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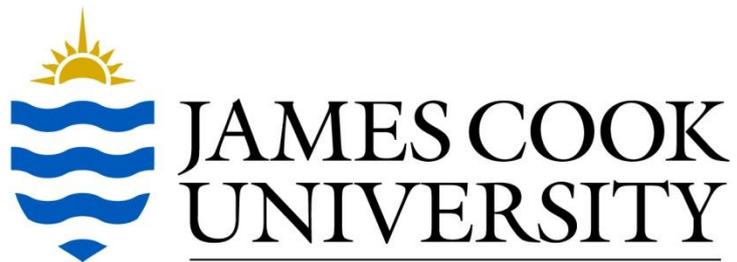
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JAMES COOK
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Short-Term Heat Acclimation Training: Effects on Performance and Inflammation

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List of publications and contributions of others

Journal Articles

Guy, J. H., Pyne, D. B., Deakin, G. B., Miller, C. M., & Edwards, A. M. (2016). Acclimation training improves endurance cycling performance in the heat without inducing endotoxemia. *Frontiers in Physiology, 7*, 318. doi: 10.3389/fphys.2016.00318.

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Conference Presentations

Guy, J. H., Edwards, A. M., Miller, C. M., Deakin, G. B., & Pyne, D. B. (7-9 September, 2016). Immediate post-exercise cooling following heat acclimation training improves cycling performance. SSEPAT conference proceedings, pg 22 (Townsville, Australia).

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Guy, J. H., Edwards, A. M., Miller, C. M., Deakin, G. B., & Pyne, D. B. (2-5 July, 2014). Reliability of serum biomarkers associated with heat stress, inflammation and immunosuppression in healthy, tropically acclimatised, active individuals. ECSS conference proceedings (Amsterdam, Netherlands).

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Statement on the contribution of others including financial and editorial help

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Intellectual Support	Proposal writing	Professor Andrew Edwards (University of St Mark & St John)
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		Dr Kate Miller (JCU)
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Chapter No.	Details of publication(s) on which chapter is based	Nature and extent of the intellectual input of each author, including the candidate
Three	Guy, J. H., Deakin, G. B., Edwards, A. M., Miller, C. M., & Pyne, D. B. (2015). Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. *Sports Medicine,* 45(3), 303-311. doi: 10.1007/s40279-014-0277-4	Guy developed the research question in conjunction with the co-authors. Guy collected the data for IAAF race performance as well as collected and synthesised relevant research papers for review. Guy undertook the primary data analysis and interpretation and wrote the first draft of the paper that was revised with editorial input from Edwards, Pyne, Deakin, and Miller.
Four	Guy, J. H., Edwards, A. M., Miller, C. M., Deakin, G. B., & Pyne, D. B. (2016). Short-term reliability of inflammatory mediators and response to exercise in the heat. *Journal of Sports Sciences*, 1-7. doi: 10.1080/02640414.2016.1227464	Guy developed the research question in conjunction with the co-authors. Guy collected the data and analysed the biomarkers with the assistance of Miller. Guy undertook the primary data analysis and interpretation and wrote the first draft of the paper that was revised with editorial input from Edwards, Pyne, Deakin, and Miller.
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I declare that all persons whom have provided sufficient contribution to this thesis have been included as co-authors or have been acknowledged in published papers or papers currently under review in peer-reviewed journals.

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Declaration on ethics

The research presented and reported in this thesis was conducted in accordance with the research guidelines of the *WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI – Ethical Principles for Medical Research Involving Human Subjects (1997)*, the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The research methodology and protocols of each study in the thesis received clearance from the James Cook University Experimentation Ethics Review Committee (H5122 and H5647).

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Abstract

Background

Extreme environmental conditions pose diverse challenges to the event preparation and competitive practices of athletes. In particular, uncompensable heat stress, whether experienced either passively or in response to exercise in the heat, influences a complex network of thermoregulatory, immune and inflammatory processes. However, there are complexities over the accurate assessment of responses to hot conditions due to factors such as the variability of blood biomarker concentrations associated with heat stress and inflammation. These may present difficulties when trying to characterise physiological responses to exercise and training in the heat. Furthermore, while the intention of endurance athletes may be to rapidly gain meaningful physical adaptation via short-term heat training prior to competition, it is currently unclear whether or not this condensed, intense process also presents an overt, acute challenge to the immune system. In addition, factors such as residual, accumulative fatigue as a consequence of intense, short duration heat training, have yet to be addressed. Therefore, interventions that may facilitate both rapid adaptation to the heat, coupled with reduced fatigue sensations and rapid recovery could be attractive. Consequently, post-exercise recovery interventions such as rapid-cooling following exercise in the heat could be a useful stratagem to minimise potential adverse residual effects of heat training if they do not compromise the adaptation process to a hot environment. These problems have been addressed through four separate research projects.

Common Methods

Study One employed a two group design (exercise and non-exercise control) with participants being sampled at different time points. Studies Two, Three and Four employed a randomised control trial design with multiple groups in each study (e.g. intervention and control). The participants recruited for this thesis were recreationally active males aged 18-30 years and all exercise was performed on a cycle ergometer. The heat stress test (HST) utilised was the same for all studies and comprised three x sub-maximal intervals (50%, 60%, and 70% of power output associated with VO_2 max) followed by a 5 km time trial (TT) on a cycle ergometer. The HST lasted for ~60 min and was performed in an environmental chamber at a temperature of 35 °C and 70% relative humidity. Venous blood samples were drawn at rest (10 min) in a seated position from a prominent superficial forearm vein and serum biomarker concentrations analysed in duplicate with commercial immunoassay kits.

Study One – Biomarker Reliability

Aims: To examine the biological variation and reliability of blood biomarkers that are associated with heat stress and inflammation at rest and in response to a strenuous cycling task in a hot and humid environment.

Method: The short-term reliability (at rest, seven days apart) and the acute responsiveness of each biomarker to a single HST was evaluated (Subject n=32). Serum was analysed for the concentration of C-reactive protein (CRP), interleukin-6 (IL-6), extracellular heat shock protein 72 (eHSP72), immunoglobulin M (IgM) and lipopolysaccharide (LPS). Test-retest reliability was determined as the coefficient of variation (CV).

Results: Biomarkers with the least short-term within-subject variation were IL-6 (19%, ±20%; CV, ±95% confidence limits) and LPS (23%, ±13%). Greater variability was observed for IgM, eHSP72 and CRP (CV range 28-38%). IL-6 concentration exhibited the largest increase in response to acute exercise (550%, ±158%, p = <0.001, percent change, ±95% confidence limits) and, although CRP concentration had a modest CV (12%, ±7%), it increased significantly post-exercise by 21% ±16% (p = 0.02). In contrast, eHSP72 and LPS exhibited trivial changes post-exercise.

Conclusions: Variation of common inflammatory markers after exercise in the heat is not always discernible from short-term (weekly) variation.

Study Two – Reponses to Exercise in the Heat between Tropical and Temperate Residents

Aims: To compare the physiological and inflammatory responses of tropical and temperate residents to repeated bouts of exercise in a hot and humid environment.

Method: Tropical and Temperate participants (n=24) were recruited based on their location of residency (Cairns, Australia or Plymouth, UK, respectively) Participants undertook three HSTs seven days apart. Venous blood samples were drawn before and after each HST and serum analysed for concentrations of IL-6, LPS, and IgM. Data are presented as between-group effects.

Results: Tropical residents reported significantly lower rating of perceived exertion than Temperate (-2, ±1 units, mean, ±95% confidence limits, RPE scale 6-20) in each of the three HSTs (p = 0.03, large difference). Tropical residents exhibited a ~1.5-fold (p = 0.05) greater concentration in post-exercise concentrations of IL-6 at HST1, a ~3-fold

greater pre-exercise concentration of LPS at HST2 (p = 0.02), and a ~2-fold greater pre-exercise concentration of IgM at HST2 (p = 0.04) and HST3 (p = 0.02) than Temperate.

Conclusions: Tropical residents reported lower levels of exertion following strenuous exercise in the heat compared with temperate residents, however, these perceptions do not appear to influence performance. Background heat acclimatisation status may influence resting concentrations of IgM and LPS following exercise in the heat; however, both populations (tropical and temperate) appear to regulate these biomarkers within safe homeostatic limits

Study Three – Inflammation during Short-Term Heat Acclimation Training

Aims: To examine the inflammatory and immune effects of heat acclimation (HA) training, as well as the performance benefits associated with short-term HA (STHA), and to determine the effectiveness of periodic "top-up" sessions following the STHA.

Method: Moderately-active males (n=24) were allocated randomly to either HOT (35 °C and 70% RH); NEUTRAL (20 °C and 45% RH); or a non-exercising control group, (CON). Over the 18 day study HOT and NEUTRAL performed seven training sessions (40 min cycling at 55% of VO_2 max) and all participants completed three HSTs. HOT and NEUTRAL undertook an initial HST followed by four training sessions on consecutive days. Participants then rested for 48 h and performed their second HST. HOT and NEUTRAL then undertook three additional "top up" training sessions every third day, rested for 48 h and completed their final HST. CON completed three HSTs at the same time points as HOT and NEUTRAL but did not undertake any training. Venous blood samples were collected before and after each HST and serum analysed for IL-6, IgM and LPS.

Results: Both HOT and NEUTRAL groups experienced substantial improvement in 5 km TT performance (HOT -33, ±20 s, p = 0.02, NEUTRAL -39, ±18 s, p = 0.01, mean, ±95% confidence limits) but only HOT were faster (-45, ±25 s and -12, ±7 s, p = 0.01) in HST3 compared with baseline and HST2. IL-6 was elevated ~4 fold after exercise for all groups, however, there were no significant changes for IgM or LPS.

Conclusions: Short- and medium-term heat acclimation training consisting of ~60 min of heat exposure exercising at ~55% of VO_2 max does not appear to pose a substantial threat to the immune system or invoke endotoxemia in healthy, recreationally active males. Additional "top up" training every three days further improves cycling time trial performance in hot conditions compared to short-term heat training only.

Study Four – Post-Exercise Cooling following Heat Acclimation Training

Aims: To examine the effect of rapid whole-body cooling as a means of recovery during STHA training.

Method: Twenty four moderately trained males were allocated to either whole-body cooling (WBC) or passive recovery control (PRC) training groups. Both WBC and PRC undertook a VO_2 max and time-to-exhaustion (TTE) tests on a cycle ergometer in a thermo-neutral condition (20°C, 50% relative humidity) and a HST, before and after four days HA training on a cycle ergometer. Participants in WBC received a 20 min post-exercise rapid cooling intervention that comprised of whole-body fanning (~3.6 m.s^{-1}) and ingestion of a 500 mL ice-slushy immediately following each exercise in the heat session.

Results: Following the HA training program WBC had a 4.0%, ±5.8% (mean, ±95% confidence limits) greater improvement in 5 km TT performance in hot conditions (p =

0.04), and a 30%, ±45% greater improvement in TTE performance (p = 0.03) compared with PRC . WBC also reported lower levels of fatigue compared with PRC following the HA training (6.5 ± 0.5 vs 8.5 ± 1.0 units, p <0.001, mean ± SD).

Conclusions: Recreational athletes can benefit from short-term heat acclimation training at a fixed intensity at ~55% of VO_2 max for 60 min.day^{-1} to improve exercise performance in the heat, although progressive increases in work intensity of ~5% each day are recommended to elicit greater performance and physiological adaptations. Short-term heat acclimation training is enhanced with immediate post-exercise cooling utilising an ice slushy (7 g.kg.bw^{-1}) and whole body fanning (3.6 m.s^{-1}) to improve performance, enhance physiological adaptations and ameliorate accumulated fatigue that can occur from a high frequency heat acclimation program.

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List of abbreviations and acronyms

ANOVA	analysis of variance
ANCOVA	analysis of covariance
CL	confidence limits
CRP	C-reactive protein
CV	coefficient of variation
eHSP70	extracellular heat shock protein 70
ELISA	enzyme-linked immunosorbent assay
ES	effect size
HA	heat acclimation
HR	heart rate
HST	heat stress test
IAAF	International Association of Athletics Federations
IgM	immunoglobulin M
IL-6	interleukin-6
LPS	lipopolysaccharide
MTHA	medium-term heat acclimation (8-14 days)
PRC	passive recovery control
PV	plasma volume
RH	relative humidity
RPE	rating of perceived exertion
SD	standard deviation
STHA	short-term heat acclimation (≤ 7 days)
T_c	core temperature
TT	time trial
TTE	time to exhaustion
WBC	whole body cooling
$VO_2\,max$	maximal oxygen uptake

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1. Introduction

1.1 Background

Exercise in the heat results in major alterations in cardiovascular, thermoregulatory, and metabolic functions (Hargreaves 2008). Both active and passive Short- and medium-term heat acclimation (HA) training protocols are widely used by endurance and team sport athletes to increase both heat tolerance and subsequent competitive performances in hot conditions via systematic exposure to a simulated hot environment (Périard, Racinais, & Sawka, 2015). This systematic approach differs to heat acclimatisation whereby athletes may live in a naturally hot environment and experience similar beneficial adaptations. Although favourable performance and physiological benefits can be realised from short-term programs (≤7 days), greater benefits are likely from longer programs (7-14 days) (Daanen, Jonkman, Layden, Linnane, & Weller, 2011; Guy, Deakin, Edwards, Miller, & Pyne, 2015; Lorenzo, Halliwill, Sawka, & Minson, 2010; Nielsen, Strange, Christensen, Warberg, & Saltin, 1997). For elite athletes, busy training and performance schedules limit the time available for strategies such as HA training. Athletes in these situations may also benefit from supplementary "top-up" training sessions to sustain and/or complement the initial adaptations. As these types of multi-day training programs also result in significant levels of muscular fatigue (Wingfield, Gale, Minett, Marino, & Skein, 2016) it is also important to consider athlete recovery throughout HA training.

Considerations during HA training not only include thermoregulatory adaptations such as reductions in exerting heart rate, core and skin temperature as well as increases in performance, but also the inflammatory adaptations and responses. The acute effects of short-term heat exposure on blood biomarkers associated with inflammation have

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been reported (Gill et al., 2015; Shing et al., 2014); however, few studies have investigated the effects of longer duration heat training. The human immune system can usually deal with mild-to-moderate inflammatory responses. When a heat stimulus is too large however, systemic inflammation can result in heat stress and heat shock (Bosenberg, Brock-Utne, Gaffin, Wells, & Blake, 1988; Bouchama et al., 2007). Athletes will generally seek a heat training stimulus that is large enough to evoke a training adaptation; however, there likely comes a point where the risk of clinical or sub-clinical levels of immune disturbance increases.

Due to exercise in the heat inducing greater demands on the metabolic and thermoregulatory system, hyperthermia is likely to occur when there is sustained increases in core temperature approaching 40 $^{\circ}$C (Hargreaves 2008). This increased core temperature during strenuous exercise in the heat can result in exercise-induced endotoxaemia, which is primarily attributed to translocation of lipopolysaccharide (LPS) from the gut into the circulation (Lim et al., 2009). An abundance of circulating LPS can evoke an inflammatory response, leading to heat shock and overwhelming anti-LPS mechanisms including immunoglobulin M (IgM) (Camus et al., 1998) and cytokines operating in an anti-inflammatory role such as interleukin-6 (IL-6) (Abbasi et al., 2013). Consequently, when anti-LPS mechanisms and rate of LPS clearance are inadequate to counter the heat-induced increase of LPS, endotoxaemia may ensue. This outcome could potentially occur during a period of HA training if the athlete is unable to cope with combinations of high volume, intensity, and thermal loads that may be presented.

As IgM is a key antibody in neutralising LPS (Camus et al., 1998), its concentration in circulating blood can reflect the body's response to endotoxin accumulation. Circulating concentrations of IgM concentration can increase significantly (~20%) after

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exercise in the heat (Hailes, Slivka, Cuddy, & Ruby, 2011). Higher resting values of this biomarker may signal the degree of protective capacity in the event of further challenges. Therefore, the response of IgM to exercise and training in the heat could provide meaningful insight into the degree of readiness that an athlete's immune system has to deal with heat stress or strenuous heat training.

The response of IL-6 to strenuous exercise has been well documented (Fischer, 2006), and many of these studies have quantified the response of IL-6 following exercise in the heat (Fortes et al., 2012; Morrison, Cheung, & Cotter, 2014; Selkirk, McLellan, Wright, & Rhind, 2008; Wright et al., 2013). However, few studies have investigated the longer term effect of HA training on concentrations of IL-6 (Hailes et al., 2011), and whether changes in either resting concentrations, or the response of IL-6 to exercise in the heat are affecting wider inflammatory processes associated with heat stress. This is surprising as exercise-induced heat stress and the subsequent inflammatory responses induced by LPS can be indicated by concentrations of IL-6 reaching a critical threshold (Vargas & Marino, 2014). This raises questions as to whether the current practice of short- ($\leq$ 7 days) or medium-term (8-14 days) HA training results in unsafe levels of fatigue and inflammation, or if these processes are necessary to attenuate physiological adaptation to the heat.

Recreationally-active healthy adults often participate in one-off events such as an ironman triathlon, marathon and week-long sporting events such as the Masters' Games. It also appears that the threshold for the onset of exercise-induced endotoxaemia is lower in untrained than trained individuals (Selkirk et al., 2008). As individuals seeking to use HA training as an additional training stimulus may choose either a short- or medium-term program, the training intensity, volume, frequency, or recovery should be taken into

consideration. Training programs can often be very demanding, with some studies implementing challenging protocols for their participants, e.g. 90 min of cycling for 10 consecutive days (Gibson et al., 2015). Therefore, it is prudent to account for both training load and accumulated inflammation from heat stress over the training period. As longer heat training sessions (>60 min) are likely fatiguing for recreationally-trained athletes, and can increase peripheral fatigue compared with shorter protocols (Wingfield et al., 2016), the addition of shorter and supplementary training sessions could yield similar benefits, but with lower overall stress. Both recreational and elite athletes are often time-poor in the lead up to events and may not have the ability to schedule 10-14 day HA training programs. Therefore, the implementation of initial short-term HA training followed by supplementary "top up" training to preserve their initial adaptations may provide performance and physiological benefits. As the beneficial effects of HA training are known to decay within a few days after the cessation of training (Garrett, Rehrer, & Patterson, 2011), the use of "top up" sessions can allow athletes to undertake periodic HA training, with the intention of preserving their initial adaptations.

The potential ergogenic effects of HA training may also have transferable benefits to exercise in more temperate conditions, making it an attractive short-term training option for teams or athletes seeking to gain a competitive advantage by using an intensive programme. HA can improve 1 h cycling time trial (TT) performance by 5-8%, as well as improve anaerobic threshold, VO_2 max and cardiac output at an ambient temperature of 13 °C (Lorenzo et al., 2010) and running time-to-exhaustion (TTE) by ~29% (Scoon, Hopkins, Mayhew, & Cotter, 2007). Adaptations from HA include improved plasma volume expansion, cardiac and skeletal muscle efficiency, ventricular compliance and thermoregulatory adaptations such as lower resting core temperature, increased sweating and cutaneous blood flow (Minson & Cotter, 2016). Physiological adaptations such as

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these can result in large improvements to cardiac stability, as well as moderate-to-large beneficial effects to core temperature and skin blood flow during exercise in the heat (Tyler, Reeve, Hodges, & Cheung, 2016). The suitability of HA training as an ergogenic aid to improve performance in thermo-neutral environments remains contested and unclear (Minson & Cotter, 2016). Therefore, more studies are required to further the knowledge of this intervention.

Athletes intending to supplement their training with HA sessions should also carefully consider their recovery strategies between HA sessions to ensure they are fully recovered leading into competition, while maintaining the benefits that heat exposure brings. Recovery methods following strenuous exercise include whole-body cooling such as cold water immersion (CWI), phase change garments or whole-body fanning. As HA training relies on the development of specific heat adaptations as a result of increased core and muscle temperature such as improved sweating responses, cardiac frequency and substrate utilisation (Garrett et al. 2011), it is not clear whether the rapid decrease in core temperature as a result of recovery-cooling following HA training could potentially blunt these important adaptive processes.

The use of rapid whole-body cooling by way of cold water immersion can facilitate recovery within 24 h between bouts of intermittent cycling (Lane & Wenger, 2004). A recent review suggests that the dominant mechanism by that CWI facilitates short-term recovery is via ameliorating hyperthermia and, consequently CNS-mediated fatigue, and by reducing cardiovascular strain (Ihsan, Watson, & Abbiss, 2016). However, cold water immersion is often not feasible and may only cover a small surface area compared with other cooling techniques. Use of whole-body fans reduces core temperature faster than phase change garments and CWI techniques given an increase in evaporative cooling

(Barwood, Davey, House, & Tipton, 2009). Additionally, crushed ice and ice-slushies are also a simple, effective means to reduce core temperature either pre- or post-exercise in hot conditions (Brearley, 2012; Ross et al., 2011). Although pre-exercise ice-slushy ingestion can delay the rise in core temperature associated with prolonged exercise in the heat, these types of interventions are generally utilised to benefit performance in the short-term. Similarly, recovery-cooling via ice ingestion post-exercise is a simple strategy to rapidly reduce elevated core temperature (Brearley, 2012). Therefore, ingestion of ice or ice-slushies post-exercise may be useful as a recovery tool during intense training blocks such as those experienced during HA training.

Many studies have investigated the usefulness of HA training in non-heat acclimated individuals, although it appears that tropical natives display greater signs of heat tolerance and physiological adaptations to the heat compared with their counterparts who reside in the temperate zone (Saat & Tochihara, 2008; Taylor & Cotter, 2006). However, the differences between expressions of adaptation when exposed to the heat such as resting core temperature and cardiac frequency during exercise are not always clear between people who reside in tropical and temperate zones (Wijayanto, Toramoto, Wakabayashi, & Tochihara, 2012). Furthermore, while individual differences in biomarker responses to heat stress and acclimation have been reported (Racinais et al., 2012), whether or not underlying heat acclimatisation status influences these responses is not known. Given that athletes often compete in various events around the globe. It is therefore important to understand and consider the consequences of strenuous exercise in unfamiliar environments. The comparison of inflammatory responses between recreational athletes who reside in tropical and those in temperate zones is yet to be investigated. Residing in a tropical climate is likely to influence the inflammatory and heat stress response following exercise in the heat.

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1.2 Statement of the problem

While HA training has received increased attention over the last decade, most studies have focused on the acute physiological effects immediately following exercise in the heat, or the performance benefits that HA training can provide. Very few studies have investigated inflammatory responses that can occur in consecutive day training programs that last five days or more. Importantly, studies that have considered the inflammatory responses to HA training have not taken into consideration the normal biological variation of these blood biomarkers. When determining the clinical relevance of the response of blood biomarkers to exercise in the heat and heat adaptation, these responses need to be compared with normal biological variation.

Heat acclimation training often utilises consecutive day training programs and the increased heat and training load experienced by athletes undertaking HA training could result in high levels of fatigue. However, the development of suitable recovery strategies to aid athlete recovery specifically during HA training are yet to be investigated. The implementation of rapid cooling following exercise in the heat may provide the necessary recovery required to allow athletes to undertake multi-day training programs.

There has also been little attention paid to the background acclimatisation status of athletes, and whether or not acclimatisation influences the degree of inflammation and immune responses following exercise in the heat. Recreational and elite athletes now compete in events all over the globe, therefore, the response of non-acclimatised individuals are important considerations.

1.3 Aims of the project

The project was separated into four studies with the following aims:

1. To examine the biological variation of blood biomarkers associated with heat stress and inflammation at rest and in response to a strenuous cycling task in a hot and humid environment;

2. To compare the physiological and inflammatory responses to repeated bouts of exercise in a hot and humid environment between recreational athletes who reside in the tropical and temperate zones;

3. To examine the inflammatory and immune effects of HA training by;

   a. Following a short-term HA training program;

   b. and; Following periodic "top-up" sessions following the short-term training.

4. To examine the effect of rapid whole-body cooling as a means of promoting recovery and exercise performance with HA training.

1.4 Hypotheses

It was hypothesised that:

1. Normal biological variation of blood biomarkers (noise) would be smaller than the variation observed following a 1 h strenuous cycling task in a hot environment (signal), resulting in clear "signal" to "noise" ratios.

2. Athletes who reside in a tropical climate would experience less inflammation and physiological stress during a matched cycling task in hot and humid conditions compared with those that live in the temperate zone.

3. Athletes undertaking HA are unlikely to experience significant risks to health and immune function.

4. The implementation of "top up" HA training every third day following an initial short-term HA program would result in the retention of initial physiological adaptations and cycling performance in the heat.

5. Whole-body cooling following exercise in the heat would result in enhanced recovery and improved cycling performance in a hot and thermo-neutral environment when compared with passive recovery.

1.5 Significance of the thesis

The series of studies in this thesis enhances the current knowledge around HA training, immune function and athlete health. Chapters Four and Five can inform sports scientists of the inherent biological variability of blood biomarkers and the need to determine an individualised response to exercise in hot and humid conditions, particularly for athletes who live in varying climates and geographical locations. Chapters Three, Six, and Seven show HA training and recovery strategies that can be implemented to better prepare athletes for competition. This could be accomplished by

1) understanding the degree of inflammation associated with short-term heat acclimation training, 2) determining the balance between load and overload, thereby optimising training to elicit performance adaptations without putting the athlete at risk of heat stress or illness, and 3) providing advice on recovery techniques that can be utilised during HA training to reduce fatigue and enhance performance leading into competition.

This thesis collectively provides information to coaches, athletes, and sports scientists to review and where necessary make adjustments to HA training programs and recovery strategies to maximise training adaptations. Additionally, this thesis furthers the understanding of the complex interplay of hyperthermia, endotoxemia and the immune response during strenuous exercise in the heat.

1.6 Format of the thesis

The format of this thesis is as follows:

- Chapter One provides a brief introduction of exercise and training in the heat and its effects on performance, and immune function and inflammation.
- Chapter Two is a Leading Article that establishes when elite athlete performances are affected by hot conditions, as well as a review of the physiological and performance benefits that are realised through HA training. This chapter also provides a brief review of post-exercise recovery cooling following exercise in the heat.
- Chapter Three is a review of the literature of relevant biomarkers that are associated with heat stress, inflammation, immune function and acclimation.
- Chapter Four reports the reliability of selected serum biomarkers, both at rest and following exercise in the heat, providing methodological guidance for the ensuing chapters Five and Six.

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- Chapter Five compares the physiological and inflammatory responses to strenuous exercise in hot and humid conditions between recreational athletes who reside in the tropical and temperate zone.

- Chapter Six evaluates the effects of short-term HA training on blood biomarkers relating to heat stress, inflammation, and endotoxaemia.

- Chapter Seven reports on the effectiveness of post-exercise recovery cooling and its usefulness at enhancing performance during short-term HA training.

Chapter Two is an expanded version of a "Brief Review" and Chapter Three is in its original form as a "Leading Article", both were accepted for publication in international peer-reviewed journals. Chapters Four and Six are original investigations that have been accepted for publication in international peer-reviewed journals. Chapters Five and Seven have been written as original research articles to fit with the format of the thesis.

The four studies that make up Chapters Four, Five, Six, and Seven were conducted to ensure logical progression from one study to the next. This was made possible by firstly robust investigation of selected measures and reliability testing of methodology. Furthermore, the findings of each study helped develop pertinent scientific research questions and influenced the methodology of subsequent studies.

Chapter Eight summarises and discusses the key findings of the research, identifies possibilities for future avenues of research, and concludes with a brief paragraph of the key points addressed throughout this thesis. Figure 1.1 provides a schematic outline of the thesis structure.

