiological and perceptual adaptations to heat, resulting in improved self-paced maximal cycling performance in heat.

6.3 Materials and Methods

Ten male cyclists and/or triathletes (mean±SD: age=35.2±8.2 yrs; BM=83.72±16.40 kg; height=177.04±4.78 cm; body surface area=2.01±0.19 m^2 [Du Bois & Du Bois, 1916]; training distance=390±201 km per week; training frequency = 5±2 sessions per week) provided written informed consent to participate. Participants in this study consisted of the same individuals which participated in a previous study (Ramos et al., 2024). All athletes corresponded with an athlete classification 2 or 3 (trained/highly trained; McKay et al., 2022). Participants were not involved in a HA protocol, did not train in a heated environment (>30°C), and had not resided in a summer environment in the previous month (i.e., non-heat acclimatised). One participant dropped out of the study due to personal reasons not relating to study participation. Despite this, all data collected prior to withdrawal was retained for analysis. The study was approved by Curtin University Human Research Ethics Committee (HRE2022-0638).

This study was a cross-sectional study design. Participants attended a familiarisation session and completed a HA program with heat stress tests completed on the first (HA1) and final (HA10) sessions. The HA protocol consisted of 10 IHE sessions where participants completed 6 x 10 min exercise intervals of maximal self-paced cycling in a heat chamber (~36°C, ~50 – 80% RH; Ramos et al., 2024). Exercise intervals were followed by 3 min of passive rest in a thermoneutral environment (22.2±1.5°C, 54.2±7.3% RH) where a mixed-method PerC intervention was applied (cooling vest plus crushed-ice ingestion). The HA program was periodised by increasing RH between sessions to increase the thermal stress. Session 1 and HA10 were matched to enable comparisons between performance, physiological, and perceptual variables. Testing occurred during the cooler months in Western Australia (March – November) to control for exposure to high environmental temperatures and RH which may induce adaptations to the heat, and therefore affect the cooling capacity of the PerC intervention. Participants avoided vigorous exercise, alcohol intake (24 h) and caffeine consumption (6 h) prior to testing. They were also instructed to replicate a similar diet from 24 h prior to their first experimental session using a food diary for nutritional consistency. To ensure adequate hydration, participants were instructed to drink 6 $\text{mL}\cdot\text{kg}^{-1}$ BM of water every 2.5 h on the day before experimental sessions.

The HA program was part of a larger study where participants completed two pre- and post-HA experimental sessions prior to HA1 (two 20-km cycling time-trials in 35.8±0.4°C, 78.8±3.9% RH conditions). Standing height (Holtain Limited, Crymych, United Kingdom, ±0.01 cm) and BM (Seca 360° Wireless, Hamburg, Germany, ±0.01 kg) were recorded. To familiarise to the IHE protocol, participants performed a 10 min exercise interval on a cycle ergometer (BikeErg, Concept 2, Queensland, Australia) in the heat chamber followed by 3 min mixed-method PreC in a thermoneutral environment (22.2±1.5°C, 54.2±7.3% RH). Participants were instructed to work at the highest sustainable intensity for the duration of each exercise interval.

Eight hours prior to HA1 and HA10, participants ingested a T_c pill (E-Celsius© Performance, Bodycap Medical, Caen, France, ±0.01°C) to measure T_c. Upon arrival at the laboratory, participants' hydration status was determined using urine specific gravity (USG; Portable Refractometer, RS PRO, RS Components, New South Wales, Australia). Participants with a USG reading >1.021 ingested 600 mL of water and then re-tested after 5 min. This process was repeated until USG readings were ≤1.021. Nude BM was measured pre- and post- the HA training sessions on a set of calibrated electronic scales (Seca 360º Wireless, Seca, Birmingham, United Kingdom, ±0.01 kg) to calculate sweat loss (pre – post nude BM + fluid ingested). Surface T_{sk} was measured by placing dermal temperature sensors (DS1922L; iButton™, Maxim Integrated Products, California, USA) on the mesosternale, mid-forearm, mid-quadriceps and medial calf to calculate mean T_{sk} (Ramanathan, 1964). A HR monitor (A300 Polar Electro, Kempele, Finland) was placed on the participants' chest to measure HR. Capillary blood samples were collected from the fingertip in 75 mm heparinised sterile tubes (Haematokrit-Kapillaren, Hirschmann Laborgerate, Eberstadt, Germany). The blood sample was then centrifuged for 6 min at 3,500 rpm to obtain haematocrit (Hct; as per manufacturer's instructions, E8 Centrifugre, LW Scientific, Georgia, USA). Haemoglobin (Hb) analysis was also obtained (Hb-801 Analyzer, HemoCue, Angelholm, Sweden). Changes in PV was calculated using the equation: 100 x (pre-Hb/post-Hb) x (1 –post-Hct/1 – pre-Hct) – 100 (Strauss et al., 1951). Participants then entered the heat chamber and completed a 10 min cycling warm-up at a perceptually regulated exercise intensity (RPE of 13, 15 and 17 for 5, 3 and 2 min, respectively; 6-20 scale, Borg, 1982) on the same cycle ergometer as the familiarisation session. This was followed by performing 6 x 10 min of self-paced cycling in the heat chamber. Training sessions were periodised by maintaining ambient temperature at 36°C but increasing RH between sessions (i.e., session 1-2 = 50% RH, session 3-5 = 65% RH, session 6-9 = 80% RH and session 10 = 50% RH). After each work interval, participants exited the heat chamber and moved to a thermoneutral environment to perform the PerC intervention during the 3 min rest interval. The PerC intervention involved wearing an activated cooling vest (as per manufacturer's instructions, Arctic Heat, Queensland, Australia). At the same time, crushed-ice (using an ice shaver, Avalanche Model IS6800, Sunbeam, New South Wales, Australia) was ingested (0.7 $g{\cdot}kg^{-1}$ BM per rest break to achieve a session total of 3.5 $g{\cdot}kg^{-1}$ BM which represents 15 min of previously utilised ingestion rate (Ihsan et al., 2010). Upon completion of the rest interval, participants re-entered the heat chamber and repeated the process until the completion of all intervals. Water (retrieved from a fountain supplying ~16°C water and kept in non-insulated water bottles in the heat chamber), was ingested *ad libitum* during the HA sessions.

Heart rate, T_c, T_{sk}, Th_c, and Th_s data were collected every 5 min completed during the exercise intervals, and pre- and post-rest intervals in HA1 and HA10 (**Figure 6.1**). Similarly, RPE was obtained every 5 min completed during the exercise intervals in HA1 and HA10. Exercise performance was assessed by measuring mean power output and split-distance every 5 min completed and total distance completed for the exercise protocol.

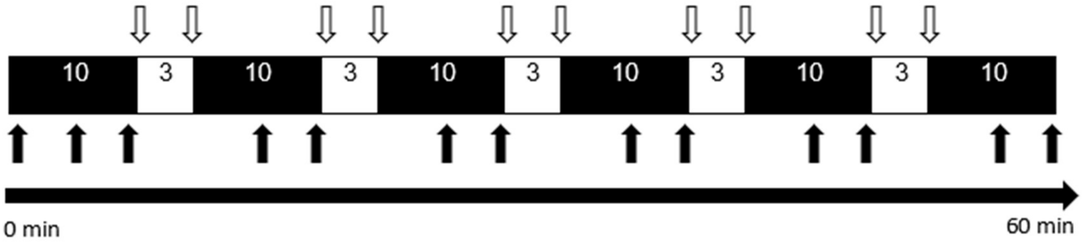

Figure 6.1. Data collection time-points every 5 min total time completed during the 10 min exercise intervals (⬆), and pre- and post-rest 3 min intervals during the intermittent-heat exposure protocol (⇧). Note: ■ = Exercise interval 10 min, □ = rest interval 3 min.

Participants performed 10 sessions over 14 days at the same time of day (± 1–2 h) with no more than 72 h between each session. The HA training sessions (Session 2 – Session 9) were conducted exactly the same as HA1 and HA10. Only mean power output, 5 min split distance, and total distance were collected during these training sessions.

6.4 Statistical Analyses

Body temperature (T_b) was calculated using the equation: $0.65 \times T_c + 0.35 \times T_{sk}$ (Burton, 1935) and core-to-skin temperature gradient (T_g) was calculated using the equation: $T_c - T_{sk}$ (Ely et al., 2009). Intraclass correlation coefficients (ICC) were calculated for all variables to determine the percentage of variance explained by individual differences between participants. Data sampled repeatedly was not independent and ICCs in 8 of 19 outcomes were >.5 (see supplementary material, Table S6.1) thus multilevel modelling was used to analyse data (Bryk & Raudenbush, 1992).

Bayesian multilevel modelling was utilised to analyse the relationship between T_c, mean T_{sk}, T_b, HR, RPE, Th_c, Th_s and total and split distance completed, with estimates reported as unstandardised regression coefficients (b), with 95% credible intervals. Additionally, Bayesian multilevel regression modelling was used to determine differences in hydration, total fluid ingested, sweat loss, change in PV, and total distance completed. This analytical approach is advantageous for small sample sizes as it enables the researcher to integrate prior expectations (priors) about typical effect sizes, thus increasing the precision of estimated effects (Zondervan-Zwijnenburg et al., 2017). We derived priors for the current study from meta-analyses and previous studies which utilised similar PerC methodologies and self-paced intermittent exercise (Alhadad et al., 2019, Best et al., 2014, Bogerd et al., 2010, Brown et al., 2023, Choo et al., 2020, Ihsan et al., 2010, Lorenzo et al., 2010, Naito et al., 2020, 2022, Roussey et al., 2021, Schmit et al., 2017, Sekiguchi et al., 2021, Zimmermann et al., 2017). Posterior distributions derived from the Bayesian estimation process describe the relative probabilities of different values for a parameter, the peak of this distribution represents the highest likelihood of the true value of the parameter. Credibility intervals provided by Bayesian analysis are interpreted similarly to that of frequentist confidence intervals: if an interval does not contain zero, the true value of the parameter is with 95% certainty non-zero. We defined statistically meaningful effects as coefficients that did not include zero and are reported in brackets alongside unstandardised regression coefficients (b). Key findings are summarised in text and full results as well as prior values are provided as supplementary material. Sensitivity analysis was conducted for all models with non-informative priors (priors that assume little to no preexisting knowledge about typical effect sizes; see supplementary material, Table S6.3) to assess the stability of our results. We controlled for the influence of PerC on physiological, perceptual and performance variables. The sensitivity analysis indicated substantive changes in the interpretation of the main effects of condition (i.e., time above T_c ≥38.5°C and total distance completed for sessions 3-5). Substantive changes were observed during the exercise intervals in the interpretation of the main effects of condition (i.e., 5 min split distance completed for sessions 3 and 7, and T_g), as well as two-way interactions for time and condition (i.e., 5 min split distance completed for session 4). During the rest intervals, substantive changes were observed in the interpretation of the main effects of condition (i.e., T_c and Th_s) and time (i.e., T_b). Priors are preferred when sample sizes are small but the sensitivity analysis indicates that some degree of caution is required when interpreting results (Mcneish, 2016). All analyses were conducted using R (R Foundation for Statistical Computing, Austria), and Bayesian multilevel models were implemented using “brms” package (Burkner, 2017).

6.5 Results

Heat Acclimation

No meaningful direct relations were observed between HA1 and HA10 for sweat loss (2.23±0.75 vs. 2.42±0.78 L, b=0.11[-0.24,0.45]). Bayesian multilevel modelling analysis indicated no meaningful direct relations between HA1 and HA10 for change in PV (n=6, -1.63±7.97%, b =-1.02[-9.49,8.38]). Of note, capillary blood sample analysis (n=6) showed a ~4.6% average increase in PV for three participants and an average decrease of ~7.9% for three participants. There were no meaningful changes between HA1 and HA10 for HR (b=1.79[-7.38,9.16]), T_c (b=-0.02[-0.30,0.22]), and T_{sk} (b=-0.47[-1.16, 0.12]) at the start of each session (Table 6.1).