2. Adaptations to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes.

2.1 Abstract

Aim: Extreme environmental conditions present athletes with diverse challenges; however, not all sporting events are limited by thermoregulatory parameters. The purpose of this leading article is to identify specific instances where hot environmental conditions either compromise or augment performance and, where heat acclimation appears justified, evaluate the effectiveness of pre-event acclimation processes.

Method: To identify events likely to be receptive to pre-competition heat adaptation protocols, we clustered and quantified the magnitude of difference in performance of elite athletes competing in International Association of Athletics Federations (IAAF) World Championships (1999-2011) in hot environments (>25ºC) with those in cooler temperate conditions (<25ºC).

Results: Athletes in endurance events performed worse in hot conditions (~3% reduction in performance, Cohen's d >0.8; large impairment) while in contrast, performance in short-duration sprint events was augmented in the heat compared with cool conditions (~1% improvement, Cohen's d >0.8; large performance gain). As endurance events were identified as compromised by the heat, we evaluated common short-term heat acclimation ($\leq$ 7 days, STHA) and medium-term heat acclimation (8-14 days, MTHA) protocols. This process identified beneficial effects of heat acclimation on performance using both STHA (0.8%, ±1.2%) and MTHA protocols (6.5%, ±3.7%). These effects

were differentially greater for medium-term acclimation, which also demonstrated larger reductions in both end-point exercise heart rate (STHA: -3.9%, ±3.4% vs. MTHA: -7.6%, ±2.1%), and end-point core temperature (STHA: -0.4%, ±0.5% vs. -0.7%, ±0.3%). **Conclusion:** It appears that worthwhile acclimation is achievable for endurance athletes via both short and medium length protocols but more is gained using MTHA. Conversely, it is also conceivable that heat acclimation may be counterproductive for sprinters. As high performance athletes are often time-poor, shorter duration protocols may be of practical preference for endurance athletes where satisfactory outcomes can be achieved.

2.2 Introduction

It is popularly perceived that performance in the heat is compromised compared with thermo-neutral conditions and that pre-competition adaptation to this environment is a necessity (Ely, Cheuvront, Roberts, & Montain, 2007; Racinais et al., 2012). However, this may not be the case for all events, depending on the intensity and duration of performance. For elite athletes, there are also issues of time-efficiencies to be considered when determining event preparation within busy training and performance schedules. Therefore, although some recent articles have added some useful information on this underserved area (Garrett et al., 2011; Sawka, Wenger, & Pandolf, 2011), the purpose of this leading article is to now take this issue forward and describe instances where heat adaptation may be useful, to identify protocols that lead to meaningful adaptations and finally to suggest future directions for this important area of research.

Endurance events in particular have often been described to be compromised in the heat (Ely et al., 2007; Racinais et al., 2012). This effect is most likely mediated as an integrated thermoregulatory response associated with exposure to the heat, including increased exercising heart rate (HR), elevated core (T_c) and skin temperatures, greater perception of effort, thermal strain, thirst, and water loss leading to dehydration (for reviews see (Garrett et al., 2011; Sawka et al., 2011b). It is therefore important for athletes to prepare themselves for events that may take place in environmentally challenging conditions. This strategy is particularly important in both team sports (Racinais et al., 2012) and endurance events (Taylor, 2000), which require performances to be sustained for extended periods of time potentially increasing the likelihood of athletes developing substantial dehydration, or a potentially critical T_c (Gonzalez-Alonso, Calbet, & Nielsen, 1999). This scenario often results in fatigue, down-regulation of effort, performance impairment and, in extreme cases, heat illness (Gonzalez-Alonso

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et al., 1999). However, particular scenarios where heat-induced decrements to performance are most prevalent, and the most effective evidence-based strategies of minimising these effects, are seldom described.

Almost fifty percent of the world's population now live in the Torrid Zone, close to the Earth's equator where temperatures are hotter and more physically challenging than in the Temperate or Frigid Zones (Harding, 2011). Consequently, many major sporting events are now scheduled to be held in geographical locations that experience hot and humid environmental conditions. These locations include the 2015 International Association of Athletics Federations (IAAF) World Championships (Beijing, summer), the 2016 and 2020 Olympic Games (Brazil and Tokyo, summer), and the 2022 Fédération Internationale de Football Association (FIFA) World Cup (Qatar). It is therefore critical that competitive athletes are adequately prepared for such competitions, particularly individuals more used to living and exercising in temperate environments and unaccustomed to hot conditions. For athletes not living and regularly training in the Torrid Zone, most would likely require some form of preparatory heat training prior to embarking on competition in this region. It is often reported that 10-14 days of heat exposure (Sawka et al., 2011b) is ample heat acclimation; however, these extended interventions might not be viable for most sporting programmes. This period may be particularly challenging for time-poor high performance athletes in terms of availability, timing, training and/or logistical reasons. To combat this, there have been recent efforts to evaluate the effectiveness of shorter heat training programs of seven days or shorter duration (Chalmers et al., 2014). The priority for coaches and athletes in such cases is determining the minimum number of days of heat training needed to provide some benefit, within their busy training and performance schedules.

Both short- and medium-term heat adaptation protocols can elicit changes in important physiological parameters such as plasma volume (PV) expansion, reductions to exercising HR, T_c, and sweating commences at a lower T_c with a more dilute concentration of metabolites (Sawka, Leon, Montain, & Sonna, 2011), which that could be useful for subsequent performances in the heat and also in cool conditions where potential fluid loss is substantial (Corbett, Neal, Lunt, & Tipton, 2014). It is important to understand how these physiological changes occur, and the potential effects they have on athletes' performances. For example, an expansion of PV can promote improved performance in aerobic events, most likely by reducing plasma protein loss (Harrison, 1985; Senay, 1972) and increasing blood volume, thus mediating a decreased exercising HR in the heat through adaptive gains in central venous return and preload (Garrett et al., 2011; O'Sullivan, 2003; Sawka, Hubbard, Francesconi, & Horstman, 1983). Consequently, an increase to stroke volume mediated by gains in PV and blood volume lowers cardiac frequency (Cadarette, Sawka, Toner, & Pandolf, 1984; Shapiro, Hubbard, Kimbrough, & Pandolf, 1981). As heat adaptation increases PV, the body more effectively regulates blood pressure in the face of fluid loss as a consequence of increased levels of sweat (Taylor & Cotter, 2006). Collectively these adaptations lower HR, promote reduced thermal strain and more efficient transfer of heat (Taylor & Cotter, 2006). Therefore, as PV expansion plays an important role in extending endurance exercise performance, heat training programmes promoting greater PV expansion are of benefit. Nevertheless, this adaptive response may only be of relevance for athletes undertaking endurance events, where fluid loss and heat dissipation mechanisms play a meaningful role in race or competition performance. For example, athletes competing in events that require only short bursts of anaerobic power (e.g. 100 m sprint) are unlikely to experience a decrement in performance in hot conditions as they are under

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substantially less sustained thermal load compared with their endurance counterparts. Furthermore, when the humidity of the surrounding environment is high, athletes will have substantially greater fluid requirements compared to those that are competing in a hot and dry environment due to the reduced efficiency in evaporative cooling. Subsequently, strenuous exercise in a hot and humid environment will cause a more rapid rise in core temperature, as well as the increasing the likelihood of cramping, compared to hot and dry environments (Larose et al., 2014).

2.3 Comparison of running performances in hot and temperate conditions: IAAF track and field performances (1999-2011).

Numerous studies have examined the effects of environmental conditions on performance in controlled, isolated laboratory experiments. However, to fully ascertain whether or not environmental conditions influence elite field-based performance it is useful to consider the magnitude of change in outcomes of regularly scheduled events over a longitudinal period performed in different conditions. This type of analysis can be performed by examining secondary data from scheduled major events such as those organised by the IAAF. These data are publicly available and facilitate rapid and meaningful comparisons when appropriately clustered for analysis of data trends. To address the question of where and which events are most affected by environmental conditions we collated and analysed the mean of the top 10 performances in distance events (top 60% of track events) and top 6 performances in sprint performances (top 60%) for males and females in the 100 m, 200 m, 400 m, 800 m, 1500 m, 5,000 m, 10,000 m and marathon events from seven consecutive IAAF world championships (1999-2011). Events were categorised as either temperate (n= 41) or hot (n=44) conditions based on the heat index/humiture of that event, separated using a standardised threshold temperature of 25 ºC as an index of comfortable working temperature (Cândido, de Dear,

& Ohba, 2012). It was determined to utilise 25 °C as it further represented the full cohort (n=85) mean temperature (24.5°C) and resulted in a temperate condition mean of 18.5 °C (±1.01°C; humidity 59.6%, ±7.0%) and a hot condition mean temperature of 30 °C (±1.32 °C; humidity 61.3%, ±4.9%), which are both in range of common specifications for these conditions. Although 25 °C is a relatively high threshold temperature, outdoor exercise benefits to a greater extent from convective cooling than laboratory exercise (Saunders, Dugas, Tucker, Lambert, & Noakes, 2005). Therefore, we sought to recognise this in contrast to laboratory exercise observations where convective cooling is minimal (Galloway & Maughan, 1997).
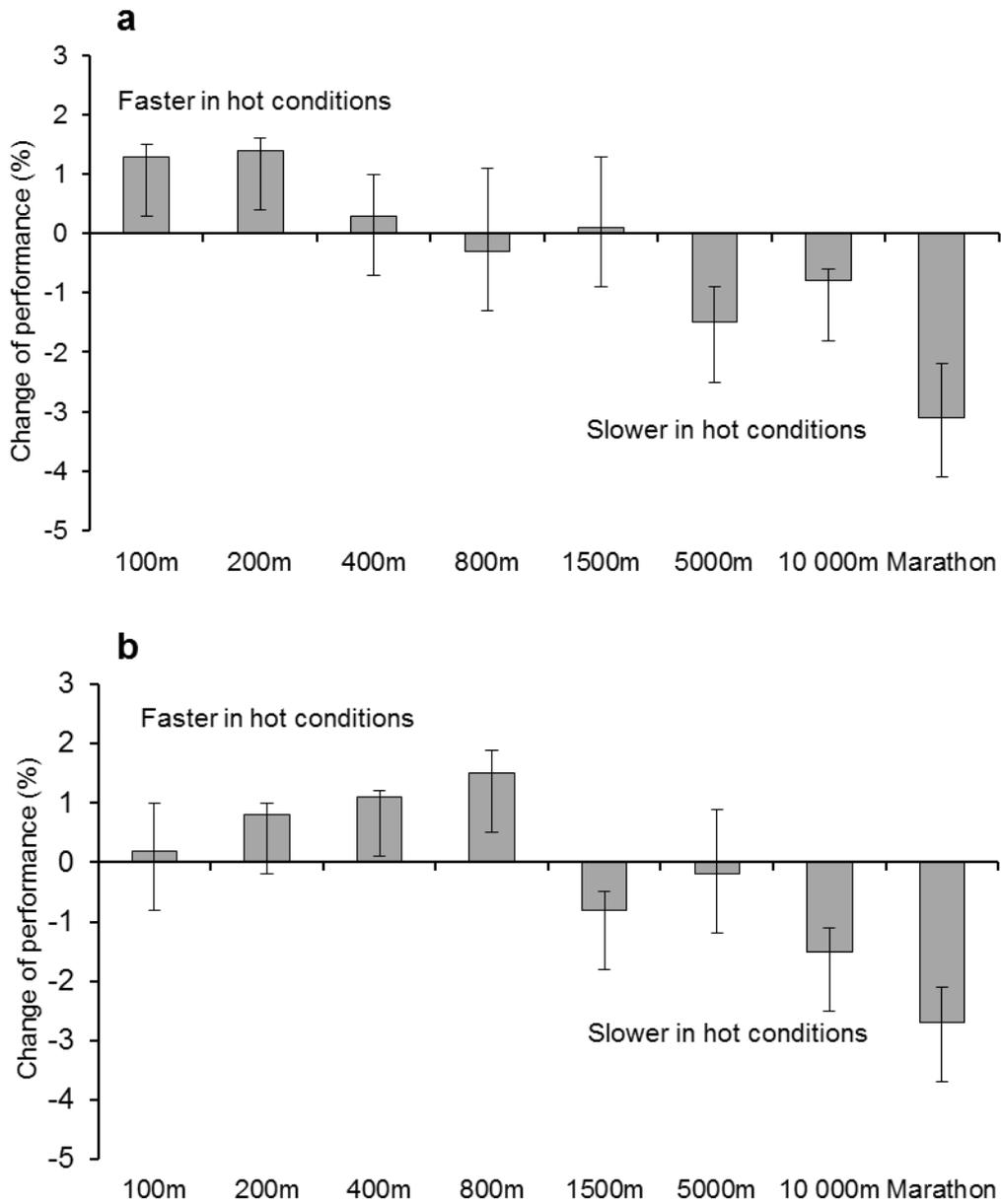
Figure 2.1 Comparative mean ± 95%CL percentage change of performance in temperate (<25°C) vs hot (≥ 25°C) conditions; from International Association of Athletics Federation World Championship track events from 1999-2011 for (a) males and (b) females. Positive percentage indicates faster performance, and negative percentage indicates slower performance in hot conditions.

Brief analysis of performances identified that the cool conditions (<25°C) resulted in faster performances in endurance events (>5,000 m) (~2% mean gain, medium effect) (Table 2.1). Conversely, the sprint events (≤ 200 m) demonstrated the opposite effect with athletes performing better in hot conditions (~2% gain, moderate to large effect)

compared to the cool (<25°C). As might be expected, middle distance events were less affected by ambient conditions and considerable variation between performance gains and losses were observed for males and females, probably due to the influence of other factors such as race tactics (Figure 2.1).

Table 2.1. **Comparative mean effect (Cohen's** d) of performance in temperate (<25°C) vs hot (**≥ 25°C**) conditions; from IAAF world championship track events from 1999-2011.

IAAF event	Men	Women
100 m	Large ↑ (2.4)	Medium ↑ (0.7)
200 m	Large ↑ (2.3)	Large ↑ (0.9)
400 m	Trivial (-0.1) ↓	Large ↑ (1.0)
800 m	Medium ↓ (-0.4)	Large ↑ (1.4)
1500 m	Medium ↑ (0.6)	Medium ↓ (-0.7)
5,000 m	Medium ↓ (-0.7)	Medium ↓ (-0.5)
10,000 m	Medium ↓ (-0.6)	Medium ↓ (-0.7)
Marathon	Large ↓ (-2.0)	Large ↓ (-2.4)

Effect sizes are reported as: trivial (<0.2), small (0.2-0.5), medium (0.5-**0.8), or large (>0.8).**↑ (positive effect) indicates faster performance, and ↓ (negative effect) indicates slower performance in hot (≥ 25°C) conditions. IAAF: International Association of Athletics Federation.

The marathon exhibited the largest performance impairment in the heat, with a reduction of 3.1% for males (also a large effect; ES = -2.0) and 2.7% mean change for females (large effect; ES = -2.4) (Table 2.1). Although inferences from this observation are limited due to absence of knowledge in relation to race tactics, it is most likely that these reductions in performance were primarily related to the ambient temperature and absolute humidity that the athletes were competing.

There are logical physiological and behavioural explanations for the differential effects of environment on performance variations in endurance and sprint events For example, in endurance events thermoregulatory response associated with exposure to the heat, including increased exercising heart rate (HR), elevated core (T_c) and skin

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temperatures, greater perception of effort, thermal strain, thirst, and water loss leading to dehydration would compromise performance (Sawka et al., 2011a). However the underlying observation that hot conditions do not necessarily compromise all events is an important consideration for athletes and coaches in their preparation for competition based in the heat. It is also important to consider that while this analysis demarcated "hot" and "temperate" events at above and below 25 °C, other studies have chosen a greater level of demarcation in environmental temperatures (Ely et al., 2007). However, this analysis also took into account the relative heat index of each event, this representation of apparent temperature was deemed most appropriate in the absence of wet bulb global temperature, rather than comparing events on dry bulb temperature alone. This information should be useful for evidence-based decisions on prescribing appropriate pre-event acclimation for endurance-type activities where performance is most likely to be impeded in the heat.

2.4 Comparison of short- and medium-term heat acclimation models

Defining the optimum length of a heat acclimation protocol will be influenced by two factors; i.e. Firstly in physiological performance terms, the number of sessions needed to attain appropriate adaptations, and secondly the practical issues of logistics related to the competition such as a one-off tournament or an ongoing seasonal competition combined with player availability. Research has primarily focused on the acute effects in response to a single stressor, or in preparation for a one-off event, with little practical recognition of preparatory time restrictions commonly experienced by athletes across a competitive season. In most sports, teams and athletes need to compete in various conditions across a season, and hot condition events might only constitute a short period within the competitive cycle (Garrett et al., 2011). As such, it is important

to consider both the acute effects of acclimation and secondary (residual) factors that might influence the magnitude and time course of benefits.

The majority of heat acclimation research has to date examined either short-term heat acclimation (≤ 7 days, STHA) (Table 2.2), or medium-term heat acclimation (8-14 days, MTHA) (Table 2.3) protocols. Clearly, for elite athletes performing in a congested competitive season, a shorter acclimation period would be advantageous and less disruptive to routine training. Therefore, we have made a brief practical comparative analysis to identify the degree of benefit derived from both STHA vs. MTHA protocols (Garrett et al., 2011). As this is a current opinion article it was not deemed necessary undertake a thorough systematic review of the literature, therefore the results that are reported should be interpreted witch that in mind. The studies that were selected for review were found through the use of the search terms heat+acclimation+acclimatisation+training via PubMed and Google Scholar in 2014. Articles were included on the basis of reporting data from before and after STHA or MTHA where the data could be easily extracted for the purposes of effect size comparisons.

Table 2.2 **Representation of studies that investigated short term heat acclimation protocols (≤7 days)**

Study/participants/design	Training status	Days/sessions	Heat training protocol	Reported outcome measures
Aoyagi et al (1994) n=16, no CON	Trained and untrained	6 d	60 min walking or running (40 °C, 30%)	Walk TTE in NBC (T: 2% ↑, UT: 2% ↑) PV (T: 1% ↑, UT: 8% ↑)
Aoyagi et al (1995) n=8, no CON	Moderately trained	6 d	60 min walking (40 °C, 30%)	Walk TTE in NBC (15% ↑), PV (7% ↑)
Brade et al (2013) n=10	Moderately trained	5 d	50 min cycling (35 °C, 60%)	Cycle work (J.kg^{-1}) (5% ↑), endpoint T_c (1% ↓)
Buchheit et al (2011) n=15, no CON	Well trained	7 d (acclimatisation)	60-90 min soccer training (35 °C, 25%)	YoYo IR1 (6% ↑), endpoint HR (1% ↓)
Buono et al (2010) n=9, no CON	Moderately trained	7 d	120 min walking and cycling (35 °C, 70%)	HST endpoint HR (2% ↓) and T_c (2% ↓)
Chen et al (1998) n=14	Moderately trained	5 d	25-45 min cycle (39 °C, 52%)	TTE cycle, (TN: 5% ↑, HT: 7% ↑), endpoint HR (TN: 5% ↓, HT: 5% ↓)
Cotter et al (1997) n=8, no CON	Healthy	5 d	70 min cycling (40 °C, 60%)	Cycle work (kJ) (1% ↑), endpoint HR (6% ↓)
Garrett et al (2009) n=10, no CON	Moderately trained	5 d	90 min cycling (40 °C, 60%)	Cycle TTE (14% ↑), endpoint HR (9% ↓ PV (4% ↑)
Garrett et al (2011) n=8, no CON	Highly trained	5 d	90 min cycling (40 °C, 60%)	Rowing TT (1% ↓), endpoint HR (1% ↓) PV (4% ↑)
Marshall et al (2007) n=7	Healthy	3 d	120 min cycling (38 °C, 60%)	HST endpoint HR (0.5% ↓), TC (0.5% ↓)
Petersen et al (2010) n = 12	Moderately trained	4 d	30 min cycling (30 °C, 60%)	Repeat sprint test (no change)
Sunderland et al (2008) n=6 (F)	Well trained	9 d, 4 sessions	30-45 min of LIST (30 °C, 24%)	LIST run to volitional exhaustion (33% ↑), endpoint HR (3% ↓) and Tc (1% ↓)

F: Female. CON: control group. HST: heat stress test. TT: time trial. TTE: time to exhaustion. NBC: nuclear biological and chemical suit. ↑: increase. ↓: decrease HR: heart rate. T_c: core temperature. PV: plasma volume. T: trained. UT: untrained. LIST: Loughborough intermittent shuttle test. TN: thermo-neutral environment. HT: hot environment. PPO: peak power output. YoYo IR1: YoYo intermittent recovery test level 1. kJ: kilojoules.

Table 2.3 Representation of studies that investigated medium term heat acclimation protocols (8-14 days)

Study/participants/design	Training Status	Days/sessions	Heat training protocol	Reported outcome measures
Aoyagi et al (1995) n=8, no CON	Moderately trained	13 d, 12 sessions	60 min walking (40 °C, 30%)	Walk TTE in NBC (11% ↑), PV (1% ↑)
Burk et al (2012) n=16, no CON	Moderately trained	10 d	110 min walking (32 °C, 18%)	Walk TTE (83% ↑), endpoint HR (4% ↓), PV (11% ↑)
Castle et al (2011) n = 8	Moderately trained	10 d	60 min cycling (33°C, 53%)	Cycle PPO (2% ↑)
Cheung et al (1998) n=15, no CON	Moderately and highly fit	14 d, 12 sessions	60 min walking wearing NBC clothing (40 °C, 30%)	TTE walk (MF, 3%, HF, 10% ↑) endpoint HR (MF, 4% ↓, HF, 6%↓) and T_c (MF and HF 0.5% ↓)
Daanan et al (2011) n=15, no CON	Moderately trained	12 d	120 min cycling (35-41°C, 29-33%)	Cycle TTE (24% ↑) endpoint HR (6%↓) and T_c (1%)
Houmard et al (1990) n=9, no CON	Trained	9 d	60 min running (40 °C, 30%)	HST run endpoint HR (8.4% ↓) and T_c (1% ↓)
Lorenzo et al (2010) n=12 (2F)	Trained	10 d	90 min cycling (40 °C, 30%)	Cycle work (kJ) (8% ↑), LT power (7% ↑), endpoint HR (9% ↓) and T_c (1% ↓), PV (7% ↑)
Magalhaes et al (2010) n=9, no CON	Healthy	11 d	60 min running (40°C, 50%)	HST endpoint HR (7% ↓) and T_c (1% ↓)
Nielsen et al (1993) n=13	Well trained	9-12 d	40 min cycling (40°C, 10%)	Cycle TTE (67% ↑), endpoint HR (7% ↓), PV (13% ↑)
Nielsen et al (1997) n=12	Trained	8-13 d	45 min cycling (35°C, 87%)	Cycle TT (17% ↑), endpoint HR (4% ↓), PV (9% ↑)
Racinais et al (2014) n=15, no CON	Elite	14 d (acclimatisation)	90 min AFL training (29-33 °C, 37-50%)	YoYo IR2 in temperate conditions (44%↑)
Sawka et al (1985) n=13, no CON	Moderately trained	9 d	120 min walking (49°C, 20%)	Cycle power output (TN 4% ↑, HT 2% ↑), endpoint HR (TN 4% ↓, HT 2% ↓)
Voltaire et al (2002) n=9	Highly trained	12 d (acclimatisation)	50 min running and 70 min swimming (33°C, 78%)	Maximal anaerobic velocity (4% ↑), mean HR (16% ↓) and T_c (1% ↓)
Weller et al (2011) n=16, no CON	Moderately trained	10 d	110 min walking (32°C, 18%)	HST endpoint HR (14% ↓) and T_c (1%↓), PV (1% ↓)

F: Female. CON: control group. HST: heat stress test. TT: time trial. TTE: time to exhaustion. NBC: nuclear biological and chemical suit. ↑: increase. ↓: decrease HR: heart rate. T_c: core temperature. PV: plasma volume. MF: Moderately fit. HF: Highly fit. MAV: maximal anaerobic velocity. LT: lactate threshold. YoYo IR2: YoYo intermittent recovery test level 2. TN: thermo-neutral environment. HT: hot environment. HD: hydrated. EU euhydrated. AFL: Australian football

Table 2.4. **The mean change and effect size (mean Cohen's** *d*) of short- and medium-term acclimation training derived from protocols in Tables 2.2 and 2.3

Acclimation period	TTE	Athletic performance	Heart rate	Core temperature	Plasma volume[a]
STHA (≤ 7 days)	11 ± 8% med ↑ **(0.5)** (n=7)	2.4 ± 3.5% small ↑ (0.3) (n=5)	-3.5 ± 1.8% large ↓ (-1.0) (n=9)	-0.7 ± 0.7% **large ↓ (-0.9)** (n=5)	3.5 ± 2.6% (↑) (n=7)
MTHA (8-14 days)	31 ± 29% large ↑ (1.0) (n=7)	10.2 ± 14.0% large ↑ (0.6) (n=7)	-7.0 ± 1.9% large ↓ (-1.0) (n=14	-0.8 ± 0.3% large ↓ (-1.1) (n=12)	7.1 ± 3.7% (↑) (n=7)

Data is expressed as mean change ± 90% confidence limits with effect size descriptor and (value). STHA: short term heat acclimation. MTHA: medium term heat acclimation. TTE: time to exhaustion. med: medium. ↑: increase ↓: decrease. Effect sizes are reported as: trivial (≤ 0.19), small (0.2-0.49), medium (0.5-0.79), or large (≥ 0.8), unclear = unclear finding. [a]Effect size not applied as the selected studies did not report pre-post values

From this brief comparison of available data, it is evident that there are merits to both STHA and MTHA strategies. Both strategies appear to result in some positive effects on subsequent performance outcome, HR adaptations, and reductions to exercising T_c (Table 2.2). However, it is also evident that MTHA protocols are more beneficial for eliciting plasma volume expansion (~9.5% gain, n = 7) when compared with STHA (~2.3% gain, n = 7) (Table 2.4). This is also supported by changes in performance outcomes that demonstrate greater gains in response to MTHA compared with STHA protocols. The extent of any possible gain will be acutely meaningful among high performance athletes for whom the smallest advantage represents a competitive edge. It is plausible that elite athletes may also adapt more rapidly to a hot environment and several studies (Garrett et al., 2011; Racinais et al., 2012) suggest short-term protocols are capable of evoking beneficial adaptations to athletic performance but greater consistency of protocol design, and a considerably larger volume of data, is required to fully elucidate this area of athletic preparation. The balance between time effectiveness

of the protocol and gaining meaningful adaptation should be the focus of future investigations. Nevertheless, it is important for a leading article such as this, to identify important current deficiencies in contemporary practice and research literature, and propose areas where more empirical data is required.

Based on current evidence and utilising a limited range of protocols, MTHA acclimation periods (>7 days) are of more benefit for both performance and physiological indices such as plasma volume expansion, lower exercising T_c, and lower end-point exercise HR. These observations are likely to be particularly meaningful for the preparation of athletes competing in particularly long duration events such as marathon or triathlon that would be most challenging to heat dissipation mechanisms, or athletes required to continue with high quality training regimes with minimal disruption. For example, hot environmental conditions may diminish training intensity among non-acclimated athletes if they are still acclimating, which could induce a detraining effect. However, as many of the selected studies in this brief review encompassed different exercise modes such as running, cycling, and walking (Table 2.2 and 2.3), it is difficult to determine the overall effects of STHA and MTHA on specific situations of athletic performance. Therefore, the specific individualised requirements and periodisation of athletic preparation must be carefully considered.