Table 6.1. Mean ± SD for physiological variables during the exercise intervals, start, and finish of the first (HA1) and final (HA10) heat acclimation training sessions.

Variable		HA1	HA10
Core temperature (°C)	Mean	37.47 ± 0.65	37.34 ± 0.77
	Start	36.99 ± 0.36	37.00 ± 0.33
	Finish	37.84 ± 0.74	37.41 ± 1.14
Mean skin temperature (°C)	Mean	35.08 ± 0.78	34.96 ± 0.90
	Start	33.90 ± 0.55	33.56 ± 1.13
	Finish	35.28 ± 0.98	35.13 ± 0.70
Body temperature (°C)	Mean	36.62 ± 0.79	36.50 ± 0.78
	Start	35.88 ± 0.37	35.80 ± 0.53
	Finish	36.72 ± 1.05	36.61 ± 0.88
Core-to-skin temperature gradient (°C)	Mean	2.31 ± 1.00	2.21 ± 1.02
	Start	3.16 ± 0.46	3.44 ± 1.02
	Finish	2.22 ± 1.04	2.28 ± 1.02
Heart rate (bpm)	Mean	140 ± 22	144 ± 21
	Start	84 ± 20	89 ± 11
	Finish	158 ± 20	165 ± 20

Hydration Status

No meaningful direct relations were observed between HA1 and HA10 for baseline USG (1.017±0.008 vs. 1.015±0.005 µ, b=-0.003[-0.009,0.004]). There was evidence of greater total fluid ingestion (total ice + water ingested) in HA10 compared to HA1 (1.31±0.55 vs. 0.95±0.45 L, b=0.40[0.10,0.71]).

Cycling Performance

Out of ten participants, six showed an improvement in total distance achieved in HA10 compared to HA1, three observed a decrease in the total distance achieved, and one person did not complete the last session. Bayesian multilevel modelling analysis indicated greater total distance completed in HA10 compared to HA1 (+1.07-km or +3.97% mean change, b=1148.05[328.32, 2146.53]; Table 6.2). There was evidence of a two-way interaction between time and condition for 5 min split distance completed in HA10 (b=1.29[0.32,2.25]; Figure 6.2), indicating greater split distance completed over the course of HA10 compared to HA1 which decreased over the course of the session. Similarly, greater split distance completed was observed over the course of the protocol in HA4 compared to HA1 (b=1.29[0.10,2.39]). Alternatively, lower split distance completed was observed over the course of the protocol in HA6 (b=-2.17[-3.27,-1.02]), HA7 (b=1.23[-2.43,-0.07]), HA8 (b=-2.36[-3.49,-1.17]), and HA9 (b=-1.87[-3.10,-0.72]).

Table 6.2. Mean total and 5 min split distance completed during the intermittent-heat exposure training program.

Session	Total Distance (km)	5 min Split Distance (km)
1	26.98 ± 2.55	2.26 ± 0.22
2	26.94 ± 2.43	2.25 ± 0.21
3	26.68 ± 2.76	2.23 ± 0.23
4	26.47 ± 2.70	2.23 ± 0.23*
5	26.60 ± 2.77	2.24 ± 0.23
6	25.83 ± 3.41	2.16 ± 0.28
7	25.72 ± 3.22*	2.16 ± 0.27*
8	25.44 ± 3.77*	2.15 ± 0.32
9	25.73 ± 2.91*	2.16 ± 0.32
10	28.05 ± 2.55*	2.34 ± 0.25

Note: *meaningfully different compared to first heat acclimation session (HA1).

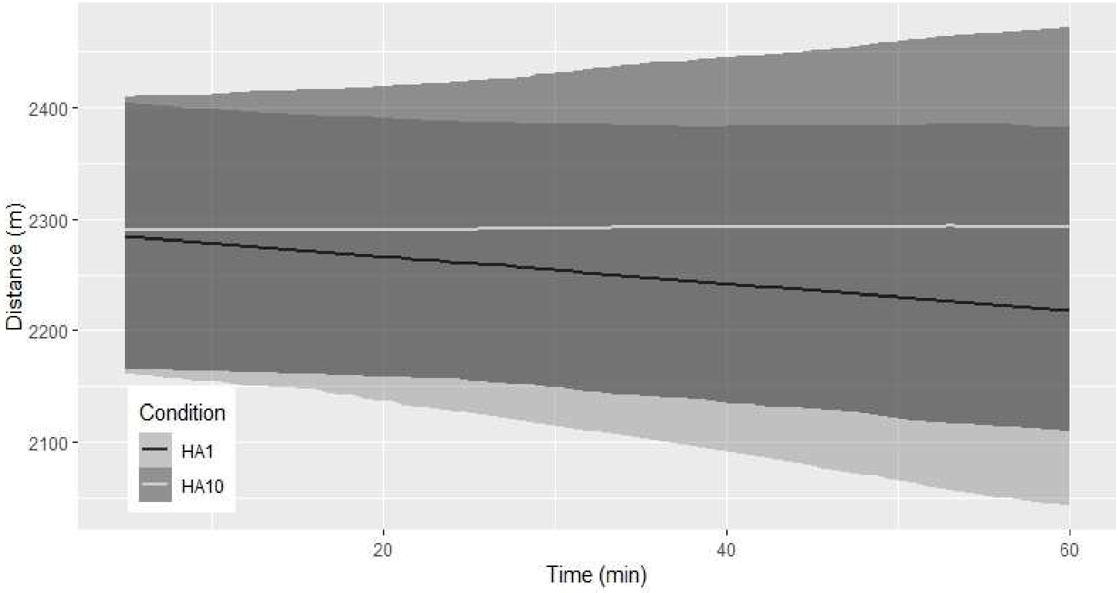

Figure 6.2. 5-min split distance completed as a function of total-time cycled in the first (HA1) and final (HA10) heat-acclimation sessions. *Note: Shaded areas indicate 95% credible intervals.*

Physiological

During the exercise intervals, there was evidence of a main effect of condition for T_g (b=1.2935[0.1391,2.8452]), indicating greater T_g in HA10 compared to HA1. There was also evidence of a main effect for time within the HA sessions for HR (b=0.42[0.20,0.60]) and T_c (Figure 6.3A; b=0.0078[0.0004,0.0142]), indicating an increase over the course of session. No meaningful direct relations were observed for mean T_{sk} (Figure 6.3B; b=-0.05[-0.38,0.26]) and T_b (b=0.12[-0.19,0.44]) during the exercise intervals between HA10 and HA1.

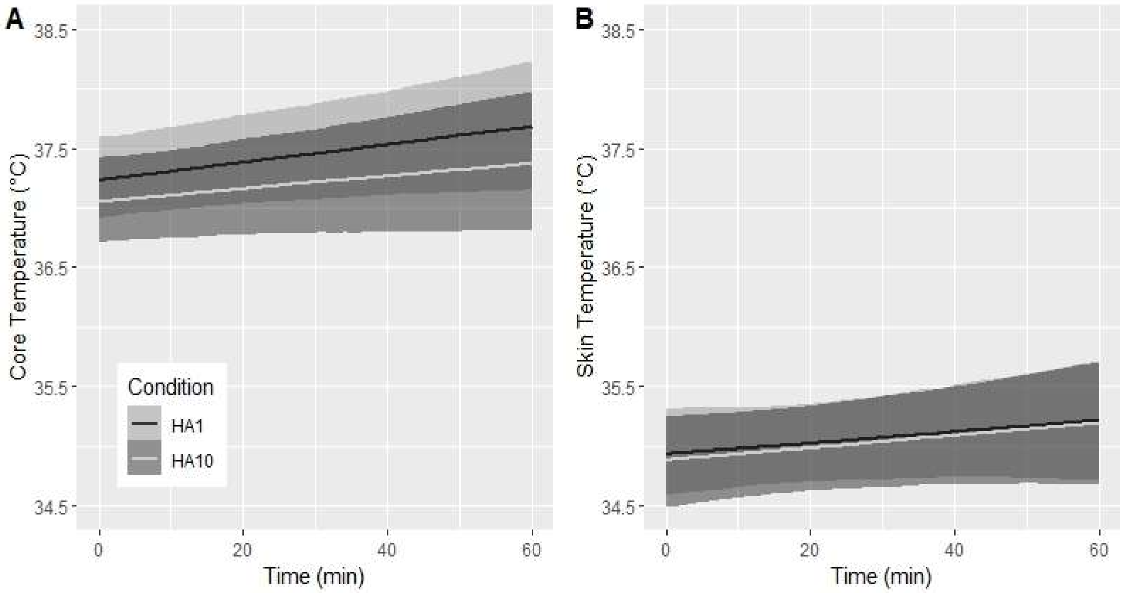

Figure 6.3. (A) Core and (B) mean skin temperature as a function of total-time cycled in the first (HA1) and final (HA10) heat-acclimation sessions. *Note: Shaded areas indicate 95% credible intervals.*

During the rest intervals, no evidence of meaningful direct relations were observed for HR, and T_c. There was evidence of a main effect of time within sessions for mean T_{sk} (b=-0.11[-0.16,-0.06]; Figure 6.4A), T_b (b=-0.024[-

0.048,-0.001]; Figure 6.4B), and T_g (b=0.08[0.02, 0.14]) indicating lower mean T_{sk} and T_b, and greater T_g over the course of the rest intervals in the HA training sessions.

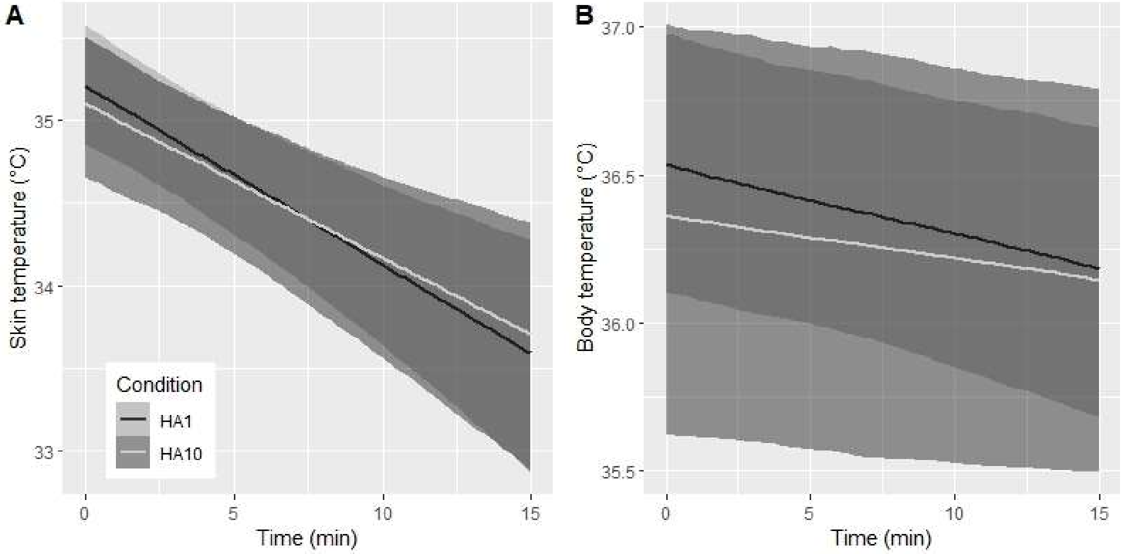

Figure 6.4. (A) Mean skin and (B) body temperature as a function of total rest time in the first (HA1) and final (HA10) heat-acclimation sessions. *Note: Shaded areas indicate 95% credible intervals.*

Perceptual

There was evidence of a main effect for time within the sessions for Th_c (b=0.04[0.02,0.07]; Figure 6.5A) and RPE (b=0.022[0.003,0.042]; Figure 6.5B) during the exercise intervals, indicating an increase over the course of the HA sessions (i.e., more uncomfortable and greater perceived effort). No meaningful direction relations were observed between HA1 and HA10 for Th_c (b=0.13[-0.06,0.31]), Th_s (b=0.11[-0.08,0.30]), and RPE (b=0.21[-0.18,0.60]).