2.5 Preparatory activities that may optimise exercise in the heat

It is often purported that for exercise-induced heat acclimation to be most effective, athletes should employ the same exercise mode that they will compete (Taylor & Cotter, 2006). One way to achieve this is to use high specification ergometry in a regulated hot and/or humid environment in a sealed heat chamber utilising the athlete's common exercise modality. Depending on the expected environmental conditions of the targeted

athletic event, mere heat exposure in the absence of (elevated) humidity is less appropriate for preparation to hot humid environments (Armstrong, Hubbard, DeLuca, & Christensen, 1987). Specific humidity exposure can form part of the acclimation strategy, if appropriate for the athlete, as high humidity is an aspect of heat exposure that is both extremely challenging and under-researched. Responsiveness to these conditions requires manipulation of training volume and intensity to ensure that the appropriate exercise and recovery strategies are applied. Quantifying the degree of thermal load throughout training sessions through devices such as ingestible T_c pills can complement this process. Simple sub-maximal heat stress tests that include physiological and performance measures can be used throughout the acclimation process to indicate the level of adaptation reached (Magalhães et al., 2010).

It is widely reported that following heat acclimation, physiological adaptations may decay after a period of non-exposure, with one walking-based study demonstrating T_c adaptations could be preserved for up to one month (Weller, Linnane, Jonkman, & Daanen, 2007). However, for elite athletic performance the period of decay may be much shorter. It is likely that athletes would, therefore, benefit from undertaking 'top-up' or supplementary heat exposure sessions periodically following heat exposure, although there is currently no systematic evidence of this type of strategy being performed. Routine and regular exposure to heat during an acclimation protocol enables the athlete to experience the heat in day-to-day training sessions (Taylor & Cotter, 2006), and athletes can gradually increase passive heat exposure related to daily living as soon as possible (i.e. live hot). Greater research in this area and manipulations of time spent training and passively recovering in hot or cool conditions may help ascertain whether residual effects of heat exposure are retained and once undertaken, whether and how often it should be repeated.

It is possible that to adapt to the heat optimally and in a time efficient way, short term protocols may best utilise a combination of active acclimation and passive acclimatisation. This could be achieved by using widely reported and effective heat tolerance training protocols in standardised conditions (acclimation), but also by promoting passive effects of the heat by living in hot conditions over the short- or medium-term period (acclimatisation). Living in the heat could enable athletes to adapt to hot conditions more rapidly while facilitating training in the cooler parts of the day. It has also recently been proposed that heat-training may prove a useful preparatory strategy for performance in thermo-neutral conditions (Corbett et al., 2014) and consequently the potential gains from STHA and MTHA could be multi-faceted. Therefore, a combined approach could prove effective and achievable in short durations protocols (<7 days), however, new research is required to clarify the interactions between STHA and MTHA and the extent that passive exposure to heat might be useful.

Training intensities in hot or humid conditions, certainly in the short-term, should not rely solely on HR or personal best times as effective markers of adaptation as these can be misleading (Edwards & Polman, 2012). Consequently, the use of scalar methods such as perceived exertion may be more effective in this context. Effective pacing strategies take time to establish in the heat and athletes should expect a degree of performance decrement in events of prolonged duration, especially when still acclimating. Knowing that elite athletic performance can be reduced by as much as 3% in endurance events such as the marathon (Table 2.1), athletes can adjust their pacing strategies to ensure maximum possible performance, taking into consideration their current level of acclimation, relevant ambient conditions (temperature and humidity) and other competitor actions. It seems likely that the shorter time spent acclimating, the faster the acquired adaptations may diminish (Sawka et al., 2011a) and, therefore, it is probable

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that undertaking pre-event acclimation, top up sessions, and living hot as soon as practical could facilitate athletes to compete at greater intensities in hot and humid conditions (Montain, Maughan, & Sawka, 1996). Potentially the combination of all three strategies (heat acclimation, top up sessions and living hot) may yield greater improvements in performance but this premise remains to be tested and may be best suited to either individual sports or tournament-like competitions that are major features of the athletes' season.

The adaptations underpinning maintenance of performance are likely consequent to the cumulative effect of the necessary heat adaptations for that particular individual or event. As discussed above, a 100 m runner may not require a lowered HR or T_c nor other body cooling capabilities for optimal performance. It is plausible that physiological factors associated with being non-acclimated to the heat, such as peripheral vasodilation, coupled with elevated pre-race muscle temperatures may actually be beneficial in the context of sprinting performance although this is a concept rarely considered (Mohr, Krustrup, Nybo, Nielsen, & Bangsbo, 2004). Minimising heat acclimation adaptations for these athletes could therefore be of benefit as it is possible that acclimation could have the opposite of the intended effect. More data are required to determine if it could be counter-productive for sprint athletes to undertake heat acclimation. It is even conceivable that sprinters may gain more from exaggerating the effects of initial heat exposure by undertaking pre-(hot) event cold acclimation to promote immediate 'fight or flight' style of responsiveness to the heat to up regulate muscle temperature, elevate HR heart rate and T_c as a means of readiness for very short duration events (Iwase, Cui, Wallin, Kamiya, & Mano, 2002). It is one of the purposes of a leading article to challenge existing concepts and stimulate new research; it is our view that new research is required to clarify the issues we have identified.

2.6 Post-exercise cooling following exercise in the heat

This brief section encompasses a review of post-exercise cooling and its application following exercise in the heat. The contemporary recommendation for short-term HA in athletes is five training sessions over consecutive days for a minimum of 60 min (Chalmers et al., 2014). However, these types of multi-day training programs have been shown to induce significant levels of fatigue (Wingfield et al., 2016). Therefore, recovery between training sessions should be carefully considered to minimise fatigue, promote heat adaptation and enhance exercise or physical performance. Although, the literature in this particular area is more extensive than presented here, the aim of this section is to provide a brief overview of the expected acute and ongoing effects of post-exercise cooling recovery. These interpretations reflect only the literature which is presented and reviewed here.

Recovery methods following strenuous exercise include whole-body cooling such as cold water immersion (CWI), phase change garments or whole-body fanning. Rapid cooling of the muscles post-exercise in the heat may assist positive aerobic adaptations (Bishop, Jones, & Woods, 2008; Heesch, Shute, Kreiling, & Slivka, 2016; Ihsan et al., 2015). Furthermore, the use of rapid whole-body cooling by way of cold water immersion can facilitate recovery within 24 h between bouts of intermittent cycling (Lane & Wenger, 2004). A recent review suggests that the likely mechanism that CWI facilitates short-term recovery is via ameliorating hyperthermia, reducing cardiovascular strain and consequently central nervous system (CNS)-mediated fatigue (Ihsan et al., 2016). However, cold water immersion is often not feasible and other cooling techniques, such as whole-body fanning, can provide meaningful cooling effects.

Simple interventions such as whole-body fans can reduce core temperature faster than phase change garments, due to an increase in evaporative cooling (Barwood et al., 2009). Furthermore, ingestion of crushed ice and ice-slushies are also a simple, effective means to reduce core temperature either before or after exercise in hot conditions (Brearley, 2012; Ross et al., 2011). Although pre-exercise ice-slushy ingestion can delay the rise in core temperature associated with prolonged exercise in the heat, most studies have focused on the utilisation of these techniques to acutely benefit performance. However, recovery-cooling via ice ingestion post-exercise is a simple strategy to rapidly reduce core temperature in a short period of time (Brearley, 2012). Therefore, utilisation of a mixed method cooling approach involving a combination of whole-body fanning and ingestion of ice or ice-slushies post-exercise may be useful as a recovery tool during intense training blocks such as those experienced during HA training. However, there are only a limited number of studies that have explored the use of rapid whole-body cooling following exercise in the heat.

Mixed method whole body cooling followed by 24 h recovery can moderately improve the mean ball speed in cricket fast bowling by 3% (Minett, Duffield, Kellett, & Portus, 2012). Furthermore, the use of CWI following an initial 30 min cycling task in the heat does not influence the total work completed on a subsequent 30 min cycling task in the heat following a 40 min recovery period (Vaile, Halson, Gill, & Dawson, 2008). The effects of cold therapy recovery techniques indicate large improvements in endurance performance that are relevant for competitive athletes (~5%), although these benefits are generally realised between 24-96 h post-recovery (Poppendieck, Faude, Wegmann, & Meyer, 2013). Therefore, using cold-therapies to positively impact performance over a short recovery period (e.g. 30-60 min) may not be beneficial.

Cold therapies following strenuous exercise can attenuate CNS fatigue (Ihsan et al., 2016). Cold water immersion can reduce impairment in maximal voluntary contraction force (MVC) following 60 min (Pointon, Duffield, Cannon, & Marino, 2011) and 70 min of intense running exercise in the heat (Minett et al., 2014). Although the use of cold therapies may mitigate CNS fatigue, no studies have investigated the effectiveness of these recovery strategies during longer duration training programs such as short-term HA training over seven days.

Undertaking exercise in a hot environment increases muscle temperature beyond that of exercising in a temperate environment (Sawka et al., 2011b). Furthermore, these elevations to muscle temperature can decrease activated protein kinase (AMPK) (Tamura et al., 2014), which attenuates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) activity (Cantó & Auwerx, 2009). As PGC-1α is a key regulator of mitochondrial biogenesis, its exercise-induced expression is an important process in improving skeletal muscle aerobic function (Ihsan, Watson, & Abbiss, 2014). Additionally, post-exercise cooling of the muscles enhances exercise-induced mRNA expression of PGC-1α, and possibly mitochondrial biogenesis following exercise in the heat (Ihsan et al., 2015). Therefore, utilisation of post-exercise cooling techniques following exercise in the heat may not only attenuate fatigue via CNS recovery, but also potentiate aerobic adaptations during strenuous training in the heat.

While undertaking HA training is becoming an increasingly popular training intervention, little attention has been paid to the effect of cumulative fatigue, nor to interventions that may accelerate recovery between training sessions. As HA training often results in significant levels of fatigue, there is potential to explore interventions such as cold therapies that may improve recovery during intense training blocks.

2.7 Perceptual responses to exercise in the heat

While physiological adaptations and responses to exercise in the heat are well documented (Garett et al., 2012; Sawka et al., 2011), perceptual responses and adaptions may also serve as indicators of heat acclimation. It appears that perceptions of both exertion and effort are regulated within various regions of the brain based on the integration of information relating to motor drive, afferent feedback and numerous other factors, including prior experience, awareness and motivation ((Abbiss, Peiffer, Meeusen & Skorski 2015). During exercise in the heat, significant relationships between core temperature and RPE ($r = 0.82$) have been reported However, these increases in RPE can be attenuated by cooling of the head and neck regions (Simmons, Mundel and Jones 2008). Furthermore, while data on the effects of HA on RPE are limited, HA appears to moderately improve RPE, and result in small improvements in thermal comfort during exercise in the heat (Tyler et al., 2016). For example, five days of HA training significantly improved the RPE of Australian Football Players undertaking high-intensity interval training in the heat (Kelly, Gastin, Dwyer, Sostaric, & Snow, 2016). Although, it appears that aerobic fitness, training history, and body composition are important inter-individual variables that can alter the perceptions of thermal stress (Cheung 2010). It has been suggested that exercise intensity is regulated based on one's perceived exertion in order to ensure that 'catastrophic' or 'critical' disturbances to homeostasis do not occur. This is supported by the relatively stable increase in perceived exertion that is typically observed during high-intensity, self-paced exercise such as time trials (Abbiss, et al., 2015). Therefore, in situations where individuals are undertaking high-intensity exercise in the heat, perceptions of effort are likely to limit exercise capacity to a similar extent to physiological capacity.

2.8 Brief update on STHA and MTHA

Given that the initial version of this chapter was published in 2015, a brief update of recent literature surrounding STHA and MTHA was required. Although several pertinent studies that warrant further discussion have recently been published, a recent systematic review has confirmed the above findings with regard to the likely effects of STHA and MTHA on performance and physiological adaptations (Tyler et al., 2016). The application of HA training in elite athlete groups has so far been limited, although recently, fourteen days acclimatisation in a hot and dry (34.5ºC, 18.5% RH) increased elite cycling performance by 5% (power output). While physiological adaptations such as improved cardiac output, reduced T_c and increased PV all occurred within the first six days of training, performance continued to improve during the second half of the acclimatisation program (Karlsen et al., 2015), suggesting improved pacing and awareness of effort. In elite Laser sailors, STHA has been shown to provide meaningful reductions in core temperature, exercising heart rate and improvements in thermal comfort (Casadio, Kilding, Siegel, Cotter, & Laursen, 2016). Importantly, Casadio and colleagues (2016) also demonstrated that the use of "top up" sessions following an initial STHA block preserve physiological adaptations and performance in subsequent heat response tests for up to two weeks.

In contrast, a five day heat acclimation program provides limited physiological improvements (e.g. lowered heart rate and core temperature during exercise in the heat) for elite Australian Football players (Kelly, et al. 2016), although significant improvements in perceived effort and thermal comfort were observed. Furthermore, it does not appear that HA training improves other physiological aspects such as increased lactate threshold that is linked with improved athletic performance (Dileo et al., 2016).

Therefore, the application and programming of HA training can be situationally dependent.

A recent study has reported that only 15% of surveyed athletes prepared specifically by training in the heat in preparation for IAAF world championships held in a hot environment (26 ºC – 33 ºC and 70% RH) (Périard et al., 2016). This is surprising as elite athlete's likely benefit from HA training, and such a large decrement in performance occurs in distance running events held in the heat (Figure 3.1), although, those distance athletes who undertook HA training reported a varied regimen of ~17±10 days of acclimation training (Périard et al., 2016). Collectively these recent reports suggest that while some elite athletes can benefit from HA training, further performance gains are likely to depend on altered pacing strategies and comfort.

2.9 Conclusion

Athletic performance for males and females participating in endurance events is likely to be impaired in very warm to hot environments. The opposite is the case for athletes competing in short distance sprint events. Short-term heat acclimation programmes of <7 days provide athletes with modest thermoregulatory adaptations and performance benefits but based on current evidence more can be gained from medium-term (8-14 day) acclimation periods. However, considerable recent evidence suggests STHA may be worthwhile (Chalmers et al., 2014) and given the practical considerations of congested training and competition schedules, coaches and athletes will most likely give preference to shorter term protocols. Importantly, the perceptual effort of exercise in the heat should also be considered, and strategies that can improve these perceptions may also confer performance benefits. Such interventions may include post-exercise cooling to encourage recovery between bouts of training. More efficient shorter term acclimation

may also be achieved through strategies such as manipulations of active and passive periods of heat exposure and top up sessions over the adaptive period.

2.9 Chapter progression

Although the findings in Chapter Two reported how elite running performance is effected by ambient temperature, the subsequent experimental chapters (Chapters Four – Seven) recruited recreationally active "untrained" males. Therefore, direct comparisons between these groups is problematic. However, the analysis of STHA and MTHA on athletic performance, exercising heart rate, and other physiological variables (Section 2.4) quantified the responses to HA training across a wide range of fitness levels (Table 2.2 and 2.3). This allows a more direct comparison of the results of the subsequent chapters to previous HA training research. Furthermore, the following Chapter (Three) discusses the effects of exercise in the heat on blood biomarkers associated with heat stress and the inflammatory cascade.

(1) Introduction

(2) Review of STHA and MTHA training programs

(3) Review of inflammatory mediators in reponse to exercise in the heat

(4) Reliability of serum biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between residents of the tropical and temperate zones

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

(7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and synthesis, future directions, and conclusions

3. Review of inflammatory mediators, endotoxaemia, and immune responses during heat exposure and training.

3.1 Introduction

This review examines the inflammatory model of heat stress, its relationships with short-term heat acclimation (HA) training, and exercise in the heat. While the classical thermoregulatory model of heat stress has been well documented (Pyne et al. 2014), the interplay between molecular mechanisms that mediate adaptive and maladaptive responses to exercise in challenging environments is not wholly understood. It is well known that exercise-induced heat production is further elevated by exercise performed in hot environments (Tyler et al., 2016), and this can affect cytoprotective mechanisms, immune function and gastrointestinal health (Horowitz, 2016; Leon, 2016; Zuhl et al., 2014). Therefore, a common stratagem to counteract the debilitating effects of heat on endurance exercise is to undertake heat acclimation (e.g. simulated environment) or acclimatisation training (e.g. natural environment) (Chalmers, Esterman, Eston, Bowering, & Norton, 2014). Nevertheless, determining optimal training load, duration and impacts on immune function have not yet been thoroughly documented, although most studies typically share common features of measures of thermoregulation such as cardiac stability, plasma volume expansion, lowered core temperature and increased efficiency of heat loss pathways (Tyler et al., 2016). This review will focus on lipopolysaccharide (LPS), immunoglobulin M (IgM) and interleukin 6 (IL-6) as these variables are of direct relevance to the inflammatory cascade in response to heat stress (Lim et al., 2009). These markers reflect the dynamic interplay between hyperthermia, the coagulation cascade, and a systemic inflammatory response occurring after transient damage to the gastrointestinal tract.

Several lines of evidence supporting the involvement of the classic thermoregulatory and inflammatory pathways in regulation of heat strain and heat stroke. Some athletes, and individuals in occupational or military settings, can perform adequately with a core temperature exceeding 40 °C (Aughey, Goodman, & McKenna, 2014; Lee, Nio, Lim, Teo, & Byrne, 2010). In contrast, athletes can suffer the effects of heat stress at moderate core temperatures below 40°C (Selkirk et al. 2008). This suggests that it is not solely hyperthermia that drives heat stress and symptoms of heat illness. Other field-based observations of individual susceptibility to heat stress point to more complex regulation involving both thermoregulatory and inflammatory processes (Sithinamsuwan et al., 2009).

The degree and pattern of biological variation of immune and inflammatory pathways associated with exercise in the heat provide valuable information on how an athlete can cope with the demands of strenuous exercise tasks in hot conditions. While there are numerous biological markers associated with heat stress and inflammation, this review will focus on lipopolysaccharide (LPS), immunoglobulin M (IgM) and interleukin 6 (IL-6) as these variables are of direct relevance to the inflammatory cascade in response to heat stress (Lim et al., 2009). The rise of circulating LPS is associated with decreased splanchnic blood flow, exertional ischaemia, and increased gastrointestinal (GI) permeability as a result of exposure to hot conditions. IgM is a key antibody in neutralising LPS (Camus et al., 1998) and its concentration in circulating blood can reflect the body's response to endotoxin accumulation, as well as the degree of protective capacity in the event of further challenges. The cytokine IL-6 acts as both a pro- and anti-inflammatory cytokine and a myokine, with much of its release during exercise coming directly from the muscle (Shephard, 2002). Furthermore, the thermal stress experienced by athletes undertaking long duration exercise in the heat could trigger

a systemic inflammatory and immune response via interplay between these, and other mediators. It is therefore important to understand the degree that the circulating concentrations of these biomarkers are affected by exercise in the heat. Hence, this review has also quantified the magnitude of change for these biomarkers following exercise in hot conditions. This analysis calculated the effect size (Cohen's *d*) for the change of each biomarker (Cohen, 1992) from selected studies that reported pre- to post-exercise change in the biomarkers IL-6, LPS, and IgM (Table 3.1).

Table 3.1. Representation of the magnitude of increase in the circulating concentration of interleukin-6, lipopolysaccharide, and immunoglobulin M from laboratory studies that reported pre- and post-exercise biomarker concentrations following exercise in the heat.

Author	Biomarker effect size (Cohen's d)			Oxygen uptake $(ml.kg^{-1}.min^{-1})$	Environmental conditions	Exercise intervention
	IL-6	LPS	IgM			
Barberio et al (2015)	0.8	3.0	-	55 ± 3, n = 9	40 °C & 40% RH	Running at 4 nM blood lactate until exhaustion or until T_c ↑ by 2 °C
Fortes et al (2012)	0.7	-	-	60 ± 5, n =13	33 °C & 50% RH	Running at 65% VO$_2$ max for 40 min
Hailes et al (2011)	2.9	-	0.4	54 ± 6, n =15	38 °C & 40% RH	Cycling at 70% VO$_2$ max until critical core temp or exhaustion
Lim et al (2009)	1.9	1.3	0.9	64 ± 7, n =18	35 °C & 40% RH	Running at 70% VO$_2$ max until critical core temp or exhaustion
Morrison et al (2014)	2.6	-	-	64 ± 4, n =7	30 °C & 50% RH	Running at near max effort for 60 min
Morrison et al (2014)	1.0	-	-	46 ± 4, n =8	30 °C & 50% RH	Running at near max effort for 60 min
Selkirk et al (2008)	4.0	2.0	-	70 ± 2, n =12	40 °C & 30% RH	Walking at 4.5 km/h at 2% incline wearing PC until exhaustion
Selkirk et al (2008)	1.2	3.2	-	50 ± 1, n =11	40 °C & 30% RH	Walking at 4.5 km/h at 2% incline wearing PC until exhaustion
Shing et al (2014)	0.8	0.5	0.1	63 ± 6, n =10	35 °C & 40% RH	Running at 80% VO$_2$ threshold until exhaustion
Starkie et al (2005)	2.5	-	-	61 ± 5, n = 7	35 °C & 30% RH	Cycling at 70% VO$_2$ peak for 90 min
Wright et al (2013)	1.4	-	-	46 ± 6, n =14	35 °C & 60% RH	Cycling at moderate – heavy intensity for 4 x 15 min
Wright et al (2013)	1.3	-	-	37 ± 6, n =14	35 °C & 60% RH	Cycling at moderate – heavy intensity for 4 x 15 min
Yeh et al (2013)	-	1.0	-	49 ± 3, n =15	33 °C & 50% RH	Running at 70% VO$_2$ max for 60 min
Mean effect	1.8 ± 1.0	1.8 ± 1.1	0.5 ± 0.4			

IL-6, interleukin-6. LPS, lipopolysaccharide. IgM, immunoglobulin M. PC, protective clothing. VO$_2$ Oxygen uptake. Environmental conditions presented as degrees Celsius and % relative humidity (RH). Mean effect presented as mean ± SD mean. Cohen's d ± SD. Effect size (Cohen's d), trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater).

3.2 Lipopolysaccharide

Increased thermoregulatory and cardiovascular strain during exercise in the heat is associated with redistribution of blood flow from internal organs to active skeletal muscle and peripheral tissues during exercise. The effects of this redistribution include disturbances to the GI epithelium (Barberio et al., 2015). Increased GI permeability can lead to the release of LPS from Gram-negative bacteria residing in the gut and its subsequent translocation to the portal system (Lim et al., 2009). Elevated levels of LPS in the portal circulation can cause a dose-dependent systemic inflammatory response (Barberio et al., 2015; Sakurada & Hales, 1998). High levels of LPS indicate that the GI mucosa is unable to fully preserve an effective barrier function, resulting in bacterial translocation from the gut lumen to the circulation (Van Wijck et al., 2012). When the rate of LPS clearance by the liver is overwhelmed by increased translocation after transient damage to the gut, endotoxaemia can occur if there are insufficient levels of anti-LPS antibodies, (Zuhl et al., 2014). Therefore, it is important to consider both the initial influx of LPS, as well as the rate of clearance after exercise.

The increase of LPS in response to exercise in the heat has been reported from several field- and laboratory-based studies. These field studies include triathlon (Camus et al., 1998; Jeukendrup et al., 2000) and ultra-marathon (Gill et al., 2015), and provide an important insight to the thermo-inflammatory response that athletes experience when competing in a hot environment. Laboratory studies have investigated the concentration of LPS in response to running at the lactate threshold (Barberio et al., 2015), running to exhaustion (Lim et al., 2009; Shing et al., 2014) and walking to exhaustion in protective clothing (Selkirk et al., 2008). These responses have primarily been investigated in moderately– trained males with peak oxygen uptake (VO$_2$ peak) ranging from 49 – 64 mL.kg^{-1}.min^{-1} (Table 3.1). Although, only one study reported the concentrations of LPS

following exercise in highly trained athletes (VO_2 peak 70 mL.kg^{-1}.min^{-1}) (Selkirk et al., 2008) (Table 2.1). With many sub-elite athletes travelling to take part in challenging sporting events around the globe, it is important to understand and investigate the possible role and consequence of elevations in LPS with documented episodes of heat exhaustion and heat stroke.

The role of LPS during strenuous exercise has been investigated since the 1980's (Bosenberg et al., 1988; Brock-Utne et al., 1988). Circulating LPS concentration in ultra-triathletes have been shown to rise ~3.5 fold following an eight hour race, coupled with a 58% reduction in anti-LPS Immunoglobulin G (anti-LPS IgG) (Bosenberg et al., 1988). This increase in LPS was also positively correlated ($p<0.01$, n = 18) with both increased body mass and incidence of cramping of the lower limbs, suggesting it may be in part attributable to physical conditioning. A majority (81%) of randomly selected runners who took part in the 1986 Comrades Marathon (89.4 km) had increased levels of LPS at the cessation of the race (Brock-Utne et al., 1988). It appears that race duration and intensity, as well as the fitness level of an athlete, influence the LPS response to exercise (Brock-Utne et al., 1988). Athletes exposed to consecutive days of exertional-heat stress during a multi-stage ultra-endurance marathon experienced a modest and sustained rise (21%, $p<0.001$) in both resting and post-stage circulatory LPS (Gill et al., 2015). In contrast, moderately-trained athletes are likely to suffer from greater rises (~50%) in circulating LPS during consecutive days of strenuous exercise in hot conditions (Barberio et al., 2015). Athletes who have had less exposure to higher intensity training, drop out due to severe gastro-intestinal complaints, dehydration, and heat shock (Bosenberg et al., 1988). Moreover, those who take longer to finish long-duration race events are more at risk of endotoxaemia than those who have pre-exposure to small amounts of LPS leakage during training (Brock-Utne et al., 1988). This exposure during

training can result in higher basal levels of anti-LPS immunoglobulins pre-exercise, and greater readiness to combat the influx of LPS that occurs during exercise.

It appears the relationship between gastro-intestinal complaints and gut ischaemia-associated leakage of LPS is not always clear. LPS leakage may be a relatively common feature of hard and sustained exercise, especially when cardiovascular and thermal strain is compounded by factors such as exogenous heat stress, upright posture, and dehydration (Morrison et al., 2014). Furthermore, the transverse areas of the large abdominal arteries are significantly greater in highly fit humans compared with those of moderate or lower fitness levels (Gabriel and Kindermann 1996), which may allow for more absolute blood volume to perfuse these areas during exercise. Therefore increased GI permeability, and the subsequent rise in circulating LPS, can exerbate thermal strain by initiating an inflammatory cascade, perfusion abnormalities, and organ dysfunction (Selkirk et al., 2008), Furthermore, 68% of 29 athletes exhibited an increase in LPS (150%) and a reduction in anti-LPS IgG (40%) following an Ironman distance triathlon reported (Jeukendrup et al., 2000). Although the majority (93%) of those athletes reported gastro-intestinal symptoms, the severity of symptoms were not directly associated with endotoxaemia (LPS >5 pg.mL^{-1}). This degree of endotoxaemia is modest in comparison to previous studies, for example, 81% of the athletes had LPS levels above 100 pg.mL^{-1} following the 1986 Comrades Marathon (Brock-Utne et al., 1988). Frequent exposure to tolerable concentrations of LPS during endurance races and training may lower plasma LPS response through enhanced LPS clearance mechanisms, such as anti-LPS antibodies and reticuloendothelial system activities (Lim et al., 2009). In contrast, individuals with lower aerobic fitness (Selkirk et al., 2008) typically have a higher plasma LPS concentration than more highly trained individuals when undertaking the same work. When intolerable heat stress occurs (Brock-Utne et

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al., 1988), severe endotoxaemia can ensue. Since endotoxaemia is a balance of LPS influx and LPS clearance, this rate of clearance is likely to be a post-LPS translocation event. Although strenuous and/or extended competition in adverse environmental conditions can precipitate LPS translocation and endotoxaemia, training to prepare for these types of events may also cause transient damage to the intestinal tract.

Undertaking a short-term increase in training load over 14 days has been demonstrated to elicit substantial reductions in pre- and post-exercise LPS and anti-LPS immunoglobulin M (IgM) concentrations (Lim et al., 2009). The mild-endotoxemic effect of training in the heat (30-90 min at 35°C) over 14 days yields a protective benefit, by increasing the basal level of IgM at rest. As IgM plays an important role in the defence of LPS, an increase in resting immunoglobulin levels likely protects against subsequent influxes of LPS. Importantly, in highly-trained athletes, tolerable increases to core temperature (~39.5 °C) would typically not provoke heat stress or cause sepsis to occur. Elite athletes can tolerate core temperatures >40 °C without signs or symptoms of heat stress (Aughey et al., 2014; Byrne, Lee, Chew, Lim, & Tan, 2006). Only a limited number of studies have investigated the long term effect of endotoxin leakage or anti-LPS antibody response to training, especially in preparation for competition in hot environments, or in lesser trained athletes. Thus, more studies are required to investigate these adaptations and immunoglobulin protective mechanisms.

Athletes may also experience issues with translocation of LPS as a result of diet, supplementation, and the use of certain medications. For example, supplementation with ascorbic acid can reduce post-exercise LPS concentration by ~12 fold (Ashton et al., 2003). Conversely, the use of anti-inflammatory medications such as Ibuprofen may aggravate exercise-induced intestinal injury (Van Wijck et al., 2012), thereby increasing the potential for gut leakage, leading to a greater influx of LPS to the circulation.

Therefore, the use of dietary supplementation to attenuate the rise in LPS following strenuous exercise may be beneficial for some athletes, but the use of anti-inflammatory agents such as ibuprofen could actually worsen the situation by increasing gut permeability. Ascorbic acid (a form of Vitamin C) is a naturally occurring compound with antioxidant properties, and supplementation could preserve luminal membrane integrity via an antioxidant mechanism (Ashton et al., 2003). Probiotic supplementation can reduce post-exercise LPS concentrations after a run to fatigue in hot conditions (35 – 40°C) (Shing et al., 2014). Although probiotic supplementation does not appear to alter the rise of core temperature while exercising in the heat, it has been demonstrated to decrease GI permeability as observed by large reductions in LPS translocation (15% lower compared with control), as well as increased run time to exhaustion (13.5% longer compared with control) (Shing et al., 2014). As the mechanism that results in improved performance as a result of probiotic supplementation is unknown, further work is required to further elucidate its benefits.

Other supplements such as bovine-colostrum may also curtail intestinal permeability via reduced apoptosis and paracellular permeability (Marchbank et al., 2011). However, eight weeks of bovine colostrum supplementation may increase gut permeability in recreational runners training three times a week (Buckley, Butler, Southcott, & Brinkworth, 2009). The increase in gut permeability with colostrum may have been related to greater leakiness of tight junctions between cells of the gastrointestinal tract, or by increasing macromolecular transport as it does in the neonatal gut (Buckley et al., 2009). However, seven days of bovine colostrum may have no influence on the increase in post-exercise gut permeability and pro- and anti-inflammatory cytokines (Morrison et al., 2014). Therefore, any small benefits that may be achieved with this supplementation are not known to benefit longer duration events such as a triathlon or ultra-endurance

running where there is likely greater internal and external heat load. The clinical significance and performance effects of these types of supplementation, with particular reference to their ability to attenuate gut permeability during intense exercise, remain poorly understood and more research is needed to clarify their mechanisms of action and effectiveness.

While various supplementation strategies have been investigated to increase gut integrity, many athletes may unknowingly be decreasing gut integrity via use of over-the-counter anti-inflammatory medications (Taioli, 2007). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used by athletes to reduce pain or prevent anticipated musculoskeletal pain during exercise (Alaranta, Alaranta, Heliövaara, Airaksinen, & Helenius, 2006; Garcin, Mille-Hamard, Billat, & Imbenotte, 2005). The prevalence of NSAID usage can reach as high as 90% in triathletes (Gorski et al., 2011) and professional soccer players (Taioli, 2007). It is likely that NSAIDs aggravate gastro-intestinal injury during strenuous exercise, leading to the loss of gut barrier function in otherwise healthy athletes (Van Wijck et al., 2012). NSAIDs may further promote splanchnic hypoperfusion, putting athletes at risk of serious GI compromise. As endurance athletes can have significant GI injury after endurance running without using NSAIDs (Camus et al., 1998; Jeukendrup et al., 2000), the combination of exercise and NSAIDs in scenarios where athletes undertake long-duration endurance exercise in the heat may increase the incidence of thermal injury or GI disturbances. In endurance events, athletes are likely aware of the risks of heat stroke and heat stress and would manage their race strategy accordingly. However, there appears to be limited information and advice surrounding the use of common NSAIDs in these circumstances.

Other evidence for involvement of inflammatory processes in heat stress comes from animal models. Sedated animals protected from the effects of heat load in the absence of endotoxaemia survived, whereas animals with endotoxaemia died under exactly the same environmental conditions (Gathiram, Wells, Brock-Utne, & Gaffin, 1988). Administration of anti-LPS antibodies in animal studies can reduce morbidity and mortality. Illness accompanied by a fever in the lead-up to exercising or competing in thermally-challenging environments also appears to be a risk factor for heat stroke. A pre-existing inflammatory state can exacerbate heat stress in both animals (Lim, Wilson, Brown, Coombes, & Mackinnon, 2007) and humans (Sithinamsuwan et al., 2009). A 12 year clinical study of soldiers presenting with heat stroke in a military hospital in Thailand indicated that 95% of cases were preceded by a bout of low-grade fever or upper respiratory tract infection (Sithinamsuwan et al., 2009). Although the rate of influx of LPS is usually well tolerated by well-trained athletes, further work is required to develop an understanding of the response and potential for adaptations in lesser trained athletes following heat stress.

3.3 Immunoglobulin M

IgM is a polyreactive antibody whose production can be driven by endogenous antigens such as LPS (Ehrenstein & Notley, 2010). Furthermore, individual athletes can develop specific antibody responses that can be directed towards these antigens (Camus et al., 1998). Although few studies have quantified the response of IgM, there appears to be a moderate increase in the antibody following exercise in the heat (ES 0.5 ± 0.4, mean ES $\pm$ SD, n = 3 studies, Table 2.1). However, it is interesting to note that these changes have not taken into consideration alterations in plasma volume. As IgM is a large molecule, its ability to escape the vascular will be altered as by stress-induced changes in plasma volume Increases in IgM have been observed in trained runners

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following exercise at 70% VO₂ max with termination when core temperature reached 39.5 °C for 1 min (Hailes et al., 2011). Moreover, six weeks training with a tolerable increase in training load can increase basal concentrations of anti-LPS IgM, yielding a protective effect against exercise-induced endotoxaemia (Hailes et al., 2011). The basal levels of IgM may signify an athlete's preparedness to deal with inflammatory processes when readying for competition or in performance events where heat stress is likely to occur. In addition, the degree that IgM changes following exercise may also provide important insights to an athlete's ability to cope with the demands of a strenuous exercise task in the heat.

Measurement of IgM antibodies involved in defence against increases in LPS does not account for the complex role that IgM plays in inflammation and antigen response. IgM also promotes inflammation and damage in several models of ischaemia–reperfusion injury, including myocardial infarction and intestinal ischaemia–reperfusion injury (Ehrenstein & Notley, 2010; Haas et al., 2010). Given there is an increased risk of cardiac events when performing repeat work tasks in the heat (Walker et al., 2015), it is possible that the wider role of IgM, not just its specific response to LPS, requires greater consideration. As IgM antibodies are confined mainly to the circulation, it seems reasonable to assume that changes in blood concentration might be a more sensitive indicator of their consumption associated with endotoxaemia or readiness to combat subsequent influxes. It should also be noted that the studies selected for analysis in Table 3.1 did not correct for the expected reduction in plasma volume following exercise in the heat. The confounding factor of larger molecules such as IgM not being able to escape the vascular space may result in the underestimation of its role during heat stress. Considering the importance of IgM in combating circulating LPS, more studies that

investigate its response to exercise should provide a greater understanding of athlete immune function in response to heat stress and training.

3.4 Cytokines

Cytokines exert both pro-inflammatory and anti-inflammatory effects and can act as both a mediator and protector in the resolution of inflammation (Heled, Fleischmann, & Epstein, 2013). The interaction between pro-inflammatory, anti-inflammatory, and immunoregulatory cytokines is complex and situation-specific. Furthermore, they are likely dependent on environmental conditions, demands of exercise, and individual level of fitness (Fischer, 2006). A marked inflammatory response after heat stress is involved in both damage-generating processes and repair mechanisms during the recovery phase in the hours and days after strenuous exercise. In normal circumstances, the inflammatory response after exercise is transient and diminishes quickly as homeostasis is re-established (Pyne, Guy, & Edwards, 2014). Uncoupling of the regulatory balance between pro-inflammatory and anti-inflammatory cytokine responses is thought to exacerbate tissue damage. LPS-dependent ex vivo cytokine release is upregulated by exercise, and only in part attributable to changes in messenger ribonucleic acid (Abbasi et al., 2013). In certain pathological states such as trauma, sepsis, and thermal injury, pro-inflammatory cytokines are released into the circulation (Martin, Boisson, Haccoun, Thomachot, & Mege, 1997). However, this effect may be blunted by repeated exposures to exercise in the heat (Hailes et al., 2011). Therefore, responses of cytokines such as IL-6 can provide meaningful insight into relationships between heat stress and the resultant inflammatory process following exercise in the heat.

Interleukin-6 acts as both a pro- and anti-inflammatory cytokine as well as a myokine; furthermore, during exercise much of its release comes directly from the

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muscle (Shephard, 2002). After a long distance triathlon, IL-6 levels can increase 27-fold correlating with severe GI complaints (diarrhoea, r = 0.50 and vomiting, r = 0.27), but not increases in LPS (Jeukendrup et al., 2000). The translocation and presence of endotoxins in circulation could initiate the release of IL-6 through an immune response for elimination (Starkie, Hargreaves, Rolland, & Febbraio, 2005; Vargas & Marino, 2016). Although IL-6 is known to increase following exercise in temperate conditions, there appears to be instances where IL-6 may be responding to prolonged increases in core temperature. Therefore, the relationship between these two markers (LPS and IL-6) may provide useful insight into the degree of heat stress suffered during exercise in the heat.

It is not unusual to see changes in IL-6 ranging from 1-100 fold following cycling or running activities (Fischer, 2006). In extreme cases, IL-6 has can increase 8000 fold following a 246 km "Spartathlon" (Margeli et al., 2005). Additionally, following 90 min of cycling in the heat (35°C) at 70% of VO_2 peak, the response of IL-6 is ~4 fold (p<0.05) greater than in a thermo-neutral condition (15°C) (Starkie et al., 2005). However, these extreme inflammatory responses appear to return to baseline within 48 h. Both long duration cycling and running at high intensities in hot conditions where high core temperatures are recorded (~39.5°C) result in large increases in IL-6 (ES 1.8, ± 1.0, n = 11 studies, Table 2.1). Therefore, IL-6 appears to be a useful biomarker to assess the acute phase response to exercise in the heat.

As much of the release of IL-6 during exercise comes directly from muscle, considerations for nutritional supplementation can play a role in attenuating the rise of this cytokine following exercise in the heat. Furthermore, increased heat load is known to elevate carbohydrate utilisation via accelerated glycogen breakdown as a result of

increased core body temperature (Steensberg et al., 2001), and, greater circulation of catecholamines (Fischer, 2006) can result in the subsequent synthesis, signalling and release of IL-6. To counter the increased glycogen utilisation and the subsequent rises in IL-6 following exercise in the heat, one logical strategy would be to consume a greater amount of carbohydrate, thus increasing the availability of muscle glycogen and potentially attenuating the rise of IL-6. However, higher carbohydrate diets have been shown to have an attenuating effect (Bishop, Walsh, Haines, Richards, & Gleeson, 2001), or no effect (Cox, Pyne, Cox, Callister, & Gleeson, 2010) on the rise of IL-6 following exercise in temperate conditions. Therefore, further work is required to determine the effect of a high carbohydrate diet on the IL-6 response to exercise in the heat where muscle glycogen utilisation is substantially increased.

The systemic effects of IL-6 may have a dose-response relationship with exercise duration and intensity. However, the exercise-induced peak plasma IL-6 concentration will usually not exceed 100 $pg.mL^{-1}$ (Fischer, 2006). Running at a -10% gradient is enough to elevate circulating IL-6 by ~4 fold, which then exacerbates the exercise-induced IL-6 response, as well as a moderate association (r =0.67) with increases in core temperature during exercise in the heat (Fortes et al., 2012). Therefore, previous work that has been undertaken by an athlete that results in significant muscle damage, can influence the degree that IL-6 will be released into circulation in subsequent exercise sessions.

When athletes undertake multi-day races such as an ultra-marathon, the preparation and ongoing health of the athlete is an obvious consideration. One study (Gill et al., 2015) suggests that IL-6 inflammatory responses to a five day ultra-marathon increase due to exertional-heat stress and remain elevated at rest throughout the competition

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period, despite overnight recovery between stages. Additionally, increases in IL-6 were probably also related to the muscle damaging nature of the tasks performed on previous days. Thus IL-6 responses were counteracted by compensatory anti-inflammatory cytokines that predominated throughout the ultra-marathon. Importantly, the increases in IL-6 were not associated with GI symptoms reported previously (Jeukendrup et al., 2000). When exercise is performed with an additional heat stress or environmental load, the IL-6 response may be exacerbated due to increased GI permeability and the associated inflammatory assault. However, this outcome may not always be the case, and could be dependent on the athlete's fitness level, individual ability to combat LPS, and the underlying health of their immune system.

Some athletes appear susceptible to illness and infection in periods of increased training load, and exercise-induced IL-6 responses are higher in illness-prone athletes compared with healthy athletes (10 fold vs 5 fold) (Cox, Pyne, Saunders, Callister, & Gleeson, 2007). Higher IL-6 concentration in illness-prone athletes following long distance running (60 min at 60% of VO_2 max) could make athletes more susceptible to heat strain and illness due to impaired anti-inflammatory responses or poorly regulated cytokine balances. Therefore, athletes with susceptibility to illness may need to demonstrate additional caution when undertaking strenuous training blocks, particularly when being exposed to high heat loads.

Overall, the combination of mode, intensity and duration of the exercise likely determines the magnitude of the exercise-induced increase of plasma IL-6. However, it is evident that eccentric exercise is not directly associated with more marked increases of plasma IL-6 than exercise involving concentric muscle contractions given similar effects were observed after both cycling and running tasks (Table 2.1). Thus, muscle

damage is not required in order to increase plasma IL-6 during exercise. External factors such as heat stress that alter substrate utilisation should be taken into account when interpreting IL-6 response to exercise. While many studies have detailed the acute responses of IL-6 to exercise in the heat, few studies have investigated the long term effects of multi-day training programs in hot conditions, and whether or not these changes in IL-6 impact performance.

3.5 Other markers of heat stress and immune response

One of the roles of natural killer (NK) cells is to upregulate the immune response during exercise in the heat. Exercise or physical activity in the heat also increases free plasma heat shock protein 72 (HSP72) concentration. HSP's are highly conserved proteins that are involved in maintaining cellular protein conformation and homeostasis during stress, and these processes can be attenuated during heat stress. As HSP can co-localize with CD94 on NK cells, this highlights the links between exercise and activation of the innate immune system (Horn et al., 2007). Therefore, the interaction of HSP's with wider immune function and inflammation may be of importance to regulatory control and physical outcomes.

Extracellular expression of HSP72 can increase in relation to both the level of hyperthermia attained and sustained, as well as the rate of increase in core temperature (Périard, Ruell, Thompson, & Caillaud, 2015). Furthermore, the upregulation of this protein has also been associated with stimulation of pro-inflammatory cytokines (Asea et al., 2000) as well as serving as a molecular chaperone to accelerate cellular repair from heat stress, ischaemia and endotoxic shock (Kregel, 2002; Lau, Griffin, & Mestril, 2000). The presence of HSP72 in circulation has been promoted as a marker of heat acclimatisation, and is particularly responsive to heat stress and exercise (Locke, 1997).

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Reductions in basal levels of HSP72 have been significantly correlated with markers of acclimatisation such as reductions in exercising core temperature (Kresfelder, Claassen, & Cronje, 2006).

A study of two bouts of treadmill running 45 min apart suggests that neutrophil and basophil counts can increase substantially after exercise in hot or cold environments; with a greater increase likely in hot environments (Mestre-Alfaro et al., 2012). Lymphocyte and neutrophil antioxidant enzyme activities and carbonyl index increased or decreased substantially after exercise only in the hot environment. The lymphocyte expression of catalase, HSP72 and superoxide dismutase was increased in the hot environment (Mestre-Alfaro et al., 2012). In addition, another study has demonstrated that 11 days of HA inhibits exercise-induced increases in HSP72 (Magalhães et al., 2010). Collectively, these results support the notion that increased core body temperature during exercise can elicit an acute phase immune response, and immune cell adaptations to counteract the oxidative stress.

Antioxidant status is another factor that can influence the inflammatory response to exercise. Adaptations to exercise and training include a higher level of antioxidants and lower concentration of lipid peroxidation products. Physical exercise at an elevated ambient temperature caused lower changes in oxidative stress indices compared with sauna bathing (Pilch et al., 2014). Exposure to sauna bathing induced a shift in pro-oxidant-antioxidant balance towards oxidation, although the shift was lower in the athletes compared with the untrained men. This outcome leads to the assertion that physical exercise increases tolerance to elevated ambient temperature and oxidative stress (Pilch et al., 2014). It has been known for a long time that physical fitness confers

some advantages in terms of improved heat tolerance. Another explanation for this benefit is possibly related to improvements in control of oxidative stress mechanisms.

3.6 Conclusion

Heat and immune stress are important considerations for athletes training and competing in many sports in challenging environmental conditions. The past decade has seen the emergence of new models and insights into thermoregulation during exercise and causes of heat illness. It is now recognised that inflammatory pathways can also contribute to heat illness in a variety of settings, and there appears to be direct interplay between gastrointestinal leakage of LPS, cytokines such as IL-6 and IgM. Experimental research has been conducted on many scenarios where heat stress and changes in immune function are evident in laboratory and field settings. Although athletes can typically tolerate transient increases in circulating level of LPS, IL-6 and decreases in immunoglobulins, the longer term effects of these immune disturbances is still poorly understood. A concern for athletes and coaches is the balance between load and overload. Therefore, athletes preparing for competition in hot environments require carefully constructed training programs that take into account the inflammatory and immune effects of heat training and exposure. Given the majority of research is focused on the acute effects of either races or one-off bouts of exercise, it is important to consider the cumulative effect of short-term training, inflammation, and heat stress during multi-day HA training programs. Therefore, part of the aims of this thesis were to investigate the inflammatory and immune effects of exercise in the heat and heat acclimation training.

3.7 Chapter progression

The following chapter (Chapter Four) investigated the reliability of serum biomarkers associated with heat stress and inflammation. This was done to determine the reliability and usability of selected markers in subsequent studies (Chapters Five and Six) as the response of these biomarkers to strenuous exercise in the heat was investigated.
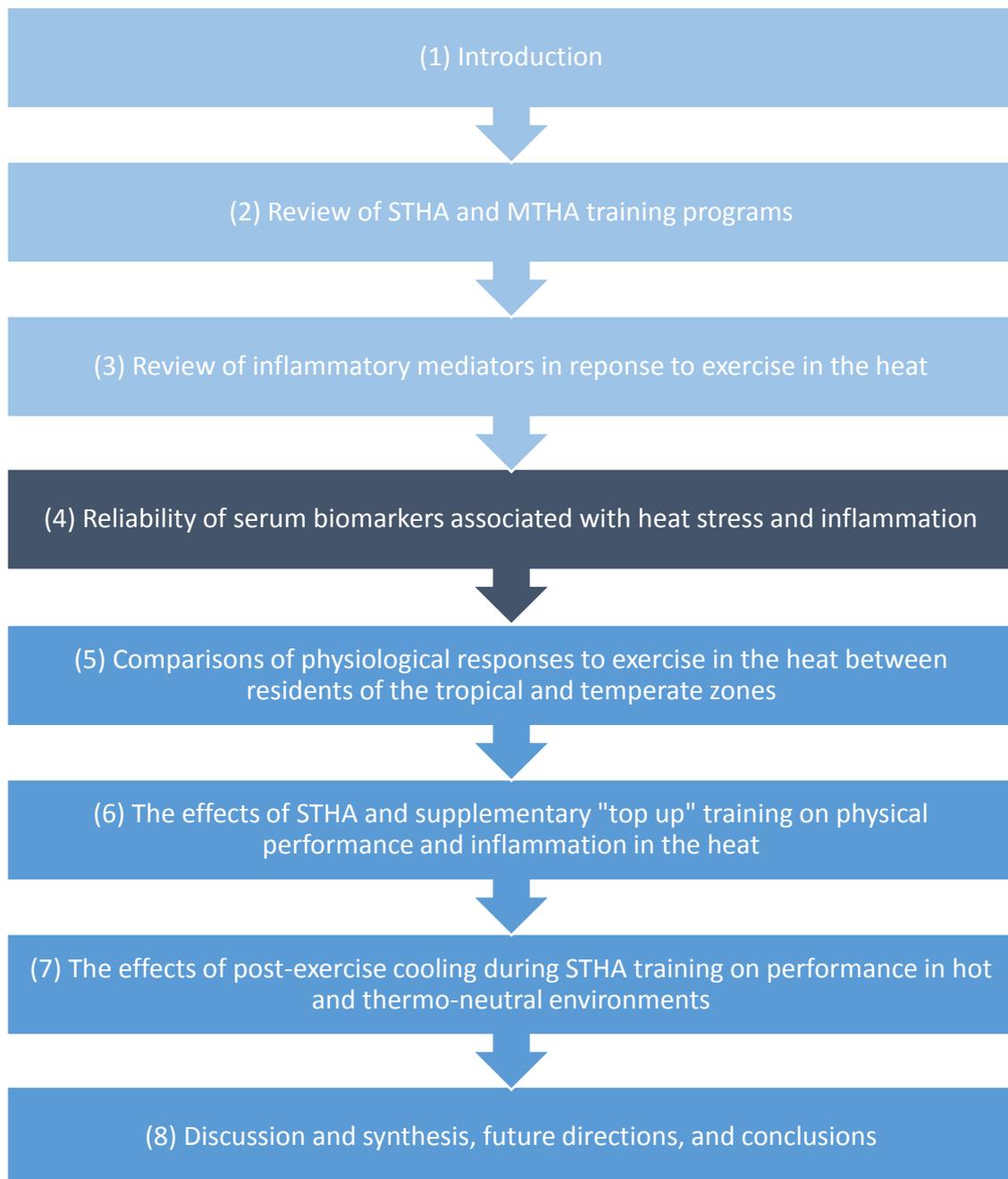
(1) Introduction

(2) Review of STHA and MTHA training programs

(3) Review of inflammatory mediators in reponse to exercise in the heat

(4) Reliability of serum biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between residents of the tropical and temperate zones

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

(7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and synthesis, future directions, and conclusions

4. Short-term reliability of inflammatory mediators and response to exercise in the heat.

4.1 Abstract

Aim: Prospective application of serum cytokines, lipopolysaccharide, and heat shock proteins requires reliable measurement of these biomarkers that can signify exercise-induced heat strain in hot conditions.

Method: To accomplish this, both short-term (seven day) reliability (at rest, n=12) and the acute responsiveness of each biomarker to exercise in the heat (pre and post 60 min cycling, 34.5 $^{\circ}$C and 70% RH, n=20) were evaluated. Serum was analysed for the concentration of C-reactive protein (CRP), interleukin (IL-6), heat shock protein 72 (eHSP72), immunoglobulin M (IgM) and lipopolysaccharide (LPS). Test-retest reliability was determined as the coefficient of variation (CV).

Results: Biomarkers with the least short-term within-subject variation were IL-6 (19%, ±20%; CV, ±95% confidence limits) and LPS (23%, ±13%). Greater variability was observed for IgM, eHSP72 and CRP (CV range 28-38%). IL-6 exhibited the largest increase in response to acute exercise (550%, ±158%, $p = <0.001$, percent change, ±95% confidence limits) and although CRP had a modest CV (12%, ±7%) it increased significantly post-exercise (21%, ±16%, $p = 0.02$). In contrast, eHSP72 and LPS exhibited trivial changes post-exercise.

Conclusion: It appears variation of common inflammatory markers following exercise in the heat is not always discernible from short-term (weekly) resting variation. 4.2 Introduction

4.2 Introduction

Uncompensable heat stress experienced either passively or in response to exercise in the heat influences a complex network of thermoregulatory, immune, inflammatory and neuromuscular factors (Pyne et al., 2014). In extreme cases this inflammation can culminate in multi-organ failure and even death (Singh, Kapoor, & Singh, 2013). In the context of exercise and physical activity, induction of an inflammatory response plays an important role in this process after transient heat can damage the gastrointestinal tract, causing it to become permeable, leading to leakage of harmful bacterial endotoxins from the gut into the circulation (Pyne et al., 2014).

Exercise-induced endotoxaemia has been attributed primarily to lipopolysaccharide (LPS) translocation from the gut into the circulation (Lim et al., 2009). An abundance of circulating LPS can evoke an inflammatory response, leading to heat shock and an overwhelming of anti-LPS mechanisms including the antibody, immunoglobulin M (IgM), (Cohen, Block, Green, Lowell, & Ofek, 1989) and cytokines such as interleukin-6 (IL-6) operating in an anti-inflammatory role (Abbasi et al., 2013). Therefore, when the anti-LPS mechanisms and rate of LPS clearance are inadequate to counter the heat-induced increase of LPS, endotoxaemia may ensue.

A rise in extracellular heat shock protein (eHSP) concentration is a consequence of an innate immune response to whole body hyperthermia (Ahlers et al., 2005). In this scenario, an acute phase immune response is evoked to counteract heat-induced oxidative stress leading to an increase in leukocyte and eHSP concentrations (Mestre-Alfaro et al., 2012). Numerous studies have demonstrated that non-critical exposure to heat may increase both tolerance to oxidative stress and effectiveness of anti-LPS mechanisms (Pilch et al., 2014; Pyne et al., 2014; Yeh et al., 2013).

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Several studies have used blood biomarkers to quantify the magnitude of adaptation to hot environmental conditions, although a comparison of short-term variability in exercise-induced biomarkers has not yet been conducted. This is surprising as there is considerable variation in the magnitude of exercise-induced change to markers such as interleukin (IL)-6, C-reactive protein, LPS and eHSP72 following a bout of exercise in hot conditions (Hailes et al., 2011; Lim et al., 2009; Marshall et al., 2007; Rhind et al., 2004; Wright et al., 2013). As a common length for a short-term heat acclimation protocol for athletes is seven days (Garrett et al., 2011), further investigation into the variation of these biomarkers is warranted. The utility of individual biomarkers may depend on typical variation (noise) under normal conditions, and the magnitude of the response to exercise in the heat (signal). The issue is whether the noise is sufficiently small so as not to mask biologically and/or clinically important changes or differences. While some biomarkers may exhibit substantial short-term variability, they could still be useful if the exercise stimulus produces a sufficiently large signal (response). This is a point often overlooked in the study of reliability of biomarkers. Therefore, it is important to quantify reliable, relevant, and objective outcome measures of the immune and inflammatory responses.