Similarly, no meaningful direct relations were observed for Th_c (b=0.03[-0.16,0.22]) and Th_s (b=0.56[-0.20,1.29]) during the rest intervals.

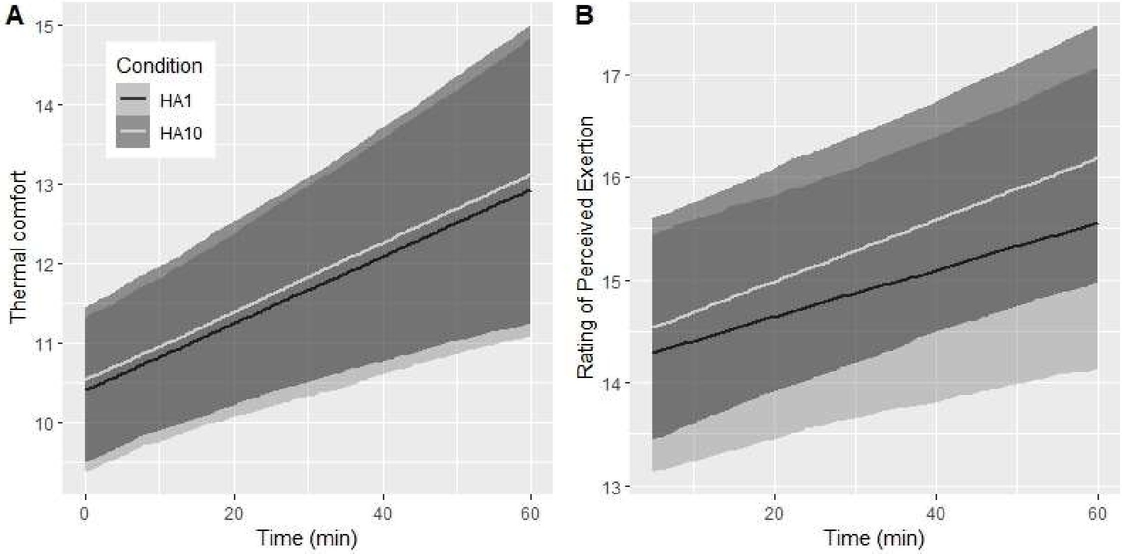

Figure 6.5. (A) Thermal comfort and (B) rating of perceived exertion as a function of total-time cycled in the first (HA1) and final (HA10) heat-acclimation sessions. *Note: Shaded areas indicate 95% credible intervals.*

6.6 Discussion

To the best of our knowledge, this is the first study to determine the efficacy of IHE with PerC as a novel HA program for non-heat acclimatised endurance athletes. One key finding was the lack of evidence to support the attainment of hallmark adaptations (e.g., greater sweat loss, PV, and lower resting and exercise T_c and T_{sk}). Despite this, session total and 5 min split distance completed was greater in HA10 compared to HA1. These results contrast our hypothesis that IHE with PerC will induce physiological adaptations resulting in improved perception and self-paced maximal cycling performance in heat.

We investigated the efficacy of IHE with PerC as a HA protocol following previous findings that it elicits comparable acute (single session) physiological responses to that of continuous exposure to heat (Ramos et al., 2024). The lack of evidence to support the attainment of physiological adaptations (i.e., sweat loss, lower resting and exercising T_c and T_{sk}, and increased PV) contrasts expected adaptations commonly noted by research utilising medium-long term (i.e., 8-14 days) traditional methods of HA (Daanen et al., 2018, Gibson et al., 2015, Périard et al., 2021). For instance, Gibson et al. (2015) utilised 10 isothermic HA sessions (90 min cycling at 65% peak oxygen consumption [$\dot{V}O_{2peak}$] at T_{re} ≥38.5°C in 40°C, 40% RH conditions), and observed hallmark adaptations on day 10 including lower resting T_{re} (~0.49°C) and HR (~18 bpm), greater sweat rate (~0.54 L/h) and PV (~15%) compared to day 1. Additionally, Naito et al., 2022 observed ~0.3°C lower resting T_{re} despite only completing a short-term HA protocol (i.e., 5 days) with the addition of PerC during training sessions. The disparity in findings may be due to the difference between our HA protocol (i.e., IHE with PerC) and continuous heat exposure protocol utilised by Gibson et al. (2015) and Naito et al. (2022), which may have led to less thermal strain experienced by participants during our HA training sessions. For instance, mean T_{re} was ≥38.5°C for ~54% (i.e., ~49 min) and ~25% (i.e., ~20 min) of the total session in the studies by Gibson et al. (2015) and Naito et al. (2022), respectively. In contrast, our study did not observe any time ≥38.5°C T_c in HA1 or HA10 despite previously observing similar accumulated time ≥38.5°C T_c (~50% [~30 min] vs. ~48% [~29 min] of total exercise time) between IHE with PerC (i.e., 6 x 10 min exercise intervals with 3 min rest in between) and continuous heat exposure (i.e., 1 x 60 min interval; Ramos et al., 2024). Yet, it must be noted that this observation was not unexpected, as we anticipated a delay in the increase in T_c and T_{sk} during the IHE protocol due to the cooling intervention and its application in a thermoneutral environment. This may indicate that the lack of observed HA physiological adaptations in our study could be attributed to an insufficient heat stimulus (i.e., T_c ≥38.5°C) during the HA program. Possibly, the cooling intensity (i.e., combination of duration and application of PerC in thermoneutral environment) of our PerC intervention may have been too great, compromising the heat stimulus during the IHE with PerC training sessions.

Despite the lack of evidence to support the attainment of hallmark physiological HA adaptations, T_g was greater in HA10 compared to HA1, which may have resulted in greater session total and 5 min split distance completed over the course of HA10. This supports previous research by Cuddy et al. (2014) who investigated the effects of T_g on incremental treadmill running to volitional fatigue in 18, 26, 34, or 42°C conditions. Their findings showed larger T_g for the 18 and 26°C conditions compared to the 42°C condition at the halfway (~2.6 and ~2.0 vs. ~1.3°C) and finish (~3.3 and ~3.5 vs. ~2.1°C) timepoints of a time-to-exhaustion trial which corresponded with longer times-to-exhaustion in the 18 and 26°C conditions compared to the 42°C conditions (~58 and ~63 min vs. ~51 min). The larger T_g in 18 and 26°C conditions were attributed to cooler chest Tsk at the halfway (~35.2 and ~35.4°C vs. 36.2°C) and finish (~35.4 and ~35.3°C vs. ~37.1°C) timepoints. No differences were observed for T_c at halfway and finish for any of the conditions. Interestingly, our findings also found no meaningful differences in HA1 and HA10 for T_c and T_{sk}. This may explain the lack of difference observed in Th_c and Th_s, which have been previously noted to reflect the changes in T_{sk} (i.e., cooler T_{sk} results in lower ratings of Th_c and Th_s), and result in greater initial exercise intensity compared to warmer T_{sk} (Schlader et al., 2011). However, T_{sk} at the end of the 18 and 26°C conditions in the study by Cuddy et al. (2013) and our study was similar (i.e., ~35.3°C, and ~35.1°C, respectively), which may explain the improvement in performance. These findings suggest that a large T_g (via cooler T_{sk} than T_c) allows for greater heat loss to the environment (via increased blood flow to the skin to transfer body heat from the core to the skin), leading to improved exercise performance in thermally stressful conditions (Ely et al., 2009, Cuddy et al., 2014).

We observed a shift in pacing profile in HA1 compared to HA10 (i.e., from positive [slower intensity over time], to flat [consistent intensity over time]), which corresponded with greater total and 5-min split distance completed in HA10. Although no difference in physiological (i.e., T_c and T_{sk}) and perceptual (i.e., Thc and Ths) responses between HA1 and HA10 were observed, starting T_{sk} was ~0.34°C lower in HA10 compared to HA1 which may have led to greater initial exercise intensity. This supports previous findings by Schlader et al. (2011) who investigated the effects of manipulating T_{sk} (via a liquid-perfused suit) over the course of a 60-min CTT from cool to hot (~-6°C to ~61°C) or hot to cool (~61°C to ~1.4°C). Their findings showed greater self-selected exercise intensity when exercise commenced with cooler T_{sk} (~258 vs. ~251 W). Likewise, our study observed greater exercise intensity evidenced by greater 5-min split distance completed in HA10 compared to HA1. This indicates that IHE with PerC improves training quality by altering pacing strategy.

Although findings of this study support the notion that IHE with PerC does not result in hallmark physiological adaptations to heat, we observed improved within-session exercise intensity, likely through altering pacing strategy. Further, it must be noted that our study only provides an assessment of this specific HA protocol design (i.e., 6 x 10 min intervals with 3 min passive rest in between). Therefore, it cannot be excluded that an appropriate heat stimulus may be achieved with different combinations of exercise and/or rest duration (i.e., longer exercise duration and/or shorter PerC duration), rest mode (i.e., passive vs. active rest), and cooling intensity (i.e., mixed-method vs. external/internal only), whilst improving within-session intensity.

6.7 Perspective

This study is consistent with previous research concluding the combination of cooling (i.e., PerC) and HA does not induce full HA physiological adaptations (Naito et al., 2022), showing that IHE with PerC (i.e., 6 x 10 min intervals with 3 min rest in between) does not result in the attainment of hallmark physiological adaptations to heat. Despite this, we observed improved within-session performance in HA10 which may be explained by a large T_g (via cooler T_{sk}) created by PerC. This indicates that IHE with PerC may allow for greater work done within a session, which may be beneficial for situations where a large training volume or maintenance of training quality is required with limited time.

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6.9 Supplementary Material

Table S6.1. Intraclass correlation coefficients for hydration and sweat loss, performance, physiological, and perceptual data during the exercise and rest-intervals.

Variable	Exercise Interval	Rest Interval
Urine specific gravity	9%	
Total fluid ingestion	50%	
Sweat loss	79%	
Total distance completed	89%	
Change in plasma volume	4%	
5-min split distance completed	83%	
Core temperature	44%	47%
Mean skin temperature	32%	18%
Body temperature	45%	40%
Heart rate	34%	20%
Thermal comfort	58%	66%
Thermal sensation	70%	66%
Rating of perceived exertion	67%	-

Table S6.2. Mean ± SD for hydration, sweat loss, and blood analysis during the first (HA1) and final (HA10) heat acclimation training sessions.

Variable	HA1	HA10
USG (μg)	1.02 ± 0.01	1.02 ± 0.01
Total fluid ingested (L)	0.95 ± 0.45	1.31 ± 0.55
Sweat Loss (L)	2.23 ± 0.75	2.42 ± 0.78
Haemoglobin (g/dL)	148.3 ± 18.2	159.7 ± 14.5
Haematocrit (%)	50.0 ± 4.6	50.0 ± 4.1
Change in plasma volume (%)	-	-1.63 ± 7.97

Table S6.3. Informative and default priors for Bayesian analysis.

	Estimates [95 % credible intervals]	Time Coefficient (Mean ± SD)	Condition Coefficient (Mean ± SD)
Between-person			
Urine specific gravity	1.017 [1.012, 1.022]	-	0.001 ± 0.01 (Schmit et al., 2017)
Total fluid ingested	0.95 [0.58, 1.31]	-	0.54 ± 0.61 (Sekiguchi et al., 2021
Sweat Loss	2.25 [1.71, 2.78]	-	-0.01 ± 0.03 (Naito et al., 2022)
Total Distance	27189.22 [25413.53, 29086.40]	-	8720 ± 2000 (Naito et al., 2022)
Change in plasma volume	-1.15 [-8.51, 6.88]	-	16.6 ± 21 (Schmit et al., 2017)
Exercise intervals			
5-min split distance completed	2289.79 [2138.70, 2435.70]	-	0.06 ± 0.3 (Naito et al., 2022)
Heart rate	127.42 [117.64, 137.99]	0.06 ± 0.003 (Morito et al., 2022)	-3 ± 6 (Naito et al., 2022)
Core temperature	37.22 [36.90, 37.55]	0.0003 ± 0.5 (Naito et al., 2022)	-0.1 ± 0.2 (Alhadad et al., 2019)
Mean skin temperature	34.94 [34.56, 35.31]	0.001 ± 0.5 (Naito et al., 2022)	-1.2 ± 0.7 (Naito et al., 2022)

Body temperature	36.36 [36.05, 36.68]	0.0004 ± 0.5 (Naito et al., 2022)	0.2 ± 0.2 (Lorenzo et al., 2010)
Thermal comfort	10.39 [9.45, 11.43]	-	0.13 ± 0.1 (Roussey et al., 2021)
Thermal sensation	11.74 [10.42, 12.95]	-	-0.02 ± 0.1 (Naito et al., 2022)
Rating of perceived exertion	14.17 [12.81, 15.43]	-	0.02 ± 0.1 (Choo et al., 2020)
Rest intervals			
Heart rate	120.10 [108.13, 133.25]	0.003 ± 0.1 (Zimmermann et al., 2017)	-6.0 ± 18.6 (Brown et al., 2023)
Core temperature	37.40 [36.96, 37.84]	-0.0003 ± 0.3 (Zimmermann et al., 2017)	-
Mean skin temperature	35.21 [34.85, 35.58]	-	-0.73 ± 0.46 (Best et al., 2014)
Body temperature	36.53 [36.10, 36.98]	-0.0003 ± 0.1 (Bogerd et al., 2010)	-
Thermal comfort	10.84 [9.82, 11.92]	0.001 ± 0.1 (Bogerd et al., 2010)	-
Thermal sensation	9.66 [7.67, 11.64]	-0.001 ± 0.5 (Zimmermann et al., 2017)	-

Chapter 7: Opinion Paper

The use of an internal-to-external load ratio to determine the efficacy of heat acclimation/acclimatisation using self-paced exercise

This paper has been published in Frontiers in Sports and Active Living.

Ramos, J. A. P., Brade, C. J., Ducker, K. J., Landers, G. J., & Girard, O. (2022). The internal-to-external load ratio: A tool to determine the efficacy of heat acclimation/acclimatization using self-paced exercise. *Frontiers in Sports and Active Living*, **3**:830378. DOI: 10.3389/fspor.2021.830378.

Presented here in accepted publication format.

7.1 Introduction

To combat the negative effects of heat on exercise tolerance, daily training for 1 – 2 weeks for 60 – 90 min in hot conditions (heat acclimation or acclimatisation; HA) is recommended (Périard et al., 2021). Briefly, HA results in physiological (e.g., lower core temperature) and perceptual (e.g., improved thermal comfort) adaptations, which may enhance exercise performance (e.g., increased power output; PO) in the heat (Périard et al., 2021). Heat acclimation protocols typically involve performing continuous or intermittent exercise, either at a fixed intensity (e.g., maintaining a PO corresponding to 60% of maximal aerobic capacity; $\dot{V}O_{2max}$) or using a physiologically controlled approach (i.e., fixed hyperthermia [core temperature ~38.5°C] or heart rate [HR; ~150 $b \cdot min^{-1}$]; Périard et al., 2021). Alternatively, self-paced exercise, whereby athletes self-regulate work rate during HA sessions to match a perceptually regulated intensity (e.g., exercise at a given rating of perceived exertion; RPE), is gaining popularity (Gibson et al., 2020, Périard et al., 2021).

7.2 Nature of the Problem

A heat stress test (HST) is performed pre- and post-HA to assess the effectiveness of a HA program by measured changes in physiological, perceptual and performance variables. Interpreting the interactions between these variables and determining what adaptations have occurred, is easier when work rate is fixed. For example, lower HR post-HA compared to pre-HA when external load is fixed (e.g., cycling at an absolute intensity of 100 W) infers a physiological adaptation to the heat. However, interpreting whether adaptations have been attained during self-paced HA (e.g., 20-km cycling time-trial) is more difficult, as external and internal loads vary (Périard et al., 2021). For example, it is harder to ascertain whether adaptations have occurred when PO (158 vs. 150 W) and HR (169 vs. 160 $b \cdot min^{-1}$) hypothetically change in similar proportion post-HA compared to pre-HA.

7.3 Proposal

Utilising an internal-to-external load ratio may be a method of objectively concluding whether a self-paced HA session or protocol is effective (Figure 7.1) compared to other methods (e.g., observing changes in sweat rate, and core or skin temperature when external load is fixed). Ratios can be applied to physiological or perceptual variables (internal load; HR, thermal comfort or sensation) and performance variables (external load; mean PO) obtained during a HST, single HA session, or throughout a HA program.

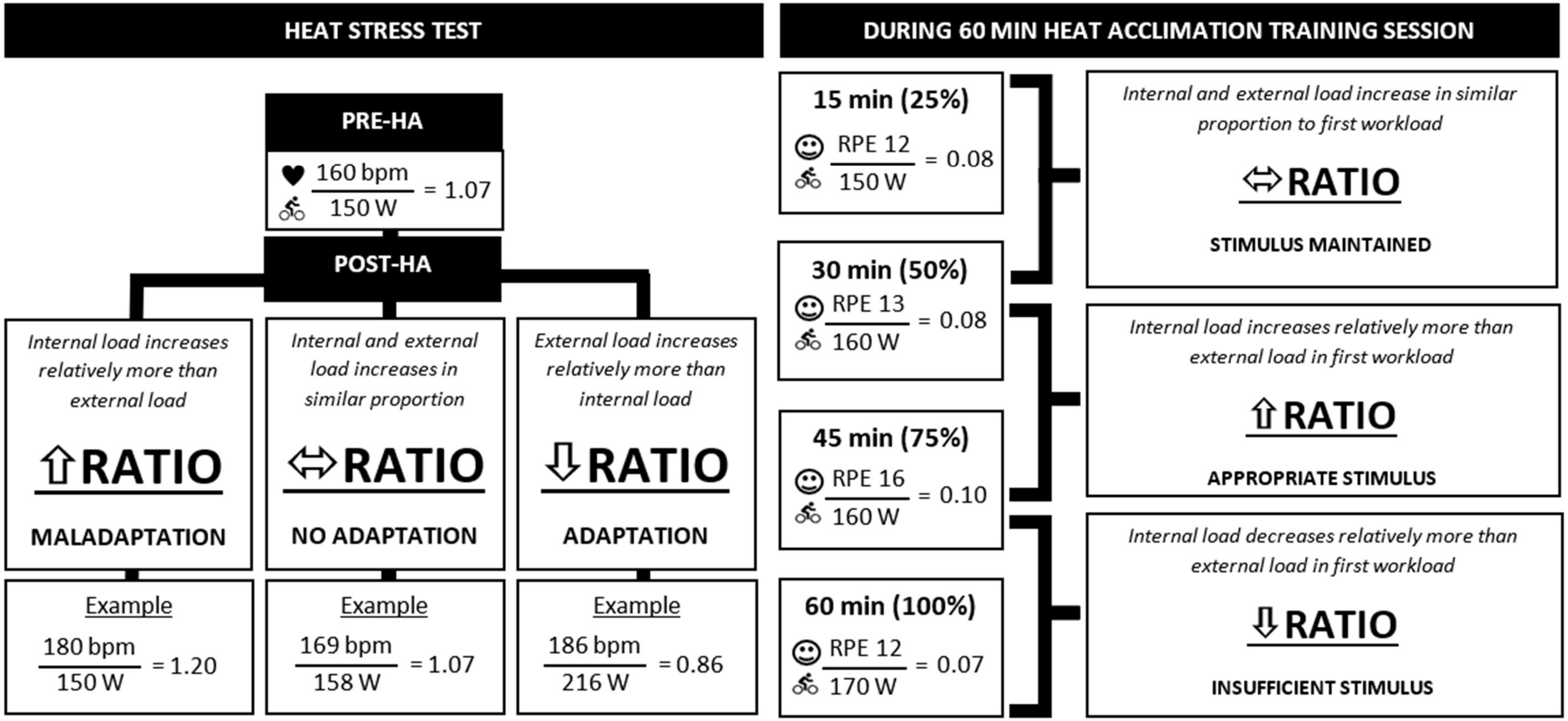

Figure 7.1. Hypothetical example of applying an internal-to-external load ratio for assessing self-paced heat stress tests and heat acclimation (HA) training sessions. ♥ = mean heart rate, 🚴 = mean power output, ☺ = rating of perceived exertion.

For example, observing a larger relative change in internal load compared to external load (thus a lower internal-to-external ratio) during a post-HA compared to pre-HA HST, may be indicative of HA. This is evident in a study by Wingfield et al. (2016) who performed a HST in the form of a 20-km self-paced cycling time-trial in the heat (33.1°C, 60.0% RH) pre- and post-HA training (five consecutive days cycling for 30 min at alternating intensities every 3 min between 40 and 70% peak PO in 32°C, RH not reported). Results showed no difference in completion time (40.46 vs. 40.45 min) pre- and post-HA HST, which may indicate that no adaptation to the heat had been attained. However, utilisation of the internal-to-external load ratio on mean PO pre- and post-HA HST (154 vs. 157 W) and HR (161 vs. 153 b·min^{-1}) show a lower ratio post-HA compared to pre-HA (0.98 vs. 1.05). This is due to a lower HR despite a higher sustained PO post-HA, suggesting that HA has occurred as a lower internal-to-external ratio compared to pre-HA HST is observed.

Alternatively, internal-to-external load ratios could be utilised to determine the efficacy of a single session of self-paced HA training. For example, if internal-to-external load ratio for a hypothetical RPE (12 vs. 13) and mean PO (150 vs. 160 W) at 25% and 50% of total exercise time completed show similar ratios (0.08), this indicates that the stimulus has been maintained throughout the session. Alternatively, reductions or failure to maintain ratios throughout a single self-paced HA session could indicate an ineffective session. This may be due to the internal load decreasing relatively more than the external load, which indicates that the athlete is not receiving the appropriate stimulus required to induce adaptations to the heat.

Finally, whole-session internal-to-external load ratios may be utilised to track whether athletes are receiving the appropriate stimulus for heat adaptation throughout a HA program. For instance, if a hypothetical whole-session rating of thermal sensation (15; 0 - 20 scale, Gaoua et al., 2012) and mean PO (180 W) were obtained for the first session of a HA program (ratio = 0.08), subsequent sessions in a simple stepwise progression will need to obtain a ratio of ≥ 0.08 to ensure athletes are receiving the appropriate progressive overload stimulus.

7.4 Conclusion

Utilisation of internal-to-external load ratios could assist with objectively concluding that a self-paced HA session or protocol is effective. This could lead to a novel addition in identifying the effectiveness of HA protocols.

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Chapter 8: General Discussion

8.1 Main Findings

The overarching objective of this thesis was to investigate how cooling can be used to enhance endurance performance and HA protocols and propose an objective method of determining the effectiveness of self-paced HA protocols. Study one (see chapter 3) determined the AT conditions where PreC provided the greatest benefit for self-paced, maximal endurance performance. Study two (see chapter 4) investigated the effect of single-session IHE with frequent and shorter cooling breaks compared to traditional continuous heat exposure. Study three (see chapter 5) determined the effect of PreC on self-paced endurance performance in heat-acclimated (Heat$_{Acc}$; via IHE) individuals. Study four (see chapter 6) determined the effectiveness of IHE plus PerC over a full (10 sessions) HA protocol. Finally, we proposed the use of an internal-to-external load ratio to objectively determine the effectiveness of a self-paced HA session/program and as a tool to monitor self-paced HA training.