The aim of this study was to quantify the reliability (short-term test re-test reliability) in the concentration of common inflammatory (blood) biomarkers at rest (twice over seven days, Part A). A second aim was to examine the acute response of those biomarkers to an exercise challenge performed in hot and humid conditions (Part B). It was hypothesised that normal biological variation of blood biomarkers (noise) would be smaller than the variation observed following a 1 h strenuous cycling task in a hot environment (signal), resulting in clear "signal" to "noise" ratios.

4.3 Methods

Experimental Design

Part A: Short-term reliability of serum biomarkers.

This phase of the study was designed to examine the weekly variation in venous blood of selected biomarkers in a non-exercise context and was conducted over 14 days (Figure 4.1). The seven days preceding the first test day were used as a "lead-in" period and participants were instructed to abstain from partaking in moderate -high intensity physical activity for the duration of the study period. Participants then had venous blood drawn on two occasions seven days apart. After being seated for 10 min, venous blood was drawn in a seated position before and after the heat stress test. Blood was sampled approximately 2 h post-prandial at a similar time of day (morning) to limit diurnal variation. At the beginning of the lead-in period all participants undertook a baseline evaluation of maximum oxygen uptake (VO_2 max) using an incremental treadmill running test to exhaustion. A seven day controlled lead-in or baseline period was used to ensure that the participants were not suffering from any residual inflammatory effects of exercise or illness prior to taking part in this study. Participants were instructed to maintain a similar dietary intake and (light) activity levels for 24 h preceding each venous blood sample. Physical activity diaries were kept by the participants to ensure that they were not undertaking strenuous exercise throughout the study.

Figure 4.1. Schematic illustration of the experimental procedures showing that blood was sampled at D 1 (day one, Part A and Part B) and D 7 (Day seven, Part A). ExH; Exercise in the heat intervention (Part B).

Part B: Acute response of serum biomarkers to exercise in the heat.

This phase of the study examined the acute response of biomarkers to exercise performed in the heat. To aid robust evaluation of biomarkers free from influence of prior exercise, this part of the study also contained a seven day lead-in period prior to assessment. At baseline, all participants performed an incremental test to exhaustion for the assessment of VO_2 max on a cycle ergometer - the same modality as the subsequent heat stress test protocol. As before, all participants were required to abstain from moderate-high intensity exercise for the remainder of the seven day lead-in period prior to further assessment of pre- to post-exercise evaluation of biomarker activity. The exercise in the heat test occurred seven days after baseline evaluation of VO_2 max. Venous blood was drawn in a seated position prior to and immediately following the heat stress test. Blood was sampled approximately 2 h post-prandial at a similar time of day for all participants (morning) to limit diurnal variation.

Participants

Participants in Part A of this study (short-term variation) comprised twelve healthy moderately-trained males (age 24.3 ± 4.1 years, VO_2 max 52.0 ± 2.7 mL.kg^{-1}.min^{-1},

height 1.78 ± 0.09 m, mass 73.9 ± 8.5 kg, mean $\pm$ SD). Part B participants (acute response to exercise in the heat intervention) comprised twenty males (age 24.6 ± 3.7 years, VO_2 max 43.2 ± 5.4 mL.kg^{-1}.min^{-1}, height 1.78 ± 0.07 m, mass 83.5 ± 11.0 kg). All participants completed a pre-screening medical questionnaire that screened for the use of NSAIDS and immunomodulating medications (none were present). After explanation of the study procedures, benefits and risks, participants provided written informed consent before inclusion in the project. This study was approved by the James Cook University Human Research Ethics Committee and conformed to the guidelines set forth by the Helsinki Declaration. Participants in Part A were also required to complete a daily physical activity diary for the duration of the study so that any exercise undertaken could be quantified for intensity and duration. All participants were also required to self-report any symptoms of illness, inflammation, or soreness.

Blood collection

For both Parts A and B, blood was drawn via a 22 gauge needle from a prominent superficial forearm vein located at the antecubital fossa, and drained directly into an 8.5 mL sterile serum separator Vacutainer tube containing a clot activator and gel for serum separation (Beckton and Dickson, USA). Samples were refrigerated at 4 °C for 30 min to allow clotting and then centrifuged at 1000 x g at 6 °C for 10 min (Rotina 420R, Hettich, Germany). Serum was removed and stored in 400 µl aliquots that were frozen immediately for a maximum of three months at -80 °C for later analysis. Levels of IL-6 (Quantikine HS600B, R&D Systems, United States, detectable limit = 0.45 pg.mL^{-1} - 9.96 pg.mL^{-1}), inducible eHSP72 (HSP72; ADI-EKS-715, Enzo Life Sciences, United States, , detectable limit = 0.09 ng.mL^{-1} - 125 ng.mL^{-1}), IgM (AB137982, Abcam PLC, United Kingdom, detectable limit = 0.45 mg.mL^{-1} - 100 mg.mL^{-1}), CRP (hsCRP

Immunoassay kit 11190, Oxis International, United States, detectable limit = 0.1 mg.mL^{-1} - 10 mg.L^{-1}), and LPS (HIT302, Hycult, Biotechnology, Netherlands, detectable limit = 0.04 EU.mL^{-1} - 10 EU.mL^{-1}) were analysed in duplicate by ELISA according to the manufacturer's instructions. The manufacturer stated intra-assay precision was <10% for all assays. Additionally, the in-house intra- and inter-assay coefficient of variations were calculated and are provided in Table 4.1. Inflammatory analyte concentration was not adjusted for the decrease in plasma volume from pre-to post-exercise so that comparisons to the available human experimental and clinical, and animal studies, none of which have adjusted for possible plasma volume shifts, could be made (Hailes et al. 2006).

Table 4.1. Intra- and inter-assay variability

Biomarker	Intra-assay CV	Inter-assay CV
eHSP70	2.2, ±2.7 %	11.9, ±7.1 %
LPS	4.2, ±2.9%	17.3, ±20.2 %
IL-6	4.7, ±3.6 %	15.4, ±15.6 %
IgM	3.1, ±1.9 %	8.2, ±5.5 %
CRP	4.1, ±4.6 %	22.4, ±11.6

Biomarkers presented as intra- and inter-assay mean coefficient of variation (CV), ±95% CI. eHSP72; extracellular heat shock protein. LPS; lipopolysaccharide. IL-6; interleukin-6. IgM; immunoglobulin M. CRP; C-reactive protein.

Exercise in the heat protocol (Part B)

Participants in Part B undertook an exercise test in the heat similar to what has been used previously The heat stress test was of similar design to earlier work (Garrett et al., 2009; Lorenzo et al., 2010a). Breifly, the test involved three sub-maximal workloads of 10 min duration (50%, 60% and 70% VO$_2$ max) on a cycle ergometer followed by a 5 km time trial (TT) at 35 $^{\circ}$C and 70% relative humidity (RH) (VeloTron Dynafit Pro and

Velotron Coaching Software, Racermate, United States). Three min rest was given between sub-maximal workloads and five min rest was given prior to the start of the TT. Participants undertook approximately 40 min of exercise and were exposed to the hot humid environment for 60-65 min. Briefly, the sub-maximal workloads required the participants to cycle at a fixed power output between 85-95 rpm. During the TT the participants were able to self-select their gearing and informed of their rpm and distance every 500 m. Participants were not aware of their gear, speed, or time elapsed during the TT. A standardised warm-up of 5 min cycling at 40% of VO_2 max followed by dynamic stretching was undertaken prior to the 50% workload. Heart rate (RS400, Polar Elektro, Finland), and core temperature (T_c) (ttec 501-3, software version 10.1, Nordex Pty Ltd, Australia; MEAS 449 1RJ rectal temperature thermistor, measurement specialities, United States, self-inserted 8 cm past the rectal sphincter) were sampled at 5 s intervals. Fluid intake (water, ad libitum) and rating of perceived exertion (Borg RPE 6 – 20) were recorded throughout the test (Borg, 1998). Nude dry body mass was recorded pre- and post-exercise and body mass was normalised for fluid loss and expressed as a percentage change.

Statistical Analysis

The concentration of each biomarker is presented as mean ± SD. Biomarker reliability was calculated as a coefficient of variation (CV) both within- and -between subjects at day zero and day seven and presented as mean CV, ±95% confidence limits (CL). CV was calculated as the standard deviation divided by the mean pre- and post-exercise or the Day O and Day 7 biomarker concentration, multiplied by 100. Day 0 to day seven and pre- to post-exercise changes in biomarker concentrations were analysed with paired t-tests and significance was accepted if p was <0.05. Effect sizes for changes

in biomarker concentrations were also calculated. The expected reference change, or signal, was estimated for each biomarker as 0.2 x between-subject standard deviation.

The criteria to interpret the magnitude of ES were: trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater) (Cohen, 1992).The signal to noise ratio score was determined by dividing the reference effect size (signal) by the within-subject test-retest reliability (noise). The utility of a biomarker was considered 'good' if the expected signal was greater than the noise, or 'unclear' where the signal was less than the noise. A minimum of eight participants was deemed sufficient to detect the smallest worthwhile change between means assuming the reference change was approximately twice the magnitude of the typical error of measurement, with a Type I error of 5% and Type II error of 20%. Biomarker concentrations and curve fit was performed using GraphPad Prism Version 6.03 (GraphPad Software Inc, United States) according to the manufacturer instructions. Statistical analyses were performed in IBM SPSS Statistics Version 20 (IBM, United States).

4.4 Results

Part A: Short-term biomarker reliability

The biomarker with the lowest within-subject coefficient of variation over the 7 day assessment period (day 0 to day 7) was IL-6 (CV; 19%, ±20%, mean, ±95% CI, ES; 0.16,). CRP had the highest CV (38%, ±21%) with a substantially lower level of serum concentration (ES; -0.28) after seven days (Table 4.2), although none of the biomarkers changed significantly over this period (p>0.05). A comparison of the within-subject variability for each biomarker with an expected reference change is detailed in Table 4.2. Biomarkers that displayed a good signal to noise ratio were IL-6 and CRP. The expected signal for LPS, IgM and eHSP72 was less than that of the typical noise estimated in this

analysis. In-house quality control procedures indicated that this variation was not due to a problem with the assay itself, and, therefore, the biomarkers were categorised as having unclear or poor reliability (Table 4.2).

Part B: Acute responses of blood biomarkers to exercise in the heat

Blood biomarkers with the largest pre- to post-exercise change were IL-6 (p <0.001) and CRP (p = 0.02). The blood biomarkers least sensitive to change following the exercise in the heat exposure were IgM, LPS and eHSP72 (p >0.05). The exhaustive nature of the exercise task was confirmed with high levels of physiological and perceptual exertion (Table 4.3). Changes in mean blood biomarker concentration in addition to effect sizes pre-to-post exercise in the heat are presented in Figure 4.2.

Table 4.2. Coefficient of variation both within (day zero to day seven) and between subjects with inferences to the reliability and usefulness (signal to noise) of selected biomarkers

Biomarker	Concentration Day 0	Within-subject CV Day 0 to Day 7	Between-subject CV Day 0	Within-subject E.S	Pre to Post E.S	Ratio Score	Inference
		Noise			Signal	Signal to Noise	
eHSP72	0.35 ± 0.07 ng.mL^{-1}	37%, ±23%	62%	-0.67	0.08	0.12	Unclear
LPS	0.29 ± 0.04 EU.mL^{-1}	23%, ±13%	41%	-0.55	-0.06	0.11	Unclear
IL-6	0.94 ± 0.45 pg.mL^{-1}	19%, ±20%	153%	0.16	1.58	9.88	Good
IgM	2.56 ± 0.29 mg.mL^{-1}	28%, ±17%	261%	0.73	-0.42	0.57	Unclear
CRP	0.93 ± 0.72 mg.L^{-1}	38%, ±21%	93%	-0.28	0.78	2.78	Good

Biomarker concentrations are presented as mean ± SD, within-subject coefficient of variation (CV) is presented as mean, ±95% CI. E.S; Effect size (**Cohen's** *d*), trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater). Within-subject effect size was calculated from the typical change in the mean (raw units) of the measured parameter from day 0 to day 7. Ratio score was calculated by dividing the pre to post effect size by the within-**subject effect size and was considered 'good' if the expected signal was greater than the noise, or 'unclear' where the signa**l was less than the noise. CRP; C-reactive protein. eHSP72; extracellular heat shock protein. IL-6; interleukin-6. LPS; lipopolysaccharide. IgM; immunoglobulin M.
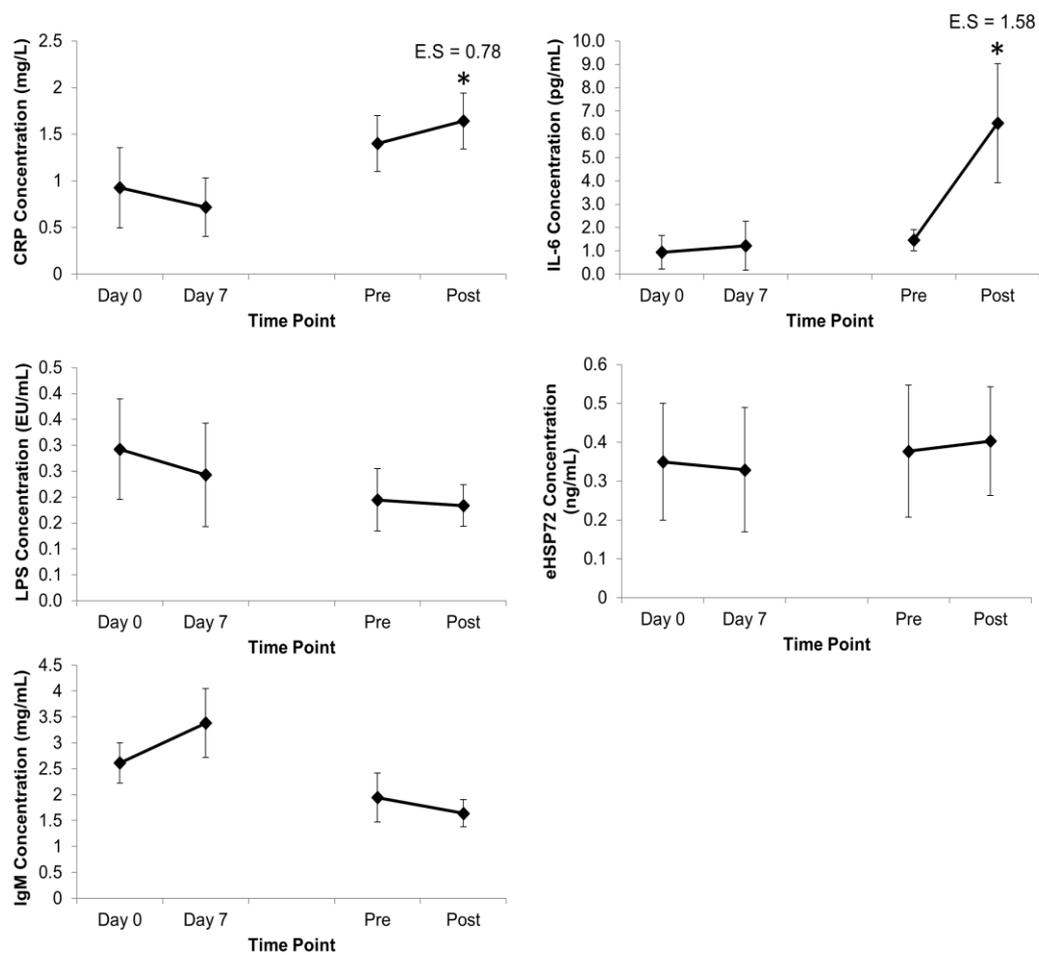
Figure 4.2. Serum biomarker concentrations presented as mean ± SD from Part A (Short-term; Day 1 and Day 7) and Part B (Exercise in the heat; Pre and Post). * = significantly different from pre concentration. CRP; C-reactive protein. eHSP72; extracellular heat shock protein. IL-6; interleukin-6. LPS; lipopolysaccharide. IgM; immunoglobulin M. E.S = Effect size (Cohen's *d*), trivial (0-0.19), medium (0.20-0.49), and large (0.80 and greater).

Table 4.3. Physiological and perceptual responses to the exercise task in the heat

Measure	Group B
5 km TT time (s)	626 ± 100
Peak HR (bpm)	187 ± 5
Mean HR (bpm)	160 ± 19
Peak core temperature (°C)	38.9 ± 0.2
Reduction in body mass (%)	1.7 ± 0.3
End point RPE (units)	17 ± 1

Data is presented as mean ± SD. TT; time trial. HR; heart rate. RPE; rating of perceived exertion.

4.5 Discussion

The biomarker IL-6 exhibited the smallest within-subject short-term variation (19%) and the greatest acute pre- to post-exercise change in the heat (4.5 fold change). For the other biomarkers, the short-term resting variation was similar to that of pre- to post-exercise evaluations in the heat, indicating minimal alteration to an acute bout of exercise. It appears only some biomarkers are potentially useful for the purpose of reliably quantifying acute physiological responses in healthy active individuals to hot environmental conditions that elicit modest rises in T_c.

Even in a resting state, considerable weekly variation was evident for each variable. The cytokine IL-6 exhibited the least within-subject variability of 19% whereas other biomarkers such as CRP varied by 38%. The magnitude of this variation is considered concurrently with the expected change in response to an exercise challenge or a period of training, and can be used to inform the decision making process on effects of heat stress (Table 4.2). Quantifying variation is an inherent part of studying biological

systems and can yield important information when seeking to determine whether or not intervention-induced change in a measured parameter is meaningful.

The exercise presented to the participants resulted in a mean core temperature rise of 1.5 °C above baseline levels and the duration of heat exposure was ~60 min, of which 40 min was dedicated exercise. Although concentrations of IL-6 and the acute phase protein CRP were elevated following exercise, other biomarkers indicative of heat stress such as LPS and eHSP72 did not rise significantly from pre-exposure levels. Serum concentration of IgM also did not rise but instead there was a small 15% reduction in circulation following the exercise bout. It seems plausible that a modest reduction in IgM concentration post exercise reflects the anti-LPS properties of this antibody in response to mild heat stress. This observation is consistent with the findings of Camus et al. (1998), but not of Hailes et al. (2011) and Lim et al. (2009). The exercise stimulus elicited a response from non-specific pro- and anti-inflammatory blood biomarkers, however, it was not sufficient to cause further inflammatory processes associated with heat stress in healthy, moderately trained males.

The significant increase of IL-6 concentration post-exercise may not signify heat stress per se, but rather the stress invoked by the exercise demand itself. IL-6 can be released into the circulation following various pathological events such as physical exercise, trauma, sepsis, and thermal injury (Moldoveanu, Shephard, & Shek, 2000). There are few studies that have investigated IL-6 as a blood biomarker during exhaustive exercise in the heat, although Selkirk and colleagues (2008) observed a large increase following 2 h of exhaustive walking in protective clothing in very hot and humid conditions. However, similar effects have been detected following exercise in the absence of a significant heat load. Moldoveanu and colleagues (2000) reported a twenty-

fold increase in plasma IL-6 concentrations following 3h of exercise at 60-65% of peak oxygen uptake in a thermo-neutral environment - this change is similar in magnitude to that reported by Selkirk et al. (2008).

The large within-subject variation observed for CRP (38%) raises the question of its suitability as a meaningful biomarker. However, in this study, the biomarker noise (short-term, within-subject variability) was less than that of the signal (response to the exercise task) and there was a medium increase in CRP concentration pre- to post-exercise (p = 0.02, ES; 0.78). Serum levels of CRP can increase rapidly during the acute phase of an inflammatory process (Pepys & Hirschfield, 2003), but this is a non-specific response that could be indicative of infection, illness or other metabolic factors not associated with a heat stimulus. A recent study (Hailes et al., 2011) that measured CRP in serum following five consecutive days of exercise in hot and dry conditions (38º C and 40% RH) reported high variability between participants and a standard deviation approximately twice that of the mean after both an acute and ongoing exposure to heat. As the presence of IL-6 is likely to cause an increase in serum levels of CRP (Petersen & Pedersen, 2005), it is possible that the exercise stimulus, and not necessarily the heat load presented to the participants was sufficient to stimulate the release of CRP from the liver. Although both IL-6 and CRP may play important roles in determining the degree of strain placed upon individuals competing or training in more extreme (hot and/or humid) conditions, it seems unlikely that this measure would present useful information in terms of responses or adaptations to the heat specifically.

The low within-subject variability of LPS (CV; 23%) was encouraging for the practical application of this biomarker for evaluating responses to hot environmental conditions. The low concentrations of LPS observed in this study indicate the

participants had the capacity to tolerate the heat load with minimal gut leakage (Pyne et al., 2014). As LPS is the primary endotoxin translocated to circulation under heat load (Yeh et al., 2013), its concentration and regulation is a primary consideration in the study of responses to the heat. The outcomes of this study indicate that LPS evaluation in circulating blood should yield reliable results provided the participants are well rested or are capable of completing a demanding exercise task. Nevertheless, measurement of LPS alone merely indicates the extent of susceptibility to endotoxaemia and not the responses of the immune system initiated by this challenge, which can be investigated using other measures such as intestinal fatty acid-binding protein (Morrison et al., 2014), tight junction proteins that indicate increased intestinal permeability (Yeh et al., 2013) or soluble CD14 (Stuempfle, Valentino, Hew-Butler, Hecht, & Hoffman, 2015). Therefore, to facilitate a comprehensive view of both the underlying endotoxin threat, and compensatory biochemical mechanisms addressing this challenge, it is worthwhile to consider the utility of other viable biomarkers such as IgM and eHSP72.

The responsiveness of the immune system to release endotoxin is a primary consideration in defence against heat shock. As IgM is a key antibody in neutralising LPS (Camus et al., 1998), its concentration in circulating blood can reflect the body's response to endotoxin accumulation, and the likelihood of protective capacity to further challenges. In this study the observed weekly variability of IgM concentration was 28%. The pre- to post-exercise change was -15%, with 13 of the 20 participants exhibiting a negative change. To our knowledge only one other study has investigated the response of non-specific IgM following exercise in hot and humid conditions (Hailes et al., 2011). However, the reference change reported by Hailes and colleagues (2011) pre- to post-exercise in the heat (CV; 16%) is smaller than the within-subject variability (noise) reported here (CV; 29%). It should also be noted that the analysis of IgM was not

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corrected for the expected reduction in plasma volume that occurs following exercise in the heat. As pilot testing within this study did not provide reliable data in regards to changes in plasma volume, this analysis was not possible. The confounding factor of larger molecules such as IgM not being able to escape the vascular space may result in the underestimation of its role during heat stress. It appears that IgM has shortcomings as a viable biomarker for quantifying the anti-LPS response, and this is possibly related to the capability of the participants to tolerate the heat load placed upon them, although these data indicate that this response could result in either an increase or decrease in circulating concentrations. Future research is needed to clarify why some individuals respond in this manner.

Inducible eHSP72 exhibited high short-term variability (37%), however, the pre- to post-exercise change was trivial. In this study, the heat load was seemingly not sufficient to induce a significant change in serum concentration of eHSP72. The usefulness of this variable must also be considered against the intended heat load and it may only be useful to quantify the magnitude of response and adaptations to hot environmental conditions, provided the heat stimulus is large enough (Ogura et al., 2008). This may be achieved through longer duration or core temperature clamping protocols and it seems likely that heat loads that cause an increase in core temperature >39 °C are needed to evoke LPS translocation and induction of eHSP72 (Pyne et al., 2014). However, the expected increases of eHSP72 following the stress of exercise induced hyperthermia display highly individual variability, and would be expected to be higher at baseline in heat-acclimatised individuals (Morton, Kayani, McArdle, & Drust, 2009). The relatively small observed changes in eHSP72 in this study would reflect the participants were not under significant heat stress as a result of the HST task.

Between-subject variation also provides useful information for researchers interested in the utility of different measurements. Low within-subject variation indicates that an individual could be expected to provide a similar result on repeated occasions under constant conditions. Therefore, on an individual basis this increases the likelihood that resting or post-exercise measurements could be useful. Conversely, low between-subject variation indicates that all individuals in a cohort exhibit similar concentrations and/or regulate the variable at a similar level. For example, the participants in this study regulated IL-6 at very low and consistent levels. The observation of large between-subject variation for biomarkers such CRP may necessitate the recruitment of more participants to compress the variation between individuals. However, this type of approach may also limit the interpretation of results and does not permit (easy) determination of an individual's response to heat acclimation (Racinais et al., 2012). Furthermore, as the intra-assay CV was better than the manufacturer stated CV of <10% for all assays (Table 4.1), it is likely that the changes and variation observed in blood biomarker concentrations were indicative of the biological variation at rest, or in response to the exercise task. Although methods such as repeat quality control of samples could be used if possible, due to plate availability limitations it was not possible to do so for all samples in this study. The use of duplicate measures in assays is a standard procedure, although triplicate measures (where possible) can aid in the compression of within-sample variation.

Although this study employed the use of an exercise task in the heat, it has been discussed that exercise in temperate environments can also result in large changes to immune biomarkers such as IL-6 and IgM, and future studies may choose to include an exercise matched task in a temperate environment to quantify the degree of change following exercise in those conditions. The use of an exercise task in the heat in this

study was chosen to place a large load on the participants, both from the physical demands of the exercise task, and the demands of thermoregulation in a hot and humid environment. Future studies should also examine whether highly-trained athletes respond differently to moderately-trained individuals, the differential effects of exercise in the heat as well as temperate conditions, and the influence of a prior history of heat acclimation or acclimatisation on concentrations of inflammatory mediators.

A limitation of this study was the differing level of aerobic fitness of the subjects in Parts A (VO_2 max 52 mL.kg^{-1}.min^{-1}) and B (43 mL.kg^{-1}.min^{-1}). Participants were convenience sampled from a local university and sporting club population, with those unable to commit to the full 14 day period protocol (Group A) allocated to Group B, due to sporting commitments that would likely interfere with resting levels of the blood biomarkers. Although the participants in each group had differing fitness levels as indicated by their VO_2 max this is more likely due to the protocol modality. Participants in Group A underwent their VO_2 max on a treadmill and participants in Group B underwent their VO_2 max on a cycle ergometer. As the vast majority of participants partook in either running or team sports such as football (soccer), this would likely account for the differences in VO_2 max, as differences of ~11% have been reported between cycling and running protocols in running athletes (Basset & Boulay, 2000). The decision to use a cycle ergometer for Group B was to limit the trips to the laboratory for each participant by using a single test for both VO_2 max and to calculate individual loads for the subsequent HST.

4.6 Conclusion

Quantifying the inherent variation of biological systems affected by exercise in hot and humid environments can help inform the choice of inflammatory biomarkers. The

utility of the selected biomarkers, IL-6 and CRP, appears useful to quantify the inflammatory responses to exercise, even when presented with a high (but tolerable) exercise load in the heat. However, the short-term variability of other biomarkers such as eHSP72, LPS and IgM overshadows the observed change following 65 min of exercise and exposure to a hot environment. The within-subject analysis also indicates that individuals consistently regulate the concentration of these biomarkers within homeostatic limits when measured seven days apart. However, the relatively high between-subject variation indicates that it is not possible to establish a standardised concentration of each biomarker suitable for all individuals. It appears that a substantial heat and exercise stimulus (i.e. T_c >39°C) is needed to evoke further responses associated with heat stress and the inflammatory cascade.