While there were no detrimental effects on maximal self-paced endurance performance in moderately hot-humid and hot-dry environments, mixed-method PreC had the greatest benefit when applied prior to endurance exercise in hot-humid conditions. Furthermore, when IHE with PerC was utilised in the same conditions, comparable acute physiological responses were observed compared to traditional continuous exposure. However, despite combining these findings to explore whether Heat$_{Acc}$ endurance athletes could still derive benefits from mixed-method PreC during self-paced maximal endurance performance in hot-humid conditions, we observed no further benefits beyond those achieved with HA alone. It is possible that the cooling intensity (i.e., combination of duration and application of PerC in a thermoneutral environment) of our PerC intervention during IHE may have been too high, compromising the heat stimulus during the IHE with PerC HA training sessions. Nevertheless, we observed improved within-session performance in the final HA training session which may be explained by a large T_g (via cooler T_{sk}) created by PerC. This indicates that IHE with PerC may allow for greater work done within a session, particularly beneficial for situations where a large training volume is required within a limited timeframe.

8.2 Enhancing the Utilisation of Mixed-method Pre-cooling in Heat

Our findings in chapter 3 showed ~95 s faster completion time when mixed-method PreC was applied prior to the 20-km cycling TT in hot-humid conditions. Interestingly, no performance benefits were observed in moderately hot-humid and was detrimental to 20-km cycling TT performance in hot-dry conditions. This supports previous claims that PreC may only enhance performance when thermal strain is high (Wegmann et al., 2012, Brade et al., 2013a, Castle et al., 2011, Brade et al., 2013b). Previous findings by Ross et al. (2011) showed ~66 s faster completion time when 15 min mixed-method PreC (14 $g \cdot kg^{-1}$ body mass [BM] crushed-ice ingestion + cooling vest) was utilised compared to no cooling prior to 46.4-km cycling TT in the heat (~35°C, ~60% relative humidity [RH], ~42°C AT). Faster cycling TT completion times observed by Ross et al. (2011) in PreC compared to no cooling in ~42°C AT condition, may be due to the longer cycling TT compared to our study (46.4 vs. 20-km) and higher RH (55 vs. 60% RH), which together may have elicited greater thermal strain. Bright et al. (2021) provides support to this claim with their investigation on the effects of different skin-to-air vapor pressure gradients on 30-km cycling TT in hot-humid conditions (≥28°C, ≥72% RH, ≥33°C AT) compared to cooler conditions (≤20°C, ≤70% RH, ≤21°C AT). Greater thermal strain was experienced by participants, evident through greater rectal temperature (~39.1–39.6°C vs. 38.7–38.8°C), heart rate (HR; 159–163 bpm vs. 153–159 bpm), and rating of perceived exertion (RPE; 15–17 vs. 15) in hot-humid compared to cooler conditions respectively. This corresponded to lower power output during the 30-km cycling TT in hot-humid conditions (~228–262 W) compared to cooler conditions (~272–275 W).

The lack of benefit to 20-km cycling TT performance in less thermally stressful conditions contrasts previous research which applied PreC in similar thermal conditions (~29-37°C AT). For instance, Ihsan et al. (2010) applied PreC (6.8 $g{\cdot}kg^{-1}$ BM crushed-ice ingestion) prior to 1200-kJ cycling TT (~40-km cycling TT) in heat (30°C, 75% RH, 37°C AT), which resulted in ~348 s faster completion time compared to no cooling. The disparity in findings may be due to greater reductions in T_c at the end of the PreC period in the previous study compared to our study (~0.46 vs. ~1.10°C; Ihsan et al., 2010). Additionally, longer exercise and exposure to heat resulted in higher final T_c compared to our study (~38.5-39.0 vs. ~37.5-38.0°C), eliciting greater thermal strain and therefore greater PreC benefits leading to faster mean split completion times compared to no cooling. Possibly the duration of the exercise task and environmental conditions in our study did not elicit sufficient thermal strain, and therefore mixed-method PreC did not benefit performance in <40°C AT. In fact, Faulkner et al. (2018) observed ~95 s faster 60 min cycling TT completion time after applying external PreC (30 min) in less thermally stressful conditions compared to our study (~27°C, ~50%RH, ~29°C AT). This suggests that PreC may only benefit self-paced endurance performance in ≤40°C AT when exercise duration is >60 min in duration.

Another possible explanation for the lack of benefit in performance in less thermally stressful conditions may be the lower mean T_{sk} at the commencement of the cycling TT in 46°C AT when PreC was applied, compared to no cooling. This observation corresponded with faster total completion times. This supports previous proposals that exercise intensity at the commencement of exercise is more closely associated with cooler T_{sk} than T_c (Schlader et al., 2011). Alternatively, warmer T_{sk} has been suggested to lead to an anticipatory response which results in lower initial self-selected exercise intensity, primarily to ensure the completion of exercise tasks (Tucker et al., 2004). Indeed, our findings support this, as we also observed lower T_{sk}, improved Th_c and RPE during the initial stages of the 20-km cycling TT in hot-humid conditions, and no difference in T_{sk} in moderately hot-humid and hot-dry conditions. It is possible that mixed-method PreC may have blunted the anticipatory response by lowering T_{sk} prior to the start of the exercise task, resulting in higher initial work outputs. We conclude that cooling is beneficial to self-paced endurance exercise in hotter environmental conditions.

8.3 Using Cooling to Enhance Heat Acclimation

Another method to combat against the negative effects of heat on self-paced endurance performance is HA, which provides similar or greater improvements than cooling to self-paced endurance performance in heat (Zimmermann et al., 2018, Alhadad et al., 2019). We proposed the use of IHE with PerC as an alternate method of HA due to its potential benefit of improving perception to the heat, which may result in greater training intensity. However, before it can be implemented into a full HA protocol, the physiological, perceptual, and performance responses to a single session of IHE with PerC needed to be investigated.

Single-session Intermittent-heat Exposure

Our findings in chapter 4 showed greater within-session exercise intensity when shorter, more frequent rest breaks with per-cooling (PerC; i.e., IHE) was utilised compared to continuous heat exposure with no cooling in hot-humid conditions. Importantly, despite shorter exercise bout durations and more frequent breaks with PerC, single session IHE achieved these performance benefits without compromising necessary acute physiological stimuli required for HA (i.e., time spent above 38.5°C T_c and sweat loss). Enhanced performance in single session IHE protocols supports previous findings by Naito et al. (2022), who observed significantly greater total work following PerC compared to control on Day 1 (~206 vs. ~198 kJ) of HA training. Performance improvements were attributed to lower T_c and T_{sk} resulting from PerC. Our findings showed that T_c increased over time for all conditions, but consistently remained lower during IHE compared to continuous heat exposure. This result aligns with previous research suggesting that PerC attenuates increases in T_c during exercise compared to scenarios without PerC, and added breaks may further contribute to blunting T_c increases (van de Kerkhof et al., 2023, Douzi et al., 2018, Bongers et al., 2017).

To induce HA, it is commonly proposed that maintaining T_c ≥38.5°C is a key factor for adaptation (Gibson et al., 2020, Fox et al., 1967). Despite cooler body temperatures evident in IHE compared to continuous exposure protocols, no difference was observed between continuous heat exposure and IHE (i.e., 6 x 10 min with 3 min passive rest). This may be due to greater total and mean 5 min split distance completed in IHE compared to continuous heat exposure, resulting in greater intensity driven metabolic heat production equalling the continuous heat exposure thermal strain. Thus, an IHE protocol with shorter and more frequent breaks (i.e., IHE-10) may enhance performance whilst promoting maintenance of high T_c closer to that of continuous exposure despite cooling rest periods.

Additionally, there was consistently lower mean T_{sk} observed over time during all IHE protocols compared to CON. This aligns with previous literature, as PerC is known to delay increases in T_{sk}, ultimately resulting in improved performance but possibly limiting heat adaptation (van de Kerkhof et al., 2023, Douzi et al., 2018, Bongers et al., 2017, Regan et al., 1996). (van de Kerkhof et al., 2023, Douzi et al., 2018, Bongers et al., 2017). Tucker et al. (2004) suggested that high T_{sk} (>35°C; Périard et al., 2021, Sawka et al., 2011) initiates an anticipatory response, leading to lower initial work rates during exercise in heat. Alternatively, lower T_{sk} corresponds with improved levels of Th_s and Th_c, resulting in improved distance completed during a 60 min cycling TT. Our findings support this, as the lower T_{sk} observed in all IHE protocols corresponded with improved Th_c, Th_s, and RPE, along with greater total and 5 min split-distance completed.

Intermittent-heat Exposure Heat Acclimation Training

Based on our previous findings (study two; chapter 4) that a single IHE with PerC session (i.e., 6 x 10 min exercise with 3 min rest in between; Ramos et al., 2024) resulted in greater training intensity while providing the necessary stimulus and physiological response required for effective HA, we applied this same protocol over repeated exposures (i.e., a full HA protocol).

Our findings in study four (chapter 6) showed that IHE with PerC as a full HA protocol did not result in attainment of hallmark physiological adaptations (e.g., greater sweat loss, plasma volume, and lower resting and exercising T_c and T_{sk}). Despite this, session total and 5 min split distance completed was greater in the final HA session (HA10) compared to the first (HA1).

The discrepancy in study four findings compared to our single session study (study 2) may be due to cooler environmental conditions in our HA training study (42 vs. 48°C AT). Additionally, the application of the cooling intervention in a thermoneutral environment may have further decreased the heat stimulus (i.e., T_c ≥38.5°C) during the HA program. For instance, our training study did not observe any time ≥38.5°C T_c in HA1 and HA10 despite previously observing comparable durations of ≥38.5°C T_c between continuous heat exposure and IHE with PerC (~50% [~30 min] and ~48% [~29 min] of the total exercise time, respectively; Ramos et al., 2024). Possibly, the cooling intensity (i.e., combination of duration and application of PerC in thermoneutral environment) of our PerC intervention may have been too great, compromising the heat stimulus during IHE with PerC training sessions. Additionally, the lack of evidence to support the attainment of physiological adaptations (i.e., lower T_c and T_{sk}) contrasts expected adaptations by reviews and research utilising medium-long term (i.e., 8-14 days) traditional methods of HA (Périard et al., 2021, Daanen et al., 2018, Gibson et al., 2015). For instance, Gibson et al. (2015) utilised 10 isothermic HA sessions (90 min cycling at 65% peak oxygen consumption at T_{re} ≥38.5°C in 40°C, 40% RH conditions), and observed hallmark adaptations on day 10 such as lower resting T_{re} (~0.49°C), lower resting HR (~18 bpm), greater sweat rate (~0.54 L/h), and greater PV (~15%) compared to day 1. Additionally, Naito et al., 2022) observed ~0.3°C lower resting T_{re} despite completing a short-term HA protocol (i.e., 5 days) with the addition of PerC during the training sessions.

Despite the lack of evidence to support the attainment of hallmark HA adaptations, we observed greater T_g in HA10 compared to HA1, which may have resulted in greater session total and 5 min split distance completed over the course of HA10. This supports our previous findings in single session IHE with PerC (Ramos et al., 2024). This also supports previous research by Cuddy et al. (2014) who investigated the effects of T_g on incremental treadmill running to volitional fatigue in 18, 26, 34, or 42°C conditions. Their findings showed larger T_g for the 18 and 26°C conditions compared to 42°C at halfway (~2.6 and ~2.0 vs. ~1.3°C) and finish (~3.3 and ~3.5 vs. ~2.1°C) of the time-to-exhaustion trial. This corresponded with longer time-to-exhaustion in the 18 and 26°C conditions compared to the 42°C conditions (~58 and ~63 min vs. ~51 min). The larger T_g in 18 and 26°C conditions compared to 42°C were attributed to cooler chest T_{sk} at halfway (~35.2 and ~35.4°C vs. 36.2°C) and finish (~35.4 and ~35.3°C vs. ~37.1°C). No differences were observed for T_c at halfway and finish for any of the conditions. Interestingly, our findings found no meaningful differences in HA1 and HA10 for T_c and T_{sk}. This may explain the lack of difference observed in Thc and Ths, which have been previously noted to reflect the changes in Tsk (i.e., cooler Tsk results in lower ratings of Thc and Ths), and result in greater initial exercise intensity compared to warmer Tsk (Schlader et al., 2011). However, T_{sk} at the end of the 18 and 26°C conditions in the study by Cuddy et al. (2013) and our training study was similar (i.e., ~35.3°C, and ~35.1°C, respectively). These findings suggest that a large T_g (via cooler T_{sk}) allows for greater heat loss to the environment (via increased blood flow to the skin to transfer body heat from the core to the skin), leading to improved exercise performance in thermally stressful conditions (Cuddy et al., 2014, Ely et al., 2009).

We observed a shift in pacing profile in HA1 compared to HA10 (i.e., from positive [slower intensity over time], to flat [consistent intensity over time]), which corresponded with greater total and 5-min split distance completed in HA10. Although no difference in physiological (i.e., T_c and T_{sk}) and perceptual (i.e., Th_c and Th_s) responses between HA1 and HA10 were observed, starting Tsk was ~0.34°C lower in HA10 compared to HA1 which may have led to greater initial exercise intensity. This supports previous findings by Schlader et al. (2011) who investigated the effects of manipulating T_{sk} (via a liquid-perfused suit) over the course of a 60-min CTT from cool to hot (~-6°C to ~61°C) or hot to cool (~61°C to ~1.4°C). Their findings showed greater self-selected exercise intensity when exercise commenced with cooler T_{sk} (~258 vs. ~251 W). Likewise, our study observed greater exercise intensity evidenced by greater 5-min split distance completed in HA10 compared to HA1. This indicates that IHE with PerC improves training quality likely by altering pacing strategy.

8.3.1 Using Cooling to Enhance Endurance Performance in Heat Acclimated Individuals

Although not detrimental to performance, mixed-method PreC did not improve 20-km cycling TT completion time beyond the improvements seen from HA (study 4, chapter 5). This supports previous studies results which also found no additional benefit from PreC in already Heat_{Acc} athletes prior to self-paced endurance performance in heat (James et al., 2018, Schmit et al., 2017). This finding has been attributed to HA-induced adaptations (e.g., increased plasma volume and sweat loss) facilitating skin and muscle blood flow and altering pacing profile, which ultimately blunt the effect of PreC (Nielsen et al., 1993, Schmit et al., 2017). Our findings observed greater sweat loss post-HA during the 20-km cycling TT, which may have reduced bodily temperatures. This resulted in decreased thermal strain experienced by individuals during the cycling TT and minimising the effects of PreC on self-paced maximal endurance performance in hot-humid conditions. Additionally, participants in our study adopted a negative pacing profile (i.e., slower over time) during the 20-km cycling TT, which was like that observed in previous research (Schmit et al., 2017). The similar pacing profiles may be explained by the similar experience level of athletes recruited (i.e., Tier 3: Highly trained/national level; McKay et al., 2022). Schmit et al. (2016) investigated the effect of familiarisation to the heat on the pacing profile of non-heat acclimated well trained triathletes (Tier 2; McKay et al., 2022) during 20-km cycling TT in the heat (35°C, 50% RH). Their findings showed a change from positive (i.e., higher intensity over time) to an even (i.e., steady intensity over time) pacing profile in the heat after an initial familiarisation session to the heat, which resulted in faster completion time (~48 s). It is possible that different pacing profiles may be adopted by elite or world-class (Tier 4-5), which may result in a benefit in performance.

Core temperature increased at a greater rate when PreC was applied compared to CON post-HA (~3 vs. ~2°C). This contrasts previous findings by James et al. (2018) who investigated the effects of 5 days HA with PreC (via iced towel on head and neck, cold-water hand immersion, and cooling vest) on 5-km running time-trial performance in the heat (32°C, 60% RH), and found greater increases in T_c in CON compared to PreC (~+2.06 vs. ~+1.86°C). The disparity in findings may be due to a difference in exercise duration and air temperature and humidity compared to our study. It is possible that the longer exercise task and greater humidity may have exacerbated heat gain in our study. Indeed, James et al. (2018) observed lower mean T_{sk} throughout the cycling TT compared to our study (34.1 vs. 36.4°C).

Despite observation of lower mean T_{sk} when PreC was applied compared to no cooling, we did not observe any changes in Th_c and Th_s. This contrasts previous suggestions by Schlader et al. (2011) that exercise intensity at the commencement of exercise is more closely associated with cool T_{sk} than T_c. The disparity in findings may be due to lower mean T_{sk} observed by Schlader et al. (2011) compared to our study when PreC was applied (~29.4 vs. ~36.4°C) compared to that observed by Schlader et al. (2011). However, it must be noted that the participants in the study by Schlader et al. (2011) were not $Heat_{Acc}$, which may have heightened the ergogenic benefits of cooler $T_{sk.}$

Our findings suggest that although not detrimental, there was insufficient evidence to support a meaningful change in performance when PreC was applied in Heat$_{Acc}$ individuals prior to 20-km cycling TT in hot-humid conditions.

8.4 Thesis Strengths, Limitations, and Future Directions

The strength of this thesis lies in its applicability to non-Heat$_{Acc}$ individuals which represents most of the athletic population training and/or competing across different environmental conditions and competitions (e.g., cold-climate residents competing in events hosted in typically hot climates). Additionally, the findings from this thesis provide practitioners (i.e., exercise and sport scientists) of the optimal conditions where cooling and/or HA training will provide the greatest benefit to self-paced endurance performance, as these techniques may not always be cost-effective, feasible, or readily available to many athletes. Another strength of this thesis lies in its utilisation of robust statistical models (e.g., multilevel modelling and Bayesian statistics), which account for individual variability (via the use of intraclass correlation coefficients) and leveraging prior knowledge to inform subsequent analyses to appropriately address the aims of this thesis.

The methodology employed in the studies within this thesis enabled me to accurately assess the impact of my cooling and HA intervention under controlled conditions. I acknowledge that this might limit the transferability of the findings of this thesis to some real-world scenarios. For example, the application of my PreC and PerC interventions in a thermoneutral environment may not always be feasible or attainable prior to competition held in extremely hot environments (e.g., due to unavailability of air-conditioned rooms). This may then overestimate the effect of our cooling interventions as cooler T_c and/or T_{sk} are expected in thermoneutral compared to hotter conditions. While the findings of this thesis may be more relevant to trained/highly-trained non-$Heat_{Acc}$ endurance athletes, I acknowledge that this may not be extended to already $Heat_{Acc}$ individuals or those who are of a higher level (i.e., tier 4-5 [elite or world class] athletes) as these individuals may already possess adaptations (e.g., lower T_c and T_{sk}) that enable them to tolerate hotter environments, potentially underestimating/negating the ergogenic benefits of cooling. Further, I only investigated male endurance athletes due to best practice methodological procedures for conducting research in women being beyond the feasibility of this thesis. The impact of the menstrual cycle phases on T_c changes (e.g., increased T_c in luteal phase or decreased T_c in follicular phase; Stone et al., 2021) may potentially limit the applicability of the findings from this thesis to female endurance athletes participating in HA training. Therefore, it remains uncertain whether the changes in T_c during the menstrual cycle can either create or diminish the necessary heat stimulus for effective HA (via IHE with PerC). This also indicates that further research is warranted to identify whether similar responses may be observed in elite male and/or female athletes. Given that endurance events are held in varying combinations of temperature and RH, future research should investigate the efficacy of cooling interventions across different temperature and RH combinations while maintaining the same AT. Furthermore, to enhance the relevance of cooling and/or HA training interventions for female athletes, future research could examine the effects of cooling and/or HA training at different phases of the menstrual cycle. Finally, despite observing no hallmark adaptations when IHE with PerC was applied over a full HA protocol, our findings only provide an assessment of the specific HA protocol design. Therefore, future research may look to determine whether different combinations of exercise and/or rest duration, rest mode, and cooling intensity may result in physiological heat adaptation.

8.5 Practical Applications

- Cooling is recommended prior to exercise in heat and the application of mixed-method PreC should be determined using a priority scale as shown in Figure 8.1.
- When overall training load permits prioritising maximal HA stimulus over training quality, traditional continuous heat exposure is still recommended. However, when training quality holds greater priority than heat stimulus, then IHE may be utilised (see Figure 8.2).
- Prior to utilisation in competition, familiarisation with PreC is recommended as it can lead to sub-optimal pacing strategies in the initial stages of a 20-km CTT.
- Mixed-method PreC may not be a priority for $Heat_{Acc}$ individuals as greater sweat losses from HA reduce the ergogenic benefit of PreC and does not improve Th_{S}.
- Intermittent-heat exposure with PerC allows for greater total work done within a session, which may be beneficial for situations where a large training volume and/or high training quality is required with limited time.

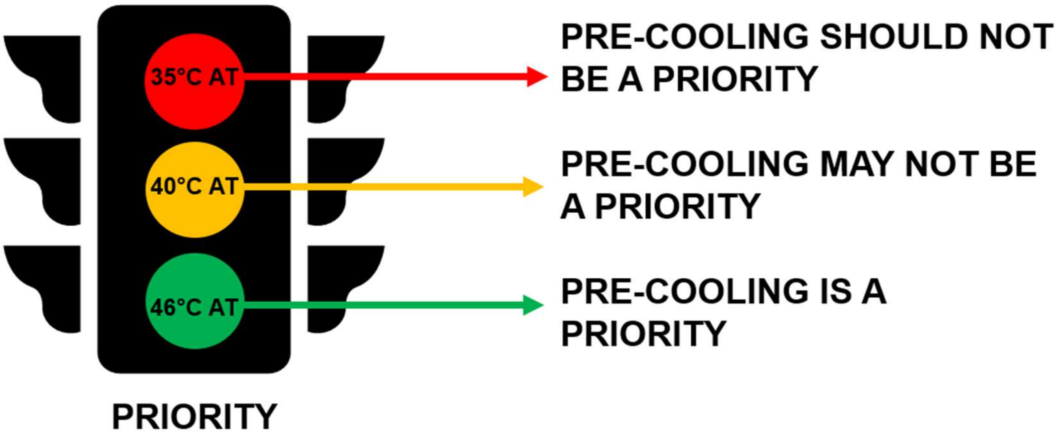

Figure 8.1. Priority scale for the utilisation of mixed-method pre-cooling at different apparent temperatures (AT).

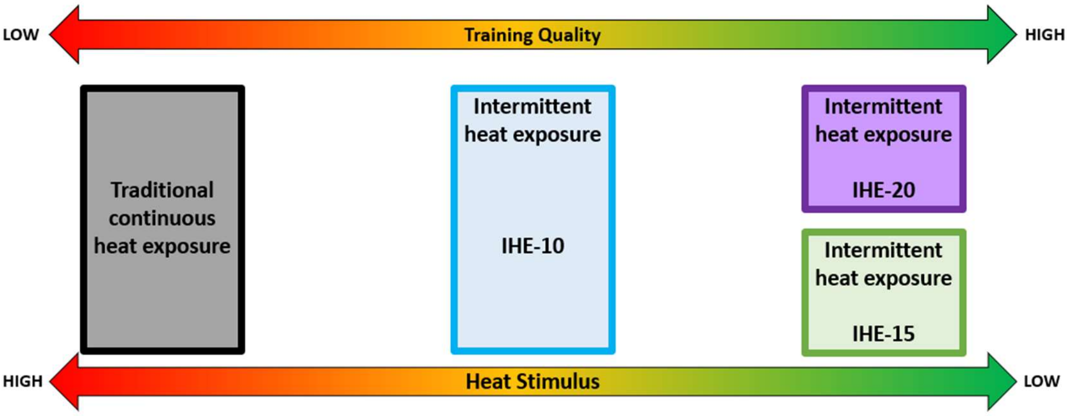

Figure 8.2. Priority scale for the utilisation of traditional continuous heat exposure training or different intermittent heat-exposure (IHE) training set structures: 3 x 20 min exercise with 7.5 min rest between sets (IHE-20), 4 x 15 min exercise with 5 min rest (IHE-15), and 6 x 10 min exercise with 3 min rest (IHE-10).