4.7 Chapter progression

These results demonstrate that some biomarkers are susceptible to large variations both at rest, and following exercise in the heat. Therefore, the results of this study informed the interpretation of biomarker responses that are reported in Chapter Five. The following chapter further investigated the effects of biomarker responses to exercise in the heat. This was achieved by investing the response of repeated bouts of exercise in a hot environment between residents of the tropical and temperate zones.

(1) Introduction

(2) Review of STHA and MTHA training programs

(3) Review of inflammatory mediators in reponse to exercise in the heat

(4) Reliability of serum biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between residents of the tropical and temperate zones

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and sythesis, future directions, and conclusions

5. Comparison of physiological responses to exercise in a hot and humid environment between residents of tropical and temperate locations.

This chapter has been written in the format of an original research article for consistency with the format of the thesis.

5.1 Abstract

Aim: Long-term residency in the tropical zone could influence inflammatory and immune processes. The aim of this study was to determine whether residency in a tropical or temperate environment influences the perceptual and physiological response to exercise in the heat between ethnically-matched recreationally active males.

Method: Two groups of adult males were recruited as either Tropical (n=8) or Temperate (n=8) based on location of residency (Cairns, AUS or Plymouth, UK, respectively). Characteristics were: age 23 ± 3 y, height 177 ± 8 cm, body mass 80.6 ± 10.9 kg, VO$_2$ max 42.1 ± 5.7 mL.kg.$^{-1}$min^{-1}, (mean $\pm$ SD). Participants undertook three Heat Stress Tests (HST) seven days apart consisting of 60 min cycling in hot conditions (35 ºC and 70% RH) at intensities of 50%, 60%, and 70% of peak power output before a 5 km time trial. Venous blood samples (8.5 mL) were drawn before and after each HST and analysed for concentrations of interleukin-6 (IL-6), liposaccharide (LPS), and immunoglobulin M (IgM). Data are presented as between-group effects as well as the coefficient of variation (CV) with standardised differences to characterise magnitudes (effect sizes, Cohen's *d*).

Results: Tropical residents exhibited significantly lower ratings of perceived exertion than Temperate (-2, ± 1 units, mean, $\pm 95\%$ confidence limits, RPE scale 6-20) in each of the three HSTs ($p = 0.03$, large difference). Tropical exhibited a ~1.5-fold ($p = 0.05$) greater concentration in post-exercise concentrations of IL-6 at HST$_1$, a ~3-fold greater pre-exercise concentration of LPS at HST$_2$ ($p = 0.02$), and a ~2-fold greater pre-exercise

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concentration of IgM at HST_2 (p = 0.04) and HST_3 (p = 0.02) than Temperate.

Conclusion: Tropical residents reported lower levels of exertion following strenuous exercise in the heat compared with temperate residents. It appears that tropical residents regulate LPS and IgM at higher resting concentrations than individuals who reside in the temperate zone. This effect may yield a minor physiological advantage to tolerate a greater degree of heat stress during longer duration and higher intensity exercise loads.

5.2 Introduction

Residents of the tropical zone purportedly display greater signs of heat tolerance and physiological adaptations to the heat than non-tropically acclimatised individuals (Lee et al., 2011; Saat et al., 2005; Taylor & Cotter, 2006). With approximately 40% of the world's population living in the tropics, a proportion projected to rise to 50% by the year 2050, many people reside in and visit these areas every year (Harding, 2011). Studies have typically focused on differences in physiological thermal adaptations such as resting core temperature, sweating response, skin temperature, and heart rate response to exercise (Wakabayashi et al., 2011; Wijayanto et al., 2012). However, to the author's knowledge, no previous studies have investigated differences in the immune and inflammatory effects of exercise in the heat between residents of the tropical and temperate zone. Furthermore, it is unknown if repeated heat exposures influence the inflammatory responses to exercise in the heat in either of these populations.

Quantifying the biological and thermoregulatory responses to exercise in the heat between tropical and temperate natives is an important consideration for safety, event management and competition preparations. Recreationally-active healthy adults often participate in one-off events such as a triathlon, marathon and week-long sporting events such as the Masters' Games. These events can take place in thermally-challenging locations around the globe, and athletes from either the tropical or temperate zones may compete in challenging environments depending on their usual residency. As the threshold for the onset of exercise-induced endotoxaemia and heat stress is lower in untrained than trained individuals (Selkirk et al., 2008), it is important to manage the risks of adverse consequences of strenuous exercise in unfamiliar environments. These processes are not only important for host defence against pathogens, but also in the

sporting and physical activity context to provide tissue healing from the acute or chronic stress of exercise, sport, physical and occupational activities.

The adaptations of tropical residents to exercise in the heat can differ between residents of the tropical and the temperate zones. Tropical natives show smaller increases in rectal temperature (0.5 ℃ lower), lower skin temperature, lower sweat rates in the forehead and thigh regions, and longer sweating onset times (Wakabayashi et al., 2011; Wijayanto et al., 2012). Despite displaying these adaptations to the heat, tropical natives can still suffer a decrement in exercise performance in a hot and humid environment of up to 30% compared with exercising in a thermo-neutral environment (Voltaire, Berthouze-Aranda, & Hue, 2003). Furthermore, tropical natives exhibit a biphasic sweating response (increased sweat rate in the middle of the training protocol followed by return to initial values by the end of it) to heat acclimation as well as moderate-large reductions in exercising heart rate (-3%) and core temperature (-1%) (Magalhães et al., 2006). While differences in thermoregulation and potential for physiological adaptation are likely between tropical and temperate natives, high heat and humidity can detrimentally affect performance and physiological responses in both of these populations. Therefore, quantifying the immune and inflammatory responses, coupled with other thermoregulatory and perceptual responses to exercise-induced heat stress, should provide further insights on the differences (and possible consequences) in thermoregulation exhibited by residents of the tropical zone compared with their temperate location counterparts.

The interaction between pro-inflammatory, anti-inflammatory, and immuno-regulatory cytokines during and after exercise is complex, situation-specific, and likely dependent on environmental conditions, demands of exercise, and individual level of

fitness. A marked inflammatory response after heat stress is involved in both damage-generating processes and repair mechanisms during the recovery phase after strenuous exercise (Pyne et al., 2014). Interleukin-6 (IL-6) acts as both a pro- and anti-inflammatory cytokine and myokine, and, during exercise, much of its release comes directly from muscle (Shephard, 2002). Both duration of exercise and heat load can influence the magnitude of the IL-6 response (Fischer, 2006). In certain pathological states such as trauma, sepsis, and thermal injury pro-inflammatory cytokines are released into the circulation (Martin et al., 1997), although this effect may be blunted by repeated exposures to exercise in the heat (Hailes et al., 2011). These post-exercise changes are not necessarily affected by heat acclimation training (Guy, Pyne, et al., 2016), however, repeated bouts of exercise in hot and challenging conditions may impose a protective inflammatory benefit (Hailes et al., 2011), as well as thermoregulatory acclimatisation effects such as reduced exercising heart rate, reduced core temperature, and improved perception of effort (Tyler et al., 2016). Furthermore, an abundance of circulating lipopolysaccharide (LPS) that has translocated from the gut into circulation due to heat stress can evoke an inflammatory response, leading to heat shock and overwhelming anti-LPS mechanisms including immunoglobulin M (IgM) (Camus et al., 1998). Consequently, when anti-LPS mechanisms and rate of LPS clearance are inadequate to counter the heat-induced increase of LPS, endotoxaemia may ensue.

IgM is a key antibody in neutralising LPS, and its concentration in circulating blood can reflect the body's response to endotoxin accumulation, and degree of protective capacity in the event of further challenges. Reports of IgM concentrations have been conflicting with claims for both substantial increases and decreases following exercise in the heat (Camus et al., 1998; Hailes et al., 2011; Lim et al., 2009). Few, if any, studies have considered the influence of long-term residence (e.g. $\geq$ 12 months) in individuals

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living and working in the tropical zone vs. those resident in the temperate zone. The response of biomarkers such as LPS, IgM and IL-6 to heat stress should indicate whether there is an underlying difference in response to heat stress between matched individuals based on their long term residency in either tropical or temperate environments. Furthermore, examination of the variability and reliability of biomarkers at rest, and also following a strenuous bout of exercise in the heat, has not yet been investigated between residents of the tropical and temperate zones. The concentrations of IL-6, LPS, and IgM may be influenced by acclimation and/or training status, however, to date, no studies have identified whether or not living in a tropical or temperate environment effects the regulation of these biomarkers, or if they respond differently following exercise in the heat.

The %coefficient of variation indicates substantial variability in these biomarkers: IL-6 (19%), LPS (23%) and IgM (28%). Furthermore, the pre- to post-exercise change in these markers is not always discernible from this normal variation (Guy, Edwards, Miller, Deakin, & Pyne, 2016). Therefore, if the "signal" of the biomarkers is less than the "noise" it is more difficult to determine whether the exercise effects are clinically/practically important. Furthermore, while increases in blood biomarkers associated with heat stress, inflammation and immune function may occur, normal variability should be considered when biological and physiological comparisons are made between residents of tropical and temperate climates in response to exercise in the heat. A comparison of these effects between populations is needed to develop climactically-relevant thresholds for these markers.

The aim of this study was to compare the thermoregulatory, perceptual, and immune and inflammatory responses to repeated bouts of strenuous exercise in the heat between male residents in tropical and temperate environments. It was hypothesised that participants who reside in a tropical climate would experience less inflammation and physiological stress during a matched cycling task in hot and humid conditions compared with those that live in the temperate zone

5.3 Methods

Study Design

Sixteen healthy males participated in this study and were allocated to two groups (Tropical and Temperate) based on their geographical residence in a repeated measures between measures design. Power analysis determined the n = 8 for each group was sufficient to detect the smallest worthwhile change in exercise performance in the heat. The Tropical group comprised of participants who resided in Far North Queensland, Australia (Longitude 16.9° S, 145.8° E) while the Temperate group resided in South West England, United Kingdom (50.4° N, 4.1° W). Briefly, both groups completed a baseline measure of maximal oxygen consumption (VO_2 max), followed by three separate Heat Stress Tests (HST) seven days apart. Three tests were chosen to determine the initial and repeated effects of exercise in the heat, while providing adequate recovery between each bout. All exercise was performed on a cycle ergometer (Tropical: Velotron, RacerMate, USA; Temperate: Wattbike Pro, Wattbike, UK) and the HSTs were performed in an environmental chamber at a temperature of 35 ºC and 70% relative humidity (RH), wind speed ~1.5 m.s^{-1}. This temperature was selected to reflect the hotter temperatures experienced during summer in Far North Queensland, Australia.

Participants

Participants were matched by age, maximal oxygen uptake (VO$_2$ max), height and mass, and drawn from local community sporting clubs and categorised as recreationally active (Table 5.1). Prior to taking part in the study all participants completed an informed consent form and a pre-screening health questionnaire including use of NSAIDS immunomodulating medications (none were present). Participants were included if they were male aged between 18-30 years, involved in exercise 3-5 times a week, and who identified as white Caucasian. This criteria was to limit the variation of responses to the heat that may occur due to ethnic differences (Taylor & Cotter, 2006). Participants were instructed to refrain from other training while taking part in the study, and not to undertake any strenuous exercise within 48 h of testing sessions. All protocols conformed to the declaration of Helsinki and were approved by both the James Cook University and the University of St Mark and St John Human Research Ethics Committees.

Table 5.1. Characteristics of Tropical and Temperate participants

Group	Height (m)	Mass (kg)	Age (yrs)	VO$_2$ max (mL.kg^{-1}.min^{-1})	PPO (Watts)
Tropical (n=8)	1.76 ± 0.10	82.7 ± 13.6	23.0 ± 3.6	42.7 ± 6.7	255 ± 38
Temperate (n=8)	1.79 ± 0.10	78.5 ± 7.7	23.0 ± 4.9	41.5 ± 4.8	263 ± 25
p value	0.36	0.45	1.00	0.92	0.65

Tropical participants resided in a tropical location (Cairns, Australia), and Temperate resided in a temperate location (Plymouth, United Kingdom). VO$_2$ max, maximum oxygen uptake, PPO Peak Power Output. Participants were matched by age, VO2 max, height and mass. There were no significant physical differences between groups

Test of Maximal Oxygen Uptake

VO$_2$ max was determined by an incremental test to exhaustion on a cycle ergometer (Tropical: Velotron, RacerMate, USA; Temperate: Wattbike Pro, Wattbike, UK) in a

thermo-neutral environment (20 °C and ~50% RH). Briefly, the test began with participants cycling at 80-90 rpm at 120 W, with the workload increasing by 20 W every min until volitional exhaustion or when cadence was unable to be maintained above 80 rpm. Throughout the VO_2 max cycling protocol the composition of expired CO_2 and O_2 was analysed by a metabolic cart (Tropical: Moxus Metabolics Measurement cart, AEI Technologies, United States, calibrated with 20.93% O_2 and 7% CO_2; Temperate: Cortex Metalyzer 3b, Biophysik GmbH, Germany, calibrated with 17% O_2 and 5% CO_2). Attainment of VO_2 max was determined by satisfaction of standard criteria (Midgley et al., 2007). Heart rate was recorded at 5 s intervals (Polar RS400, Polar Elektro, Finland). The maximum power output (Watts) reached was used to determine the cycling intensities for the subsequent Heat Stress Tests (HST).

Heat Stress Test

The HST involved the same design as described previously (Guy, Pyne, et al. 2016) and comprised cycling for three x 10 min sub-maximal workloads with a 3 min rest period between workloads, followed by a 5 km self-paced time trial to exhaustion (TT). Each HST was performed in an environmental chamber (custom built environmental chamber, Cairns, Australia, and Environmental Chamber, TESS, UK) at a temperature of 35 °C and 70% RH, airflow 1.5 m.s^{-1}. After a 5 min standardised warm-up (consisting of 3 min cycling at an RPE of 8 or 9 (Borg RPE 6 – 20 scale) (Borg, 1998) followed by 2 min of dynamic stretching), the participants completed three 10 min workloads at 50%, 60% and 70% of their peak power output corresponding to their individualised VO_2 max, with a 3 min rest between workloads. Following the completion of the 70% workload, a 5 min rest period was given before the start of the 5 km TT. Participants were able to view their rpm and informed of the distance travelled every 500 m to assist with pacing.

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Heart rate (RS400, Polar Elektro, Finland), and core temperature (T_c) (ttec 501-3 data logger and data logger software version 10.1, Nordex Pty Ltd, Australia; MEAS 449 1RJ rectal temperature thermistor, Measurement Specialities, United States, self-inserted 8 cm past the rectal sphincter) were sampled at 5 s intervals. Rating of perceived exertion (Borg RPE 6 – 20) (Borg, 1998) was recorded throughout the test and reported as end-point RPE. Nude dry body mass was recorded pre- and post-exercise on a calibrated set of scales (BF-522W, Tanita, Japan) and body mass was adjusted for fluid loss and fluid intake (water, *ad libitum)* and expressed as a percentage change from initial body mass.

Blood collection

Upon arrival at the laboratory, participants rested for 20 min before blood collection was performed. Blood was drawn in a seated position 10 min before and 10 min after each HST via a 22 gauge needle from a prominent superficial forearm vein located at the antecubital fossa, and drained directly into an 8.5 mL sterile serum separator Vacutainer tube containing a clot activator and gel for serum separation (Beckton and Dickson, USA). Samples were refrigerated at 4 ºC for 30 min to allow clotting and then centrifuged at 1000 x g at 6 ºC for 10 min (Tropical: Rotina 420R, Hettich, Germany, Temperate: BR401, Denley Instruments Ltd. UK). Serum was removed and stored in 400 µl aliquots that were frozen immediately for a maximum of three months at -80 ºC for later analysis. Serum concentrations of IL-6 (Quantikine HS600B, R&D Systems, United States), IgM (AB137982, Abcam PLC, United Kingdom), and LPS (HIT302, Hycult, Biotechnology, Netherlands) were analysed in duplicate by ELISA according to manufacturer's instructions. Inflammatory analyte concentration was not adjusted for the decrease in plasma volume from pre-to post-exercise so that comparisons to the available

human experimental and clinical, and animal studies, none of which have adjusted for possible plasma volume shifts, could be made (Hailes et al. 2006).

Statistical Analysis

Responses to exercise within and between groups

Descriptive statistics (mean ± SD) were used to summarise the physical and performance characteristics of the two groups. The percentage change after exercise has been presented as mean, ±95% confidence limits (CL). Differences between groups were evaluated with a t-test for unpaired samples or a split plot analysis of variance for within and between group analyses where appropriate. Between group differences were further investigated with a post hoc Tukey test. Significance was accepted at $p < 0.05$. Analyses were undertaken using the statistical package for social sciences (SPSS version 22, IBM, USA). Between group differences were also calculated as standardised effects (Cohen's *d*). Criteria for interpreting magnitudes were as follows: trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater) (Cohen, 1992).

Biomarker analysis

As well as determining and comparing the changes in the raw concentrations of blood biomarkers, within group biomarker reliability was calculated as the mean coefficient of variation (CV) across the three HSTs and presented as mean CV, ±95% CL. Between group CV and pre- to post-exercise CV in biomarker concentrations were analysed with unpaired and paired t-tests where appropriate and significance was accepted at $p < 0.05$. Effect sizes for differences in biomarker CV were also calculated. This was used to determine if any observed changes and variability in raw concentrations were greater

than what would be expected under normal resting circumstances (Guy, Edwards, et al., 2016).

Determination of biomarker concentrations and curve fit analysis was performed using GraphPad Prism Version 6.03 (GraphPad Software Inc, United States) according to the manufacturer's instructions. The manufacturer stated intra-assay precision was <10% for all assays. Power analysis was conducted prior to the study and a minimum of eight participants was deemed sufficient to detect the smallest worthwhile change between means in 5 km TT time (Garrett et al., 2011). The assumptions were that the reference change in TT time would be approximately twice the magnitude of the typical error of measurement with a Type I error of 5% and Type II error of 20%.

5.4 Results

Physiological responses to Heat Stress Tests

The Tropical group reported lower scores of end-point RPE (-2, ±1 units, mean, ±95% confidence limits) in each of the three HSTs (large effects, p≤ 0.03). Both groups exhibited similar heart rate responses during HST_1 and HST_2. However, in HST_3 the Tropical group exhibited moderately greater reductions in heart rate from HST_2 (-5%, ±3%, p = 0.04) (Figure 5.1 and Table 5.2).

Blood biomarker responses to exercise in the heat

Tropical residents exhibited higher post-exercise concentrations of IL-6 at HST_1 (ES = 1.01, p = 0.05) than Temperate (Figure 5.3a). Tropical had higher pre-exercise concentrations of LPS than Temperate at HST_2 (ES = 1.15, p = 0.02) (Figure 5.3b). The Tropical group also had higher resting concentrations of IgM than Temperate at both HST_2 (ES = 1.02, p = 0.04) and HST_3 (ES = 1.16 p = 0.02) (Figure 5.3c).

The variability of resting concentrations for IL-6 was significantly higher in the Temperate group (ES = 1.46, p = 0.01). In contrast, the Tropical group had substantially larger variability in post-exercise concentrations of LPS (ES = 1.68, p = 0.01) and IgM (ES = 1.14, p = 0.03) (Table 5.3).

Table 5.2. Coefficient of variation in immune biomarkers pre- and post-exercise in the heat between Tropical and Temperate residents.

	CV pre-exercise	p-value	Difference	CV post-exercise	p-value	Difference
IL-6						
Tropical	36%, ±21%	0.01*	Large	24% , ±13%	0.64	Small
Temperate	85%, ±30%			21% , ±11%		
LPS						
Tropical	38% , ±14%	0.42	Trivial	71% , ±16%	0.01*	Large
Temperate	48% , ±24%			33% , ±19%		
IgM						
Tropical	42% , ±17%	0.11	Moderate	46% , ±22%	0.03*	Large
Temperate	26% , ±16%			21%, ±9%		

Data expressed as mean coefficient of variation, ±95% CL. IL-6, interleukin-6. LPS, lipopolysaccharide. IgM, immunoglobulin M. Criteria for interpreting magnitudes of standardised effects were as follows: trivial 0.0 – 0.2; small 0.2 – 0.6; moderate 0.6 – 1.2; large 1.2 – 2.0; very large >2.0. * Significant between group difference.

5 km Time Trial Performance

Both Tropical and Temperate participants were able to complete the sub-maximal and time trial components of all three HSTs. Due to differences in cycle ergometers for the 5 km TT across locations, outcomes were evaluated by standardised unit-less comparisons using % change. Neither group improved their 5 km TT performance in the second or third HST, nor were there clear differences between groups after accounting for the different baseline performances (Figure 5.2).

Table 5.3. Physiological and perceptual responses of Tropical and Temperate residents to three Heat Stress **Tests separated by one week's recovery.**

Measure	HST_1	Between group difference (p-value)	HST_2	Between group difference (p-value)	HST_3	Between group difference (p-value)
HR $_{MEAN}$ (bpm)						
Tropical	158 ± 9	Trivial	157 ± 8	Trivial	153 ± 11	Moderate
Temperate	159 ± 11	(0.85)	157 ± 11	(0.93)	159 ± 10	(0.26)
$T_{C\,MEAN}$ (^{o}C)						
Tropical	38.22 ± 0.33	Trivial	38.14 ± 0.27	Moderate	38.12 ± 0.31	Trivial
Temperate	38.24 ± 0.34	(0.94)	38.31 ± 0.29	(0.23)	38.11 ± 0.23	(0.97)
$T_{C\,PEAK}$ (^{o}C)						
Tropical	38.80 ± 0.33	Moderate	38.76 ± 0.39	Moderate	38.76 ± 0.37	Moderate
Temperate	39.03 ± 0.40	(0.23)	39.01 ± 0.38	(0.22)	39.01 ± 0.35	(0.20)
RPE (units)						
Tropical	17 ± 1	Large	17 ± 1	Large	16 ± 2	Large
Temperate	19 ± 1	(0.01*)	19 ± 1	(0.03*)	19 ± 1	(0.01*)
Fluid Loss (% b.m^{-1})						
Tropical	1.7 ± 0.8	Moderate	1.5 ± 0.8	Moderate	1.7 ± 0.8	Moderate
Temperate	1.1 ± 0.4	(0.09)	1.1 ± 0.4	(0.15)	1.1 ± 0.4	(0.10)

Data expressed as mean $\pm$ SD. HST, heat stress test 1, 2 or 3. HR, heart rate. T_C, core temperature. RPE, rating of perceived exertion. Fluid loss is expressed as the volume of fluid lost as a percentage of body mass. Criteria for interpreting magnitude of standardised effects were as follows: trivial 0.0 – 0.2; small 0.2 – 0.6; moderate 0.6 – 1.2; large 1.2 – 2.0; very large >2.0.* Significant between group difference.
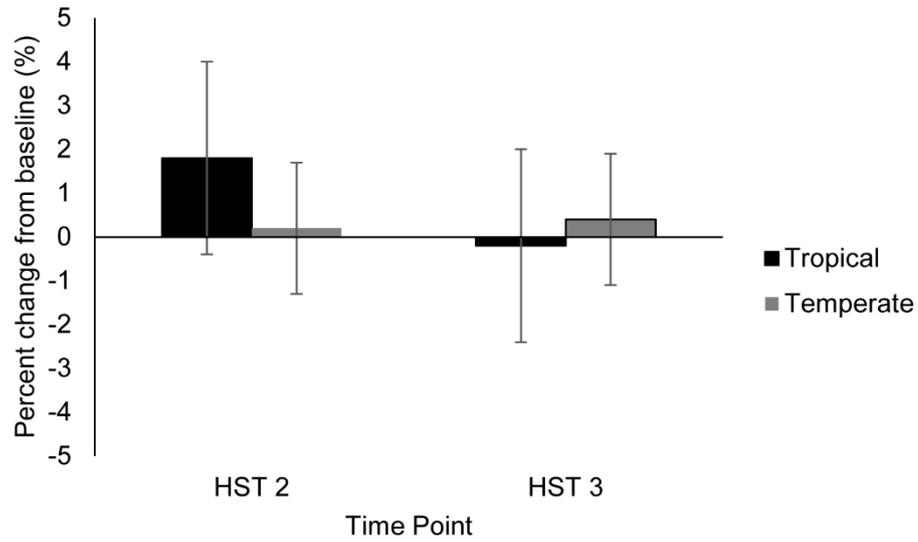
Figure 5.1. Percent change ± 95% confidence limits of 5 km time trial performance from baseline (Heat Stress Test 1) (HST) for Tropical and Temperate residents at HST 2 and HST 3. No substantial within- or between-group changes were observed.
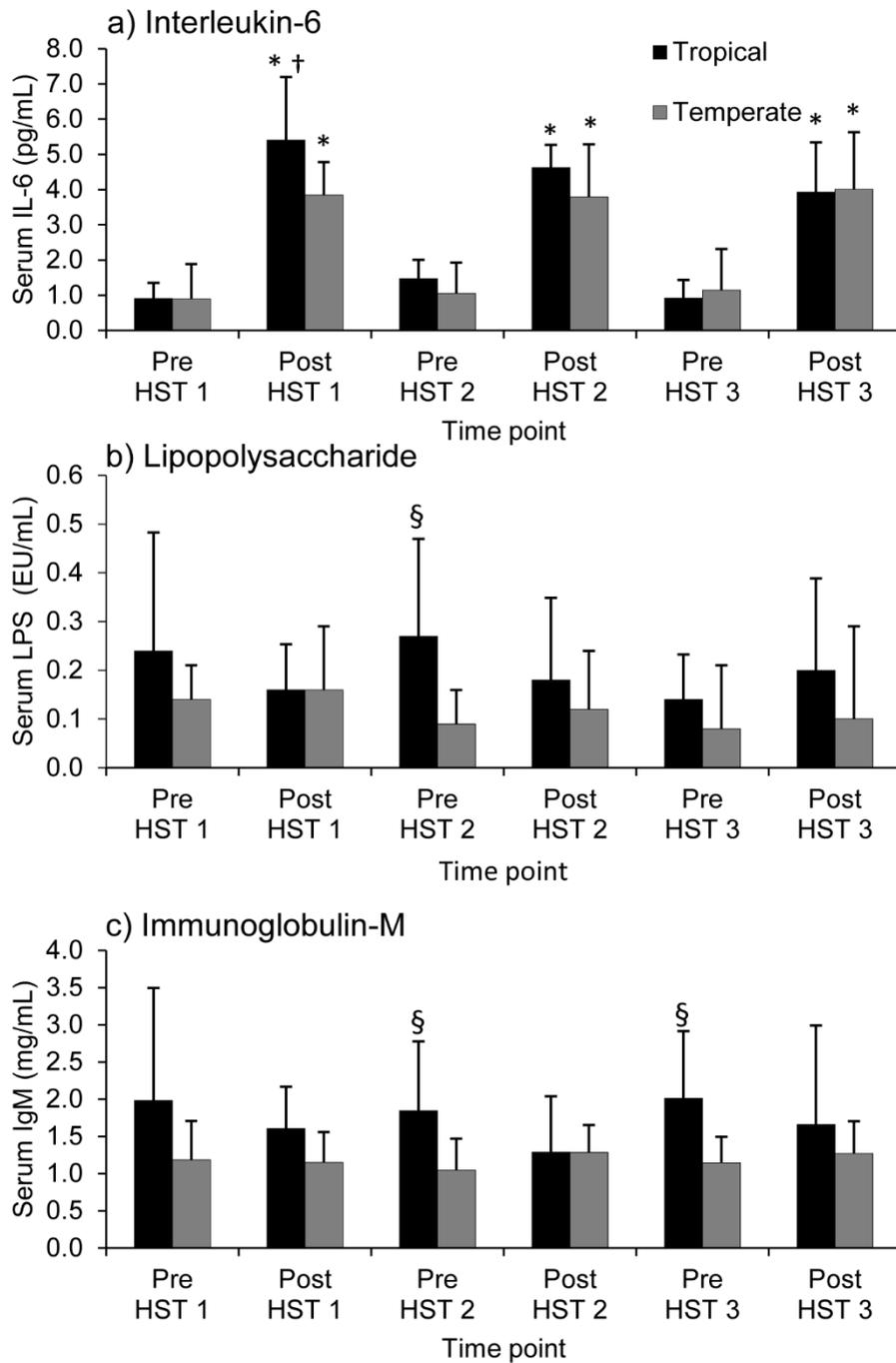
Figure 5.2. Mean blood biomarker concentrations ± SD (raw units) for (a) interleukin-6 (IL-6), (b) lipopolysaccharide (LPS) and (c) immunoglobulin M (IgM) pre and post Heat Stress Tests 1, 2, and 3 for Tropical and Temperate participants. * indicates a significant ($p<0.05$) within-group change pre- to post-HST. § indicates a significant ($p<0.05$) between-group difference in pre-exercise concentrations. † indicates a significant ($p<0.05$) between-group difference in post-exercise concentrations.