8.7 Conclusion

In conclusion, we have presented evidence that cooling may be beneficial to exercise performance and HA programs. When applied in hot-humid conditions, mixed-method PreC provides the largest benefit to endurance performance. Additionally, when we combine both HA and cooling interventions in a single session, we observe improved within-session exercise intensity, without compromising the HA stimulus and acute physiological responses to the heat. However, when applied over a full HA protocol, no evidence of physiological adaptation was observed, but exercise performance was maintained compared to HA alone.

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Appendices

Appendix A: Chapter Two Ethics Approval

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Appendix B: Chapter Two Published Article Cover Page

International Journal of Sports Physiology and Performance, **(Ahead of Print)**
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Human Kinetics
ORIGINAL INVESTIGATION

Mixed-Method Precooling Enhances Self-Paced 20-km Cycling Time-Trial Performance When Apparent Temperature Is >46 °C but May Not Be a Priority in <46 °C

Julian Andro P. Ramos,[1] Kagan J. Ducker,[1] Hugh Riddell,[1] Olivier Girard,[2] Grant J. Landers,[2] and Carly J. Brade[1]

[1]Curtin School of Allied Health, Curtin University, Perth, Australia; [2]School of Human Sciences (Exercise and Sport Science), University of Western Australia, Perth, Australia

Purpose: Precooling (PreC) may only benefit performance when thermal strain experienced by an individual is sufficiently high. We explored the effect of mixed-method PreC on 20-km cycling time-trial (CTT) performance under 3 different apparent temperatures (AT). ***Methods:*** On separate days, 12 trained or highly trained male cyclists/triathletes completed six 20-km CTTs in 3 different ATs: hot-dry (35 °C AT), moderately hot-humid (40 °C AT), and hot-humid (46 °C AT). All trials were preceded by 30 minutes of mixed-method PreC or no PreC (control [CON]). ***Results:*** Faster 2.5-km-split completion times occurred in PreC compared with CON in 46 °C AT (*P* = .02), but not in 40 °C AT (*P* = .62) or 35 °C AT (*P* = .57). PreC did not affect rectal and body temperature during the 20-km CTT. Skin temperature was lower throughout the CTT in PreC compared with CON in 46 °C AT (*P* = .01), but not in 40 °C AT (*P* = 1.00) and 35 °C AT (*P* = 1.00). Heart rate had a greater rate of increase during the CTT for PreC compared with CON in 46 °C AT (*P* = .01), but not in 40 °C AT (*P* = .57) and 35 °C AT (*P* = 1.00). Ratings of perceived exertion (*P* < .001) and thermal comfort (*P* = .04) were lower for PreC compared with CON in 46 °C AT only, while thermal sensation was not different between PreC and CON. ***Conclusion:*** Mixed-method PreC should be applied prior to 20-km CTTs conducted in hot-humid conditions (≥46 °C AT). Alternatively, mixed-method PreC may be a priority in moderately hot-humid (∼40 °C AT) conditions but should not be in hot-dry (∼35 °C AT) conditions for 20-km CTT.

Keywords*:* cooling, heat stress, self-paced exercise

Worldwide, competitive endurance events regularly take place in hot-dry (≥30 °C, ≤40% relative humidity [RH]) and hot-humid (≥30 °C, >40% RH) conditions. Compared with thermoneutral conditions (20 °C–25 °C), competing in hot-dry and hot-humid environmental conditions is often associated with slower finish times, lower sustained power output, and increased risk of exertional heat illness.[1–3]

Precooling (PreC) techniques, including external cooling (eg, cooling vest), internal cooling (eg, ice ingestion), or a combination of both (mixed-method), have been used to combat the negative effects of heat, subsequently improving exercise performance.[4] Mixed-method PreC is considered more effective in maintaining or improving exercise performance in the heat (7.3% improvement, effect size = 0.72) when compared with external (6.5%, effect size = 0.49) or internal (6.3%, effect size = 0.40) methods alone.[4] Studies show that improvements in endurance exercise performance (∼30–90 min) after PreC are observed in environmental conditions exceeding 27 °C and RH above 50%.[5–7] Conversely, no performance improvement is observed in less thermally challenging conditions (≤24 °C, ≤68% RH; ∼55–60 min).[6,7] Due to high variability in ambient temperatures and RH used in PreC literature, comparisons are difficult when 2 environmental constructs need considering and overlook how that environment feels.

Apparent temperature (AT) represents temperature in terms of how it is perceived using both RH and ambient temperature (eg, 40 °C, 40% RH feels like 46 °C).[8] Therefore, using AT improves our understanding of the thermal conditions required for PreC to improve exercise performance. For instance, a study by Faulkner et al[6] explored the effects of PreC (30-min cooling vest + sleeve) on a ∼60-minute cycling time trial (CTT) across varying temperatures. They showed faster CTT completion time under the hottest condition (35 °C, 50% RH), while no improvement was observed under the coolest condition (24 °C, 50% RH). Using AT unveils ∼16 °C AT difference between the hottest (41 °C AT) and coolest (25 °C AT) conditions compared with an absolute 11 °C air temperature difference. This outcome is unsurprising, considering that heightened RH, alongside elevated temperatures, can exacerbate thermal strain as the impaired evaporation of sweat limits heat loss due to low water vapor pressure difference between the environment and skin.[9] Consequently, several factors such as cardiovascular function (increased skin blood flow and decreased stroke volume), central neural drive (heightened perception of effort), and skeletal muscle function and metabolism (increased carbohydrate oxidation) are adversely impacted.[3]

Researchers have yet to determine the specific AT conditions necessary for mixed-method PreC to have the largest positive effect on maximal, self-paced endurance cycling performance. Therefore, we aimed to investigate the effect of mixed-method

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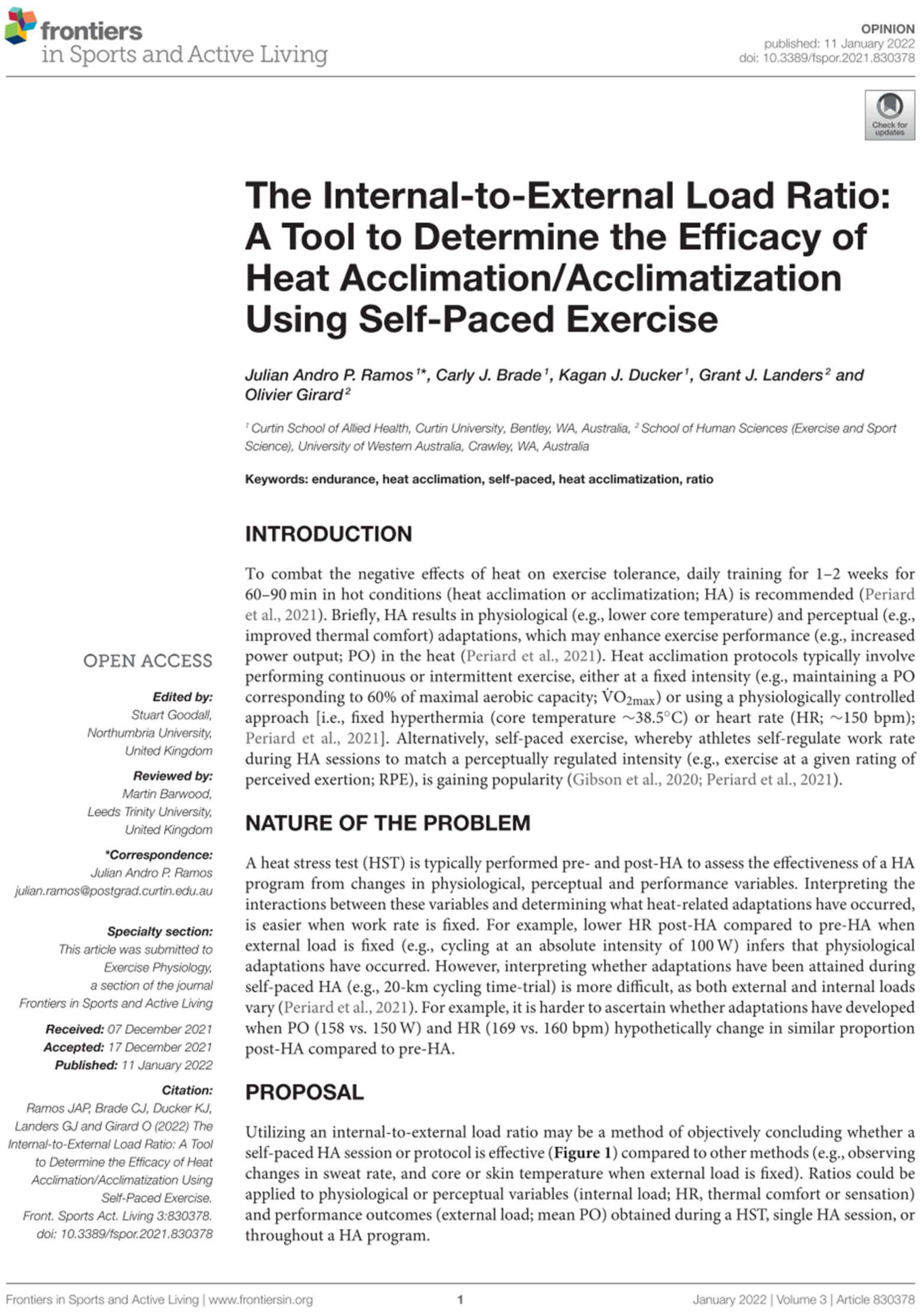
Ducker https://orcid.org/0000-0002-4251-649X
Riddell https://orcid.org/0000-0001-8218-7822
Girard https://orcid.org/0000-0002-4797-182X
Landers https://orcid.org/0000-0002-2679-4342
Brade https://orcid.org/0000-0001-9341-1412
Ramos (julian.ramos@postgrad.curtin.edu.au) is corresponding author, https://orcid.org/0000-0002-6000-4156

Appendix C: Chapter Seven Published Article Cover Page



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OPINION
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The Internal-to-External Load Ratio: A Tool to Determine the Efficacy of Heat Acclimation/Acclimatization Using Self-Paced Exercise

Julian Andro P. Ramos[1]*, *Carly J. Brade*[1], *Kagan J. Ducker*[1], *Grant J. Landers*[2] *and Olivier Girard*[2]

[1] *Curtin School of Allied Health, Curtin University, Bentley, WA, Australia,* [2] *School of Human Sciences (Exercise and Sport Science), University of Western Australia, Crawley, WA, Australia*

Keywords: endurance, heat acclimation, self-paced, heat acclimatization, ratio

INTRODUCTION

To combat the negative effects of heat on exercise tolerance, daily training for 1–2 weeks for 60–90 min in hot conditions (heat acclimation or acclimatization; HA) is recommended (Periard et al., 2021). Briefly, HA results in physiological (e.g., lower core temperature) and perceptual (e.g., improved thermal comfort) adaptations, which may enhance exercise performance (e.g., increased power output; PO) in the heat (Periard et al., 2021). Heat acclimation protocols typically involve performing continuous or intermittent exercise, either at a fixed intensity (e.g., maintaining a PO corresponding to 60% of maximal aerobic capacity; $\dot{V}O_{2max}$) or using a physiologically controlled approach [i.e., fixed hyperthermia (core temperature ~38.5°C) or heart rate (HR; ~150 bpm); Periard et al., 2021]. Alternatively, self-paced exercise, whereby athletes self-regulate work rate during HA sessions to match a perceptually regulated intensity (e.g., exercise at a given rating of perceived exertion; RPE), is gaining popularity (Gibson et al., 2020; Periard et al., 2021).

NATURE OF THE PROBLEM

A heat stress test (HST) is typically performed pre- and post-HA to assess the effectiveness of a HA program from changes in physiological, perceptual and performance variables. Interpreting the interactions between these variables and determining what heat-related adaptations have occurred, is easier when work rate is fixed. For example, lower HR post-HA compared to pre-HA when external load is fixed (e.g., cycling at an absolute intensity of 100 W) infers that physiological adaptations have occurred. However, interpreting whether adaptations have been attained during self-paced HA (e.g., 20-km cycling time-trial) is more difficult, as both external and internal loads vary (Periard et al., 2021). For example, it is harder to ascertain whether adaptations have developed when PO (158 vs. 150 W) and HR (169 vs. 160 bpm) hypothetically change in similar proportion post-HA compared to pre-HA.

PROPOSAL

Utilizing an internal-to-external load ratio may be a method of objectively concluding whether a self-paced HA session or protocol is effective (**Figure 1**) compared to other methods (e.g., observing changes in sweat rate, and core or skin temperature when external load is fixed). Ratios could be applied to physiological or perceptual variables (internal load; HR, thermal comfort or sensation) and performance outcomes (external load; mean PO) obtained during a HST, single HA session, or throughout a HA program.

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Edited by:
Stuart Goodall,
Northumbria University,
United Kingdom

Reviewed by:
Martin Barwood,
Leeds Trinity University,
United Kingdom

Correspondence:
Julian Andro P. Ramos
julian.ramos@postgrad.curtin.edu.au

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Appendix D: Chapter Three Ethics Approval

****REDACTED IN PUBLIC VERSION****

Appendix E: Chapter Four and Five Ethics Approval

****REDACTED IN PUBLIC VERSION****

Appendix F: Chapter Two Multilevel Modelling Code

Variables		Code
Between-person	**Urine Specific Gravity**	• Linear mixed-effects model **Analysis Name** <- lmer(**Variable**~ Condition * Intervention + (1|Participant), data=data) summary(**Analysis Name**) • Intraclass correlation coefficient TestModel <- lmer(**Variable** ~ (1 | Participant), control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')), data=**Variable Dataset**) RandomEffects <- as.data.frame(VarCorr(TestModel)) ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4]) print(ICC_between)
	Total Fluid Ingested	
	Sweat Loss	
	Total Time	
Pre-cooling Period	**Heart Rate**	• Linear mixed-effects model **Analysis Name** <- lmer(value ~ time * Condition * Intervention + BL_HR0 + (1|Participant), data=**Variables Dataset**) summary(**Analysis Name**) • Post-hoc analysis (3-way interaction effects) emtrends(**Analysis Name**, pairwise ~ Intervention*Condition, var="time")
	Rectal Temperature	
	Mean Skin Temperature	

	Body Temperature	- Post-hoc analysis (2-way interaction effects) *emtrends(**Analysis Name**, pairwise ~ Intervention,var="time")* - Intraclass correlation coefficient *TestModel <- lmer(value ~ (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Thermal Comfort	
	Thermal Sensation	
20-km Cycling Time-trial	**Heart Rate**	- Linear mixed-effects model ***Analysis Name** <- lmer(value ~ time * Condition * Intervention + BL_HR0 +* *(1|Participant), data=**Variables Dataset**)* *summary(**Analysis Name**)* - Post-hoc analysis (3-way interaction effects) *emtrends(**Analysis Name**, pairwise ~ Intervention*Condition, var="time")* - Post-hoc analysis (2-way interaction effects) *emtrends(**Analysis Name**, pairwise ~ Intervention,var="time")* *emtrends(**Analysis Name**, pairwise ~ Condition,var="time")*
	Rectal Temperature	
	Mean Skin Temperature	
	Body Temperature	
	Thermal Comfort	

	Thermal Sensation	• Intraclass correlation coefficient *TestModel <- lmer(value ~ (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Rating of Perceived Exertion	

Appendix G: Chapter Three Multilevel Modelling Code

Variables		Code
Between-person	**Urine Specific Gravity**	• Linear mixed-effects model ***Analysis Name <-*** *lmer(**Variable** ~ Condition + (1|Participant), data=**Variable Dataset**)* *summary(**Analysis Name**)* • Intraclass correlation coefficient *TestModel <- lmer(**Variable** ~ (1 | Participant), control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')), data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Total Fluid Ingested	
	Sweat Loss	
	Total Distance	
	Time above 38.5°C Core Temperature	
Exercise Intervals	**Heart Rate**	• Linear mixed-effects model ***Analysis Name*** *<- lmer(value ~ Time * Condition + (1|Participant), data=**Variables Dataset**)* *summary(**Analysis Name**)* • Post-hoc analysis (2-way interaction effects) *emtrends(**Analysis Name**, pairwise ~ Intervention,var="time")* *emtrends(**Analysis Name**, pairwise ~ Condition,var="time")*
	Core Temperature	
	Mean Skin Temperature	
	Body Temperature	

	Thermal Comfort	• Intraclass correlation coefficient *TestModel <- lmer(value ~ (1 | Participant), control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')), data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Thermal Sensation	
	Rating of Perceived Exertion	
Rest Interval	**Heart Rate**	• Linear mixed-effects model ***Analysis Name** <- lmer(value ~ time * Condition * Intervention + BL_HR0 + (1|Participant), data=**Variables Dataset**)* *summary(**Analysis Name**)* • Post-hoc analysis (2-way interaction effects) *emtrends(**Analysis Name**, pairwise ~ Intervention,var="time")* *emtrends(**Analysis Name**, pairwise ~ Condition,var="time")* • Intraclass correlation coefficient *TestModel <- lmer(value ~ (1 | Participant), control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')), data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Core Temperature	
	Mean Skin Temperature	
	Body Temperature	
	Thermal Comfort	
	Thermal Sensation	

Appendix H: Chapter Four Bayesian Analysis Code

Variables		Code
Between-person	**Urine Specific Gravity**	• Informative priors based on previous research/meta-analyses ***Prior(Intervention)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior(Condition)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names*** *<- c(set_prior(**Prior(Intervention)**, class = "b", coef = "InterventionPreC"),* *set_prior(**Prior(Condition)**, class = "b", coef = "ConditionPostHA"))* • Bayesian analysis model ***Bayesian model*** *<- bf(Value ~ Condition * Intervention + (1+Condition+Intervention|Participant))* ***Bayesian Analysis Name*** *<- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)* • Intraclass correlation coefficient *TestModel <- lmer(**Variable ~** (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Total Fluid Ingested	
	Sweat Loss	
	Total Time	

Pre-cooling Period	**Heart Rate**	- Informative priors based on previous research/meta-analyses ***Prior(Time)** <- "normal(**Mean**, **Standard Deviation**)"* ***Prior(Intervention)** <- "normal(**Mean**, **Standard Deviation**)"* ***Prior(Condition)** <- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names** <- c(set_prior(**Prior(Time)**, class = "b", coef = "Time"),* *set_prior(**Prior(Intervention)**, class = "b", coef = "ConditionPostHA"),* *set_prior(**Prior(Condition),** class = “b”, coef = “ConditionPostHA”))* - Bayesian analysis model ***Bayesian model** <- bf(Value ~ Condition * Intervention + (1+Time+Condition+Intervention|Participant))* ***Bayesian Analysis Name** <- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)* - Intraclass correlation coefficient *TestModel <- lmer(**Variable** ~ (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Core Temperature	
	Mean Skin Temperature	
	Body Temperature	
	Core-to-skin temperature gradient	
	Thermal Comfort	
	Thermal Sensation	
20-km Cycling Time-trial	**Heart Rate**	- Informative priors based on previous research/meta-analyses ***Prior(Time)** <- "normal(**Mean**, **Standard Deviation**)"*

	Core Temperature	***Prior(Intervention)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior(Condition)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names*** *<- c(set_prior(**Prior(Time)**, class = "b", coef = "Time"),* *set_prior(**Prior(Intervention)**, class = "b", coef = "ConditionPostHA"),* *set_prior(**Prior(Condition),** class = “b”, coef = “ConditionPostHA”))* • Bayesian analysis model ***Bayesian model*** *<- bf(Value ~ Condition * Intervention + (1+Time+Condition+Intervention|Participant))* ***Bayesian Analysis Name*** *<- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)* • Intraclass correlation coefficient *TestModel <- lmer(**Variable ~** (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Mean Skin Temperature	
	Body Temperature	
	Core-to-skin temperature gradient	
	Thermal Comfort	
	Thermal Sensation	
	Rating of Perceived Exertion	

Appendix I: Chapter Five Bayesian Analysis Code

Variables		Code
Between-person	**Urine Specific Gravity**	• Informative priors based on previous research/meta-analyses ***Prior(Condition)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names*** *<- set_prior(**Prior(Condition)**, class = "b", coef = "ConditionHA10")* • Bayesian analysis model ***Bayesian model*** *<- bf(Value ~ Condition + (1+Condition|Participant))* ***Bayesian Analysis Name*** *<- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)* • Intraclass correlation coefficient *TestModel <- lmer(**Variable** ~ (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Total Fluid Ingested	
	Sweat Loss	
	Total Distance	
	Time above 38.5°C Core Temperature	
	Change in Plasma Volume	• Informative priors based on previous research/meta-analyses ***Prior(Condition)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names*** *<- set_prior(**Prior(Condition)**, class = "Intercept")*

		• Bayesian analysis model ***Bayesian model*** *<- bf(Value ~ 1)* ***Bayesian Analysis Name*** *<- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)* • Intraclass correlation coefficient *TestModel <- lmer(**Variable** ~ (1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=**Variable Dataset**)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
Exercise Interval	**Heart Rate**	• Informative priors based on previous research/meta-analyses ***Prior(Time)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior(Condition)*** *<- "normal(**Mean**, **Standard Deviation**)"* ***Prior Names*** *<- c(set_prior(**Prior(Time)**, class = "b", coef = "Time"),* *set_prior(**Prior(Condition)**, class = "b", coef = "ConditionHA10"))* • Bayesian analysis model ***Bayesian model*** *<- bf(Value ~ Time * Condition + (1+Time+Condition|Participant))* ***Bayesian Analysis Name*** *<- brm(**Bayesian model**, prior = **Prior Names**, data = **Variables Dataset**, chains = 2, cores = 4)* *summary(**Bayesian Analysis Name**)*
	Core Temperature	
	Mean Skin Temperature	
	Body Temperature	

<table>
<tr><td rowspan="4"></td><td>Core-to-skin temperature gradient</td><td rowspan="4">• Intraclass correlation coefficient

<i>TestModel <- lmer(Variable ~ (1 | Participant),
control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),
data=Variable Dataset)
RandomEffects <- as.data.frame(VarCorr(TestModel))
ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])
print(ICC_between)</i></td></tr>
<tr><td>Thermal Comfort</td></tr>
<tr><td>Thermal Sensation</td></tr>
<tr><td>Rating of Perceived Exertion</td></tr>
<tr><td rowspan="4">Rest Interval</td><td>Heart Rate</td><td rowspan="4">• Informative priors based on previous research/meta-analyses

<i>Prior(Time) <- "normal(Mean, Standard Deviation)"
Prior(Condition) <- "normal(Mean, Standard Deviation)"

Prior Names <- c(set_prior(Prior(Time), class = "b", coef = "Time"),
set_prior(Prior(Condition), class = "b", coef = "ConditionHA10"))</i>

• Bayesian analysis model

<i>Bayesian model <- bf(Value ~ Time * Condition + (1+Time+Condition|Participant))</i></td></tr>
<tr><td>Core Temperature</td></tr>
<tr><td>Mean Skin Temperature</td></tr>
<tr><td>Body Temperature</td></tr>
</table>

	Core-to-skin temperature gradient	***Bayesian Analysis Name*** *<- brm(****Bayesian model****, prior =* ***Prior Names****, data =* ***Variables Dataset****, chains = 2, cores = 4)* *summary(****Bayesian Analysis Name****)* - Intraclass correlation coefficient *TestModel <- lmer(****Variable ~*** *(1 | Participant),* *control = lmerControl(optimizer ='optimx', optCtrl=list(method='L-BFGS-B')),* *data=****Variable Dataset****)* *RandomEffects <- as.data.frame(VarCorr(TestModel))* *ICC_between <- RandomEffects[1,4]/(RandomEffects[1,4]+RandomEffects[2,4])* *print(ICC_between)*
	Thermal Comfort	
	Thermal Sensation	