5.5 Discussion

Residents of a tropical climate reported lower levels of exertion following ~60 min of cycling in a hot and humid environment compared with their temperate counterparts. The Tropical residents also experienced higher post-exercise concentrations of IL-6 (~1.5 fold) following the first HST, higher pre-exercise concentrations of LPS (~3 fold) following the second HST, and higher pre-exercise concentrations of IgM (~2 fold) following the second and third HST. However, it appears that ~60 min of cycling in a hot and humid environment does not pose a clinically significant risk to immune function or inflammatory regulation in either of these populations. Furthermore, repeated bouts of exercise in the heat (separated by one week recovery) do not appear to alter the immuno-regulatory response following exercise, nor yield any performance benefits as might be expected by the effects of familiarity with the task.

The immuno-regulatory response to exercise in the heat can be characterised by alterations in cytokines and other inflammatory markers. While some differences were observed in the raw concentrations of some blood biomarkers, it is also important to consider whether these changes can be accounted for by normal homeostatic variability, and whether any changes are clinically relevant. Although tropical residents regulate resting concentrations of LPS (~3-fold greater) and IgM (~2-fold greater) at higher levels than their temperate counterparts, there does not appear to be a clear pattern in the response of these biomarkers following exercise in the heat, nor did these differences appear to be clinically relevant. The Tropical group experienced significantly greater variability in post-exercise concentrations of LPS (71%, ±16%, CV, ±95% CL) and IgM (46%, ±22%) compared with Temperate. Furthermore, the typical resting variation has been previously reported as 23%, ±13% for LPS, and 28%, ±17% for IgM (Guy, Edwards, et al., 2016). Therefore, the post-exercise variability in LPS and IgM observed

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in the Tropical group would appear to be greater than what would be expected to be due to normal homeostatic variation. This high CV suggests a less uniform biological response for LPS and IgM to exercise in the heat for Tropical participants. It has been previously reported that LPS concentrations can increase to ~1.1 EU.mL^{-1} following exercise in the heat (Barberio et al., 2015), therefore, the concentrations of the Tropical group (~0.3 EU.mL^{-1}) would not be considered dangerously high. The post-exercise concentration of circulating IgM for Tropical was slightly elevated compared with the normal range of ~0.5-1.5 mg.mL^{-1} for healthy male adults males aged 18-30 yrs (Gonzalez-Quintela et al., 2008). However, it is unlikely that the Tropical group would be at risk of developing endotoxaemia or a systemic inflammatory/immune response given the low level of immune disturbances observed.

Some participants in the Tropical group would have presumably experienced a small degree of heat stress on a day-to-day basis as part of their working days and recreational sporting activities in Cairns, Australia. Those individuals who worked and exercised indoors or during cooler parts of the day may not have developed underlying biological adaptations to the same extent as those that worked outdoors, resulting in more variable responses for the total cohort. Individuals who work outdoors may regulate resting concentrations of these markers at differing levels, in reaction to other external heat or pathological stressors causing greater resting concentrations of LPS. A consequence of an increased LPS concentration can be greater production of IgM as a defence mechanism against endotoxins (Lim et al., 2009), and this may have caused increased concentrations of IgM. Therefore, the variability in pre- and post-exercise concentrations may relate to individual adaptive responses within the group.

Circulating concentrations of IL-6 rose rapidly following exercise (~3 to 5-fold), an increase typical for the duration (~60 min) and intensity (incremental sub-maximal exercise followed by a short time trial) of the HST task (Guy, Pyne, et al., 2016). While Tropical residents experienced a greater post-exercise change in IL-6 following the first HST (5-fold) compared with the Temperate group (3-fold), these increases would not be considered near the critical threshold. Previous studies have reported post-exercise increases in IL-6 concentrations of 100-fold or more (Fischer, 2006), and, in this study, it would appear that the modest increases in IL-6 did not appear to be representative of a pathological insult such as the translocation of LPS. There was little variation in the resting concentrations of IL-6 in the Tropical group. However, the variability in the resting concentrations of IL-6 in the Temperate group (85%, ±30%) was approximately 4-fold greater than previously reported over a similar time period (19%, ±20%) (Guy, Edwards, et al., 2016). As elevated concentrations in this cytokine can be associated with reduced recovery from a large stress (Mihara, Hashizume, Yoshida, Suzuki, & Shiina, 2012), it is possible that some participants in the Temperate group may have been experiencing residual effects from the previous HST, resulting in greater variability in the resting concentrations of IL-6. Importantly though, this greater variability in pre-exercise concentrations did not appear to influence either the magnitude of change in IL-6 post exercise, or the post-exercise variability in IL-6 concentration. However, as both groups undertook exercise in a matched (hot and humid) environment and only experienced modest rises in core temperature (peak T_c ~39°C), it is unclear whether a similar response would be observed if matched exercise was undertaken in a thermo-neutral environment. Further work is required to clarify these responses in different ambient conditions and their biological significance for individuals.

While some moderate differences were observed in peak T_c (Tropical was lower by ~0.24 °C in each HST) and greater fluid loss (Tropical loss ~0.5% b.m^{-1} in each HST), these were not significant. Furthermore, heart rate was similar in each of the three HSTs, with Tropical experiencing a significant reduction in heart rate in the third HST compared with their first and second trials. This pattern of response indicates that the Tropical residents were beginning to show some adaptation to the exercise stimulus. Tropical natives can have lower sweat rates in the forehead and thigh regions than non-tropical individuals, but no difference in overall skin temperature or sweat rates (Wakabayashi et al., 2011; Wijayanto et al., 2012). In this study only overall fluid loss was measured, therefore, it cannot be ascertained if differences in local sweat rates or skin temperature were present. As the Tropical group also experienced a trend for moderately lower peak T_c during each of the three HSTs, it is possible this increased fluid loss resulted in greater evaporative cooling. In the absence of skin temperature data it is not possible to determine whether the increased fluid loss in the Tropical group resulted in advantageous cooling of the skin, resulting in the trend for lower T_c, however, this is the most likely explanation. Tropical natives can undergo a biphasic sweating response during heat acclimation, with an initial decrease in the sweating response during the initial period of adaptation (~5 days) followed by an increase in fluid loss following further acclimation training (~9 days) (Magalhães et al., 2006). However, as the Tropical participants in this study were not undertaking heat acclimation training, it is unlikely that any changes to the sweating response would occur. In fact, substantial changes in fluid loss do not always occur when tropical natives undertake short-term heat acclimation training (Guy, Pyne, et al., 2016). When comparing different populations, differences in morphology or cardiorespiratory fitness between tropical and temperate natives should be taken into account (Wakabayashi et al., 2011). However, in the present

study, the groups were ethnically, anthropometrically, and fitness matched. As RPE should reflect whole body effort, the effort experienced by the Tropical participants may have been affected by greater evaporative cooling due to larger fluid loss, resulting in lower T_c. Perceived effort during a self-paced task will also be largely influenced by the afferent feedback of skin and core temperature and heart rate (Abbiss et al. 2015). Therefore, the trend for increased fluid loss and reduced T_c in the Tropical group is suggestive of a distinctive adaptation to exercise in the heat compared with the Temperate participants, although further work is needed to clarify this effect. However, consideration should also be given to the sensitivity of the scale used in this study (Borg 6-20,. For example, different scales of varying sensitivity (i.e. Borg 6–20, 1–10 or 0–100), and utilizing different anchor points or descriptions, have been developed to monitor perceptions during exercise, as interpretation of any given perceptual scale is likely to depend on the anchor points or terminology used (Abbiss et al. 2015)

The three heat exposures that the participants undertook were not adequate to elicit any meaningful physiological adaptations or performance enhancement. Although the Tropical group exhibited moderate reductions in exercising heart rate compared with Temperate at the third HST, this did not translate to improved performance during the 5 km TT. Moreover, Tropical group did not exhibit reductions in either average or peak core temperature across the three HSTs. These adaptations to heat are usually realised following at least five days of training (Guy et al., 2015; Périard, Racinais, et al., 2015; Tyler et al., 2016), so it is therefore, rational that neither group improved their performance or experienced meaningful adaptations from only three heat exposures, each separated by seven days recovery. A greater and more frequent stimulus would be required to elucidate whether tropical or temperate residents experience differential thermoregulatory adaptations to heat acclimation training.

5.6 Conclusion

Recreationally active males who reside in a tropical climate report lower perceived exertion in comparison to their temperate counterparts during ~60 min of strenuous cycling in a hot and humid environment. Furthermore, repeated bouts of the same exercise stimulus appear to yield modest improvements in exercising heart rate for tropical residents. A trend for greater fluid loss and lower T_c in tropical residents during exercise in the heat suggests some differences in thermoregulation between these two cohorts may be present. Although higher post-exercise concentrations of LPS and IgM were observed in the Tropical residents, these differences do not appear to influence exercise performance in the heat, nor are they reflective of severe endotoxaemia or immune disturbances. Consequently, moderate to intense endurance exercise should not impose a substantial immune threat to recreationally active males who reside in either tropical or temperate zones.

5.7 Chapter progression

This study has shown that repeated bouts of exercise in the heat do not cause a systemic inflammatory response in residents of the tropical or temperate zones. However, multi-day heat acclimation training programs may cause a greater inflammatory response or result in exercise induced endotoxaemia due to the cumulated heat and training load that may occur as a result of multi-day training in the heat. Chapter Six investigates the physiological and performance responses to an intense short-term heat acclimation program. Furthermore, the effects of periodic "top up" sessions were also investigated to determine if they were a viable intervention to the initial adaptations that are realised during short-term program.

(1) Introduction

(2) Review of STHA and MTHA training programs

(3) Review of inflammatory mediators in reponse to exercise in the heat

(4) Reliability of Serum Biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between tropical and temperate natives

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

(7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and synthesis, future directions, and conclusions

6. Acclimation training improves endurance cycling performance in the heat without inducing endotoxaemia.

Guy, J. H., Pyne, D. B., Deakin, G. B., Miller, C. M., & Edwards, A. M. (2016). Acclimation training improves endurance cycling performance in the heat without inducing endotoxaemia. *Frontiers in Physiology*, *7*.

6.1 Abstract

Aim: While the intention of endurance athletes undertaking short-term heat training protocols is to rapidly gain meaningful physical adaption prior to competition in the heat, it is currently unclear whether or not this process also presents an overt, acute challenge to the immune system. The aim of this study was therefore to examine the effects of heat training on both endurance performance and biomarkers associated with inflammatory and immune system responses.

Method: Moderately-actively males (n=24) were allocated randomly to either HOT (n=8, 35 ºC and 70% RH; NEUTRAL (n=8, 20 ºC and 45% RH); or a non-exercising control group, (CON, n=8). Over the 18 day study HOT and NEUTRAL performed seven training sessions (40 min cycling at 55% of VO_2 max) and all participants completed three heat stress tests (HST) at 35 ºC and 70% RH. The HST protocol comprised three x sub-maximal intervals followed by a 5 km time trial on a cycle ergometer. Serum samples were collected before and after each HST and analysed for interleukin-6, immunoglobulin M and lipopolysaccharide.

Results: Both HOT and NEUTRAL groups experienced substantial improvement to 5 km time trial performance (HOT -33, ±20 s, p = 0.02, NEUTRAL -39, ±18 s, p = 0.01, mean, ±95% confidence limits) but only HOT were faster (-45, ±25 s and -12, ±7 s, p = 0.01) in HST_3 compared with baseline and HST_2. Interleukin-6 was elevated ~4 fold after exercise for all groups, however, there were no significant changes for

immunoglobulin M or lipopolysaccharide.

Conclusion: Short-term heat training enhances 5 km cycling time trial performance in moderately-fit subjects by ~6%, similar in magnitude to exercise training in neutral conditions. Three top-up training sessions yielded a further 3% improvement in performance for the HOT group. Furthermore, the heat training did not pose a substantial challenge to the immune system.

6.2 Introduction

Short- and medium-term heat acclimation training protocols are widely used by endurance athletes to increase both heat tolerance and subsequent competitive performances in the heat (Périard, Racinais, et al., 2015). Although favourable performance and physiological benefits can be realized from short-term programs (≤ 7 days), greater benefits are likely from longer protocols (7-14 days) (Daanen et al., 2011; Guy et al., 2015; Lorenzo et al., 2010; Nielsen et al., 1997). For elite athletes, busy training and performance schedules limit the time available for strategies such as heat training, and addition of supplementary training sessions may sustain and/or complement the initial adaptations.

While the acute effects of short-term heat exposure on blood biomarkers associated with inflammation have been reported (Gill et al., 2015; Hailes et al., 2011), few studies have investigated the effects of longer duration heat training. The human immune system can usually deal with mild-to-moderate inflammatory responses, however, when a heat stimulus is too large, systemic inflammation can result in heat shock and potentially fatal sepsis (Bouchama et al., 2007). Athletes will generally seek a heat training stimulus that is large enough to evoke a training adaptation; however, there likely comes a point where the risk of clinical or subclinical levels of immune disturbance increases.

Exercise-induced endotoxaemia is a potential risk of strenuous activity in the heat, primarily attributed to translocation of lipopolysaccharide (LPS) from the gut into the circulation (Lim et al., 2009). An abundance of circulating LPS can evoke an inflammatory response, leading to heat shock and overwhelming anti-LPS mechanisms including immunoglobulin M (IgM) (Camus et al., 1998) and cytokines operating in an anti-inflammatory role such as interleukin-6 (IL-6) (Abbasi et al., 2013). Consequently,

when anti-LPS mechanisms and rate of LPS clearance are inadequate to counter the heat-induced increase of LPS, endotoxaemia may ensue. This outcome could potentially occur during a period of heat acclimation training if the athlete is unable to cope with the thermal loads presented. As IgM is a key antibody in neutralising LPS (Camus et al., 1998), its concentration in circulating blood can reflect the body's response to endotoxin accumulation, and the degree of protective capacity in the event of further challenges. IgM concentration can increase substantially (~20%) after exercise in the heat, although this elevation does not occur following five days of heat training (Hailes et al., 2011). Of the few studies that have investigated IL-6 as a blood biomarker during exhaustive exercise in the heat, Selkirk and colleagues (2008) observed a twenty-fold increase in plasma concentrations following 2 h of exhaustive walking in protective clothing in very hot and humid conditions, with IL-6 inhibiting endotoxin induced increases in tumour necrosis factor alpha and cytokines. Furthermore, the neuroinflammatory response to exercise indicates that due to an increase in cytokine concentration such as IL-6 reaching a critical threshold, it is likely that sensations of fatigue develop to prevent traumatic injury of specific organs and other physiological systems within the body (Vargas & Marino, 2014). Therefore, athletes who undertake short or medium duration heat acclimation training programs could potentially be at increased risk of exercise-induced heat stress and immune disturbances associated with fatigue.

Recreationally-active healthy adults often participate in one-off events such as an ironman triathlon, marathon and week-long sporting events such as the Masters' Games. It appears that the threshold for the onset of exercise-induced endotoxaemia is lower in untrained than trained individuals (Selkirk et al., 2008). Individuals seeking to use heat acclimation training as an additional training stimulus may choose either a short- or medium-term program, to elicit the classic thermal markers of plasma volume expansion,

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lower heart rate at sub-maximal intensities and lower end point core temperature, which collectively promote aerobic performance (Guy et al., 2015). However, addition of a heat load to training can often be very demanding, with some studies implementing challenging protocols on their participants, e.g. 90 min of cycling for 10 consecutive days (Gibson et al., 2015). It is prudent to account for both training load and accumulated inflammation from heat stress over the training period. As longer heat training sessions (>60 min) are likely fatiguing for recreationally-trained athletes, and can increase peripheral fatigue compared with shorter protocols (Wingfield et al., 2016), the addition of shorter and supplementary training sessions could yield similar benefits, but with lower overall stress.

Few studies have investigated the degree of inflammation and endotoxaemia associated with short- and medium-term heat acclimation training. Therefore, the aim of this study was to investigate whether short-term heat training with the addition of supplementary sessions can improve cycling time trial performance (TT), improve sub-maximal exercising heart rate and core temperature, and to quantify the degree of inflammation associated with heat acclimation training. It was hypothesised that participants undertaking HA training are unlikely to experience significant risks to health and immune function and that the implementation of "top up" HA training every third day following an initial short-term HA program would result in the retention of initial physiological adaptations and cycling performance in the heat.

6.3 Methods

Design

This study consisted of three groups of recreationally-active male athletes: a heat training group (HOT), a matched thermo-neutral training group (NEUTRAL) and a

control (no training) group (CON), in a pre-post parallel groups design. Participants were randomly allocated into each training group by baseline aerobic capacity.

Participants

Twenty four moderately trained male participants (3 ± 1 moderate-high intensity training sessions per week, duration 60 ± 15 min; mean ± SD) aged 24.5 ± 3.8 years, height 178 ± 7 cm, mass 84.6 ± 10.8 kg, body fat 17.5 ± 6.1%, and maximal oxygen uptake (VO_2 max) of 45.0 ± 5.0 $mL.kg^{-1}.min^{-1}$ volunteered for the study. Prior to taking part, participants provided written informed consent in accordance with the Declaration of Helsinki and underwent a pre-screening health questionnaire including use of NSAIDS or immunomodulating medications (none were present). Participants were instructed to refrain from other training while taking part in the study, and not to undertake any strenuous exercise within 48 h of testing sessions. Participants were blinded to the existence of other groups as much as possible. Although, as the participants were drawn from local sporting clubs, some were able to differentiate which group they were allocated to. Both training groups (HOT and NEUTRAL) were informed that the purpose of the training was to improve heat tolerance, and the control group was informed that their heat tolerance was being assessed on three occasions to determine if the subsequent tests influenced the later tests. Participants in HOT, NEUTRAL and CON were matched based on PPO, aerobic capacity, height, age and weight. However due to the convenience sampling and availability of participants (i.e. some could not commit to the entire training program due to conflicts with other training), the CON group was comprised of participants with slightly differing physiological characteristics. The study protocol was approved by the James Cook University Human Research Ethics Council (Approval number H5647).

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Methodology

Assessment of VO$_2$ max was undertaken on a cycle ergometer (VeloTron and Velotron Coaching Software, Racermate, United States) at least 72 h before beginning the experimental trials. The intervention comprised a short-term training protocol of four training sessions on consecutive days, followed by three supplementary training sessions every three days. All participants completed three heat stress tests (HST$_{1-3}$) and seven training sessions over 18 days, with HST$_1$ performed as a baseline measure of heat tolerance, HST$_2$ completed between the end of the short-term program and before beginning the supplementary top-up training, and HST$_3$ completed 48 h after the final supplementary training session (Figure 6.1). Each group performed the HST in a custom-built environmental chamber at a temperature of 35 $^\circ$C and 70% RH (airflow ~1.5 m.s^{-1}). Participants in the HOT and NEUTRAL conditions completed exercise training sessions in hot and humid (35 $^\circ$C and 70% RH) or thermo-neutral conditions (20 $^\circ$C and 50% RH) respectively. These temperatures and workloads are similar to that as used previously to simulate exercise in a hot and humid environment (Garrett et al., 2009; Lorenzo et al., 2010). The duration and intensity of each exercise stage was selected as during pilot testing the moderately trained participants recruited for this study were unable to effectively sustain longer duration workloads at higher intensity. Participants in the CON group did not undertake exercise training but completed the three HSTs at the same intervals as HOT and NEUTRAL groups. Participants were instructed to rest and avoid moderate or high levels of physical activity on days that they were not required to attend the laboratory.
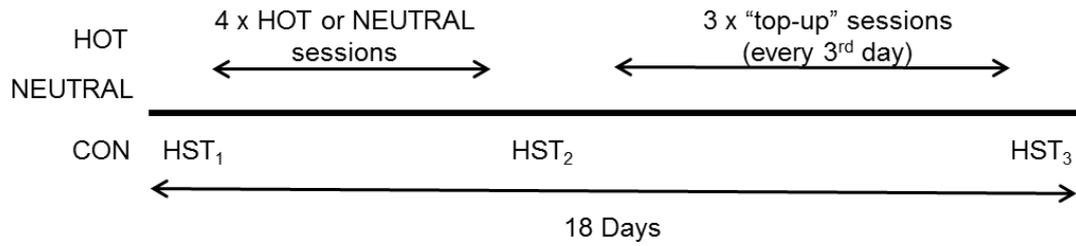
Figure 6.1. Study timeline for Heat Training (HOT), Thermo-neutral Training (NEUTRAL) and Control (CON) groups.

Test of Maximal Oxygen Uptake

Maximal oxygen uptake was determined by an incremental test to exhaustion on a cycle ergometer (VeloTron and Velotron Coaching Software, Racermate, United States). Briefly, the test began with participants cycling at 80-90 rpm at 120 W, with the workload increasing by 20 W every min until volitional exhaustion or when cadence was unable to be maintained above 80 rpm. Expired gases were collected via a one-way breathing system (Hans-Rudulph, United States) and analysed by a calibrated Moxus Metabolics Measurement cart (AEI Technologies, United States). Attainment of $\dot{V}O_2$ max was determined by the satisfaction of standard criteria (Midgley et al., 2007).

Heat Stress Test

The heat stress test was of similar design to earlier work (Garrett et al., 2009; Lorenzo et al., 2010) and comprised cycling for three x 10 min sub-maximal workloads with a 3 min rest period between workloads, followed by a 5 km self-paced time trial (TT). Following a 5 min standardised warm-up, the participants completed three 10 min workloads at 50%, 60% and 70% of their peak power output corresponding to their individualised VO_2 max. After the 70% workload was complete, a 5 min rest period was given before the start of the TT. Participants were able to view their rpm and were informed of the distance travelled every 500 m to assist with pacing. Heart rate (RS400,

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Polar Elektro, Finland), and core temperature (T_c) (ttec 501-3 data logger and data logger software version 10.1, Nordex Pty Ltd, Australia; MEAS 449 1RJ rectal temperature thermistor, Measurement Specialities, United States, self-inserted 8 cm past the rectal sphincter) were sampled at 5 s intervals. Fluid intake (water, *ad libitum*), rating of perceived exertion (Borg RPE 6 – 20) (Borg, 1998) and thermal comfort (ThC) were recorded throughout the test. Nude dry body mass was recorded pre- and post-exercise on a calibrated set of scales (BF-522W, Tanita, Japan) and body mass was adjusted for fluid loss and expressed as a percentage change.

Blood collection

Upon arrival at the laboratory, participants rested for 20 min before blood collection was performed. Blood was drawn in a seated position 10 min before and 10 min after each HST via a 22 gauge needle from a prominent superficial forearm vein located at the antecubital fossa, and drained directly into an 8.5 mL sterile serum separator Vacutainer tube containing a clot activator and gel for serum separation (Beckton and Dickson, USA). Samples were refrigerated at 4 ºC for 30 min to allow clotting and then centrifuged at 1000 x *g* at 6 ºC for 10 min (Rotina 420R, Hettich, Germany). Serum was removed and stored in 400 µl aliquots that were frozen immediately for a maximum of three months at -80 ºC for later analysis. Serum concentrations of IL-6 (Quantikine HS600B, R&D Systems, United States), IgM (AB137982, Abcam PLC, United Kingdom), and LPS (HIT302, Hycult, Biotechnology, Netherlands) were analysed in duplicate by ELISA according to manufacturer's instructions. Inflammatory analyte concentration was not adjusted for the decrease in plasma volume from pre-to post-exercise so that comparisons to the available human experimental and clinical, and

animal studies, none of which have adjusted for possible plasma volume shifts, could be made (Hailes et al. 2006).

Aerobic Interval Training

Participants in HOT and NEUTRAL undertook matched (absolute) aerobic interval training on a cycle ergometer (Monark Ergomedic 828 E, Sweden) in hot and humid (35 °C and 70% RH) or thermo-neutral conditions (20 °C and 50% RH) respectively. The exercise-training intervention included seven training sessions comprised of a standardised 3 min warm-up followed by 4 x 10 min interval at a fixed workload of 55% VO_2 max. A three min rest period was given between each workload and water was consumed *ad libitum*. A shorter duration interval-based protocol was chosen to better reflect the training status of the recreationally-trained participants; interval-based training has been shown to be beneficial for heat acclimation (Dawson, Pyke, & Morton, 1989; Kelly et al., 2016), and shorter duration training can reduce cumulative fatigue (Wingfield et al., 2016) while promoting performance (Nielsen et al., 1997). Heart rate was recorded at 5 s intervals and RPE and ThC recorded at the end of each interval. Participants self-reported symptoms of illness, infection, soreness or inflammation prior to the start of each training session. No symptoms of illness or infection were reported.

Statistical Analysis

Data that passed tests for homogeneity of variance were analysed using a mixed-model analysis of variance or t-test (where appropriate) and significance accepted when $p \leq 0.05$. Where significant differences were indicated they were identified with the *post hoc* Tukey Test. Data is expressed as mean ± SD and change scores expressed as mean, ±95% confidence limits (CL). The baseline TT performance (s) was not normally distributed and therefore analysis of covariance was used to investigate between-group

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differences with participant VO_2 max employed as the covariate - TT results are expressed as adjusted mean ± SD or 95% CL where appropriate. Standardised effect sizes (ES) were calculated to indicate the magnitude of change and/or difference within- and between-groups. The criteria to interpret the magnitude of ES were: <0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large, and >2.0 very large (Hopkins, 2004).

Determination of biomarker concentrations and curve fit analysis was performed using GraphPad Prism Version 6.03 (GraphPad Software Inc, United States) according to the manufacturer's instructions. The manufacturer stated intra-assay precision was <10% for all assays. Statistical analyses were performed in IBM SPSS Statistics Version 22 (IBM, United States). Power analysis was conducted prior to the study and a minimum of eight participants was deemed sufficient to detect the smallest worthwhile change between means assuming the reference change in 5 km time trial performance was approximately twice the magnitude of the typical error of measurement (Garrett et al., 2011)., with a Type I error of 5% and Type II error of 20%.

6.4 Results

Heat Stress Test

Between group analyses

At HST_3 a significant between-group effect for TT was evident between HOT and CON (HOT was faster by 8.2%, ±5.2%, 95% CL, p = 0.03). Time trial performance is presented in Figure 6.2 as adjusted means from the analysis of covariance. No significant between-group effects of short-term heat training were observed for T_c (0.3%, ±0.6%, Figure 6.3), RPE, ThC, sweat loss, or HR (Table 6.1).

Within group analyses

Both the HOT and NEUTRAL group significantly improved TT performance in HST$_2$ at the end of the seven days short-duration protocol (after four heat training sessions) compared with HST$_1$, with HOT 33, ±20 s (adjusted mean, ±95% CL) faster (p = 0.02) and NEUTRAL 39, ±18 s faster (p = 0.01) than baseline. After conclusion of the post-training top-up period, only HOT had a significant improvement in their TT performance at HST$_3$ compared with HST$_1$, completing the course 45, ±25 s faster (p = 0.01) compared with their HST$_1$ performance. The performance of HOT in HST$_3$ was also significantly improved from HST$_2$ (12, ± 7 s, p = 0.01).
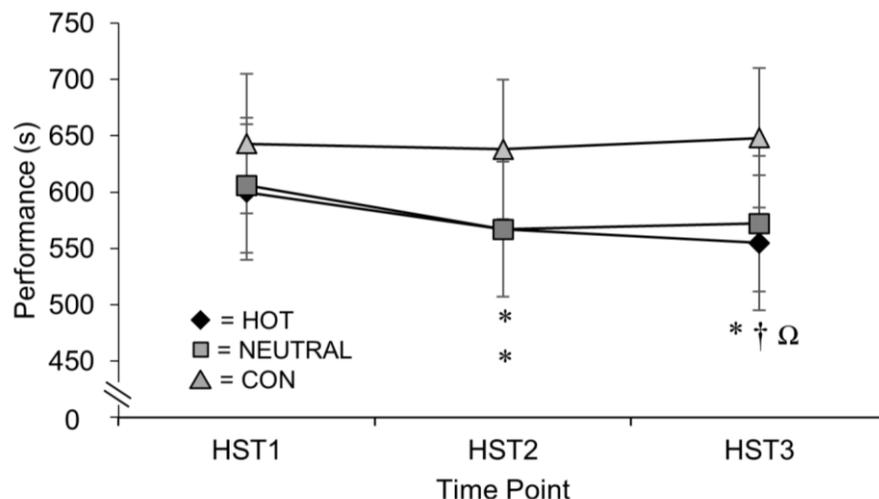

Figure 6.2. Adjusted means ± SD of 5 km time trial performance (s) across heat stress tests (HST) 1, 2 and 3 for Heat (HOT), Thermo-neutral (NEUTRAL) and Control (CON) groups following an initial short-term training block, followed by three top up training sessions. * Faster from baseline. † Faster than HST 2. Ω HOT was faster than CON.

There was a small but significant mean reduction in exercising T_c observed in the HOT group from HST$_1$ to HST$_2$ during the 60% workload of -0.22, ±0.14 °C (mean, ±95% confidence limits, p = 0.02, ES = -0.53). Additionally, there was a trend for lower T_c during the 70% workload (-0.25, ±0.21 °C, p = 0.06, ES = -0.53) and during the TT (-0.25, ±0.24°C, p = 0.09, ES = -0.45). Small to moderate significant reductions in T_c were

observed in the HOT group from HST_1 to HST_3 at the 50%; -0.18, ±0.10 °C (p = 0.016), 60%; -0.23, ±0.18 °C (p = 0.04) and 70%; -0.34, ±0.27 °C (p = 0.05) workloads. The HOT group also experienced a small reduction in peak T_c during HST_2 compared with HST_1; -0.25, ±0.21 °C (p = 0.057) (Figure 6.3). Neither the NEUTRAL nor the CON group experienced meaningful reductions in T_c in any of the HSTs (Figure 6.3).

The HOT group exhibited a moderate improvement in thermal comfort in HST_3 compared with HST_1 (p = <0.01). Thermal comfort was also improved in HOT during HST_2 and HST_3 compared with NEUTRAL (p = 0.04 and p = 0.03, respectively). There were no meaningful within group reductions of HR during the HSTs (Table 6.1).

Inflammatory biomarker responses

Between-group analyses

No significant differences between groups in any of the biomarker responses were observed either at rest or in response to any of the three HSTs. Between groups there was a ~8% ± 32% difference in post HST IL-6, ~52% ± 111% in LPS, and ~35% ± 36% in IgM.

Within-group analyses

There was a large to very large (~4 ± 2 fold) rise in serum IL-6 concentration for all groups following each HST. Serum concentrations of IgM and LPS were not substantially different following the HST for each group and there were no significant time interactions observed in any group. However, there was a trend for a small reduction in post-exercise concentrations of IgM in all participants (n=24) following the first HST (p = 0.08, ES = 0.40). There were no constant within-group changes observed in serum

concentration of LPS (44% ± 208%) or IgM (6% ± 61%) neither pre nor post each HST.

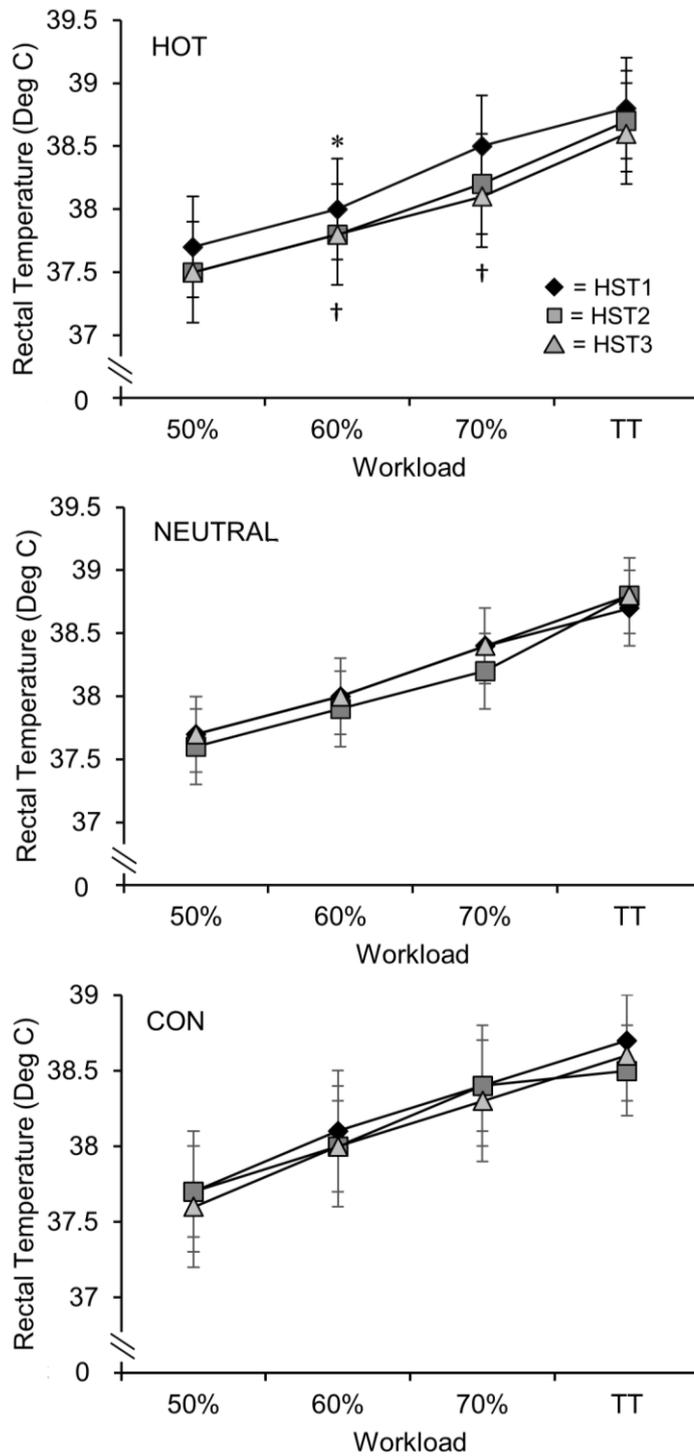Blood biomarker concentrations are presented in Figure 6.4.

Figure 6.3. Core temperature (mean ± SD)for Heat Training (HOT), Thermo-neutral Training (NEUTRAL) and Control (CON) groups during Heat Stress Tests (HST) 1, 2, and 3 following an initial short-term training block, followed by three top up training sessions,. * Reduced from baseline at HST 2. † Reduced from baseline at HST 3.

Table 6.1. Physiological and perceptual responses to Heat Stress Tests

	HST$_1$			HST$_2$			HST$_3$		
	HOT	NEUTRAL	CON	HOT	NEUTRAL	CON	HOT	NEUTRAL	CON
HR$_{50\%}$ (bpm)	139 ± 15	135 ± 12	137 ± 14	136 ± 15	133 ± 11	138 ± 13	136 ± 17	133 ± 10	133 ± 13
HR$_{60\%}$ (bpm)	162 ± 15	159 ± 9	157 ± 9	155 ± 14	154 ± 9	156 ± 9	155 ± 16	154 ± 11	153 ± 11
HR$_{70\%}$ (bpm)	175 ± 13	178 ± 7	170 ± 8	169 ± 13	172 ± 9	170 ± 6	168 ± 13	171 ± 9	167 ± 7
HR$_{TT}$ (bpm)	177 ± 11	178 ± 9	169 ± 10	176 ± 9	179 ± 6	168 ± 7	179 ± 10	175 ± 10	164 ± 12
RPE$_{Avg}$ (units)	14 ± 1	14 ± 1	15 ± 1	13 ± 2	14 ± 2	13 ± 1	13 ± 2	15 ± 3	13 ± 2
RPE$_{End}$ (units)	17 ± 2	17 ± 2	17 ± 2	17 ± 2	18 ± 2	17 ± 3	17 ± 2	17 ± 2	16 ± 3
ThC$_{Avg}$ (units)	3.0 ± 0.5	3.0 ± 0.5	3.5 ± 0.5	2.0 ± 1.0*	3.0 ± 0.5	3.0 ± 1 $^\Omega$	2.0 ± 1.0*†	3.0 ± 0.5$^\infty$	3.0 ± 0.5* $^\Omega$
ThC$_{End}$ (units)	4.0 ± 0.5	4.5 ± 0.5	4.5 ± 0.5	3.0 ± 1.0	4.5 ± 1.0$^\infty$	4.0 ± 1	3.0 ± 1.0*	4.0 ± 1.0	3.5 ± 1.0

Data are expressed as mean ± SD. HOT = Heat training group, NEUTRAL = Thermo-neutral training group, CON = Control group. HR = Heart rate. Sweat loss (%) is expressed as the amount of sweat lost (kg) divided by the persons pre-exercise mass (kg) x 100. RPE and ThC$_{Avg}$ are the mean Rating of Perceived Exertion and Thermal Comfort rating across the entire Heat Stress Test (HST). RPE and ThC$_{End}$ represent the values recorded at the cessation of the HST. *Significantly different from HST$_1$. †Significantly different from HST$_2$. $^\infty$ Significant difference between HOT and NEUTRAL. $^\Omega$ Significant difference between HOT and CON.
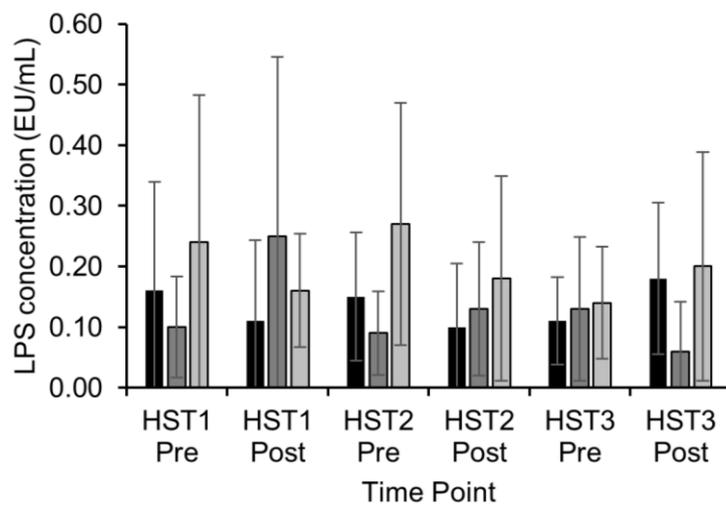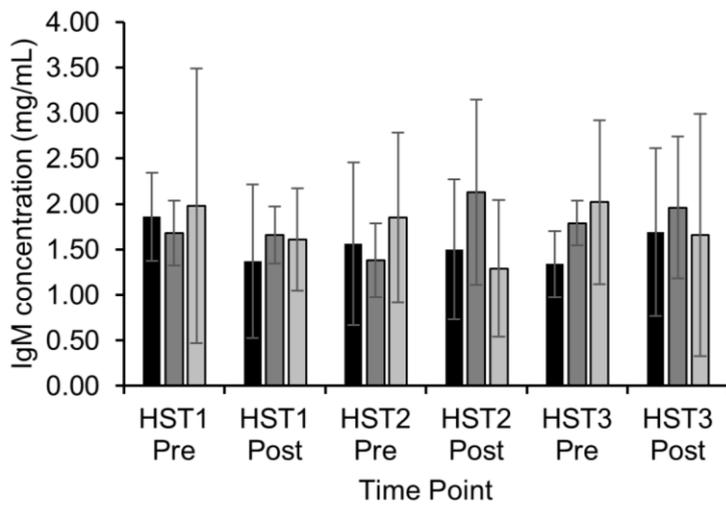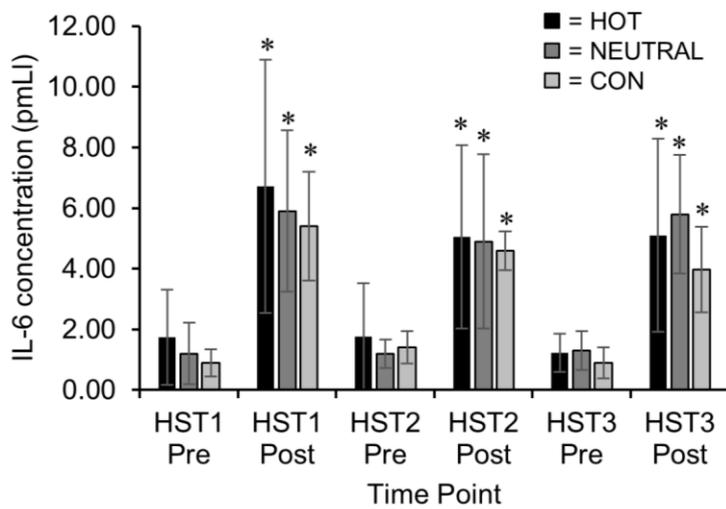
Figure 6.4. Serum concentrations of interleukin 6 (IL-6), Immunoglobulin M (IgM) and Lipopolysaccharide pre and post Heat Stress Tests 1, 2, and 3. * Increased from pre-exercise concentration.

Training sessions

There were no within-group changes observed in exercising heart rate during each of the training sessions for the HOT or NEUTRAL groups, although the HOT group exhibited higher HR in all training sessions compared with NEUTRAL. Table 6.2 outlines the physiological and perceptual variables collected during the interval training sessions.

Table 6.2. Physiological and perceptual observations during sub-maximal aerobic interval training from training sessions one, four, and the third top up session

	TR_1		TR_4		TU_3	
	HOT	NEUTRAL	HOT	NEUTRAL	HOT	NEUTRAL
HR (bpm)	161 ± 13	**145 ± 9∞**	157 ± 12	**145 ± 6∞**	154 ± 15	140 ± 13
RPE_{Avg} (units)	15 ± 1	15 ± 2	14 ± 2	15 ± 2	13 ± 3	**13 ± 1†**
ThC_{Avg} (units)	3.0 ± 1.0	3.0 ± 1.0	3.0 ± 1.0	3.0 ± 1.0	2.0 ± 1.0	3.0 ± 1.0

Data is expressed as mean ± SD. HOT = Heat training group, NEUTRAL = Thermo-neutral training group. TR_1 = Training session on day one, TU_3 = Top up training session on day 15. HR = Mean heart rate across four x 10 minute intervals. RPE_{Avg} and ThC_{Avg} are the mean Rating of Perceived Exertion and Thermal Comfort rating across the training session. * Significantly different from TR_1. † Significantly different from TR_4. ∞ Significant difference between HOT and NEUTRAL.

6.5 Discussion

Short-term heat training followed by supplementary top-up sessions (seven training sessions over 18 days) improved time trial cycling performance, reduced exercising core temperature, and improved thermal comfort during a strenuous cycling task in the heat. In contrast, participants in the thermo-neutral (exercise) and control conditions did not experience these physiological and perceptual improvements. However, as the thermo-neutral group also improved their 5 km TT performance after the initial short-term block of heat-training (five training sessions in seven days), it is likely a greater stimulus in terms of intensity and duration is required to elicit greater gains from heat training in

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shorter time periods. Although mean IL-6 concentration increased four-fold following each HST, the exercise stimulus did not elevate other biomarkers of systemic inflammation such as IgM and LPS. As biomarker activity was largely unaffected by short-term heat training, as evidenced by IL-6 returning to basal level prior to each HST, it appears that it is possible to gain useful performance and thermoregulatory adaptations from short-duration training without compromising the immune system. Therefore, coaches and athletes can use short-term heat acclimation training coupled with supplementary heat training sessions to improve time trial performance, in the confidence there is little likelihood of impairing immune system functionality.

Improvements in time trial performance with short-term heat training have been reported by Lorenzo et al. (2010) in cycling and Garrett et al. (2012) in rowing. However, Garrett and colleagues (2012) did not include a control group undertaking matched training over the five day heat training program. It is possible that the improvement (-4 s) observed in 2000 m rowing time in that study could have been similar to that of an exercise alone control/placebo group. In our study the effects of heat training were largely similar to that of the exercise-alone group during the first week of training. However, the supplementary top-up sessions appeared to elicit further gains, indicating that while short-term training offers some benefits a longer program offers additional benefits. In the study by Lorenzo and colleagues, one third of the experimental group (four out of twelve) were participants who had already completed the control condition of the experiment, consequently, the pre-exposure to exercise in the heat and heat stress test protocols. This prior exposure may have conferred a small degree of acclimation prior to taking part in the experimental portion of that study. In the present study, the inclusion of both an exercise matched (NEUTRAL) and control (CON) group allows clear interpretation of whether the heat acclimation training was responsible for the

reported changes in performance and physiological adaptations. Adaptations and improvements reported previously (Lorenzo et al. 2010; Garret et al. 2012) may relate to the increased frequency of training within a given training period. It is likely that the heat exposure resulted in ergogenic performance and thermoregulatory adaptations at the end of the 18 day period beyond that of exercise training alone.

The improved time trial performance by participants in HOT was matched by those in NEUTRAL at HST_2, indicating that the stimulus for performance gain over 7-days of short-duration training in moderately-trained individuals is exercise per se rather than the environmental conditions under which it is performed (i.e. hot or neutral). However, there were additional performance gains for the HOT group after completing the three supplementary training sessions over 10 days that increased HOT's total heat load to nine exposures (two HSTs and seven training sessions, approx. nine hours). Clearly, exercise in temperate conditions results in heat production that elevates body temperature (Gleeson, 1998), and, among recreationally-active participants, it seems probable that this heat production is a sufficient stimulus to generate modest adaptations over seven days. The observation of continued adaptation and performance improvement only in the HOT group after the post-training top-up period (after the full 18 days) suggests that the generic adaptive responses experienced by NEUTRAL after seven days had most likely run their course and plateaued. These additional gains experienced by HOT may also be related to the additional recovery that they received following the initial adaptation period and between each of the subsequent "top up" session. However the absence of objective measurements of fatigue such as Maximum Voluntary Contractions or Vertical Jump in this study makes these assertions difficult. As this study recruited participants who were recreationally-active it is possible that elite endurance athletes, already well-accustomed to performing regular heat producing exercise, would

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differentially experience greater gains compared with a matched neutral exercising group, although this remains to be determined.

Although a greater number of heat exposures (than imposed in this study) could yield more substantial physiological adaptations and performance improvements (Lorenzo et al. 2010), it is also possible that this increase could trigger systemic inflammation (Lim et al., 2009). The ~4 fold increase of IL-6 concentration in all participants after the HST may not signify heat stress per se, but rather the stress invoked by the exercise demand itself. IL-6 can be released into the circulation following various pathological events such as physical exercise, trauma, sepsis, and thermal injury (Moldoveanu et al., 2000; Natelson et al., 1996). There are few studies that have investigated IL-6 as a blood biomarker during exhaustive exercise in the heat, although one study reported a very large increase in IL-6 following 2 h of exhaustive walking in protective clothing at 40 °C (Selkirk et al., 2008). However, a different study reported a very large increase in IL-6 following 3 h of exercise at 60-65% of VO_2 peak in typical laboratory conditions (Moldoveanu et al., 2000). Prolonged elevation of IL-6 may signify cumulative fatigue or a neuroinflammatory response (Vargas & Marino, 2014), however, in the present study IL-6 returned to basal concentration prior to each HST. It appears the training load was adequate to elicit some physiological and performance benefits over the 18 day period, but not enough to elicit wider systemic or prolonged inflammation. Although IL-6 appeared to be the most sensitive blood biomarker to the exercise task, its usefulness in specifically signifying heat stress or acclimation status is limited given the non-heat specific nature of its response.

The low concentrations of LPS observed in this study indicates the participants tolerated the moderate-high heat load that was presented to them, and in doing so

experienced minimal gut leakage (Pyne et al., 2014). As LPS is the primary endotoxin translocated to circulation under heat load (Yeh et al., 2013), its concentration and regulation is a primary consideration in study of responses to the heat. It appears that undertaking ~40 min of strenuous exercise in the heat is not sufficient to evoke a systemic inflammatory response in healthy, moderately active individuals. Furthermore, as IgM is a key antibody in neutralising LPS (Camus et al., 1998), its concentration in circulating blood can reflect the body's response to endotoxin accumulation and as protection against further challenges. In this study the pre- to post-exercise change in IgM concentration in the heat was not significant, however, following the first HST there was a trend (p = 0.08) towards reduced concentrations in all participants. It is likely that a substantial heat and/or exercise stimulus may be required for IgM concentrations to be substantially affected, although in this case it seems possible that there was some degradation of the antibody occurring. Only one other study has investigated the response of non-specific IgM following exercise in hot and humid conditions (Hailes et al., 2011). During that study, a 20% increase of plasma IgM was reported pre- to post-exercise at day one of the heat acclimation program. This change was not present at day five, with post-exercise IgM not varying from basal levels (Hailes et al., 2011). The initial change of IgM in Hailes and colleagues' study may relate to the participants being required to reach a terminal core temperature of 39.5 °C, whereas in the present study core temperatures did not consistently rise to that level. Despite a substantial exercise and heat load (60 min HST), participants in the present study were able to cope with the demands of the exercise task with limited inflammation and immune disturbances.

6.6 Conclusions

Short-term heat training with the addition of supplementary top-up training sessions over 18 days enhanced time-trial performance by ~9% in recreationally-active healthy

adults, although exercise training in a temperate environment was also a sufficient stimulus for performance gains of ~6% over seven days. The effects of heat training appear to become more worthwhile between 7-18 days. Nevertheless, training in either the heat or neutral conditions proved beneficial to performance and thermoregulatory responses compared with a control (non-exercise) condition. However, none of the experimental groups exhibited substantial changes in LPS, IgM, or IL-6, indicating the training and heat load did not elicit an immune response. It is possible that a more intense heat training protocol may lead to greater physical and immune responses.

6.7 Unpublished results

In the published version of this chapter some data were omitted that while in the context of the thesis is important, was not deemed as necessary for the published manuscript. These data indicate the perception of fatigue that participants in the HOT and NEUTRAL groups experienced as a result of taking part in the HA training program.

Method – Fatigue Rating

At the cessation of the training program participants recorded a perception of fatigue measure on a visual analogue scale and were given a score from 0-10. Participants marked their fatigue level on a 20 cm line with an x. Responses were measured to the nearest cm and are express as fatigue units. No markings were present on the scale with the exception of "0" marked as "No fatigue" and "10" marked as "Extremely fatigued".
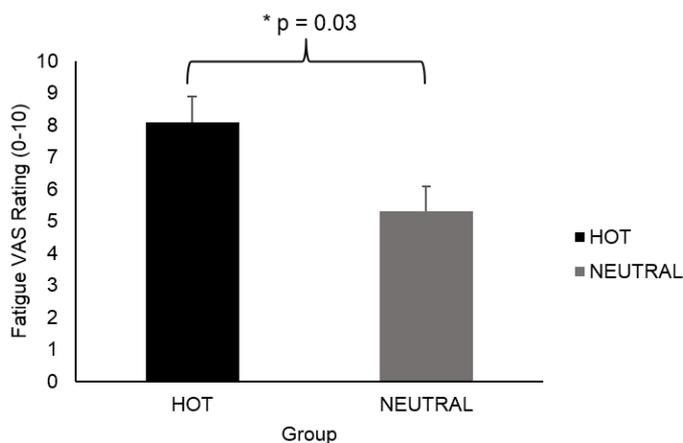
Results – Fatigue Rating

Figure 6.5. Visual analogue scale for fatigue following the heat acclimation training program for HOT and NEUTRAL groups. Data presented as mean score ± SD.

Although these perceptual data were not aligned with greater inflammation (i.e. larger increases in IL-6) for the HOT group compared to NEUTRAL, recovery between training sessions during HA training was deemed an important consideration.

6.8 Chapter progression

As participants in Chapter Six that undertook HA training reported higher levels of fatigue, but had no difference in inflammatory or LPS responses than those that undertook their training in the thermo-neutral environment, it was deemed appropriate to investigate the usefulness of a recovery intervention during HA training. Therefore, Chapter Seven included an intervention based randomised control trial to investigate the effectiveness of immediate post-exercise cooling following heat acclimation training. Furthermore, a progressive increase (overload) of external workload of ~5% per day was utilised in Chapter Seven to provide adequate resistance to participants as they progressed through the HA training.

(1) Introduction

(2) Review of inflammatory mediators in reponse to exercise in the heat

(3) Review of STHA and MTHA training programs

(4) Reliability of serum biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between residents of the tropical and temperate zones

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

(7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and synthesis, future directions, and conclusions

7. Immediate post-session cooling improves cycling performance and reduces residual sensations of fatigue following heat acclimation training.

This chapter has been written in the format of an original research article for consistency with the format of the thesis.

7.1 Abstract

Aim: To investigate the effect of immediate whole-body cooling following heat training sessions as a means to optimise performance, promote rapid recovery and reduce residual sensations of fatigue after intense, short duration (7 days) heat acclimation (HA) protocol.

Method: Twenty four moderately trained males (mean ± SD; age, 23.8 ± 4.4 years, height, 1.76 ± 0.10 m, body mass, 76.5 ± 8.7 kg, VO_2 max 46.4 ± 5.3 $mL.kg^{-1}.min^{-1}$) were randomly allocated to either whole-body cooling (WBC) (n=12) or passive recovery control (PRC) (n=12) training groups by aerobic power and peak power output. Both WBC and PRC undertook VO_2 max and time-to-exhaustion (TTE) tests on a cycle ergometer in a temperate environment (20 °C, 50% relative humidity) and a heat stress test (HST), that also included a 5 km time trial (TT), in a hot condition (38°C, 60% RH) before and after four days heat acclimation training. Participants in WBC received a 20 min post-exercise rapid cooling intervention that comprised of whole-body fanning (~3.6 $m.s^{-1}$) and ingestion of a 500 mL ice-slushy immediately following each exercise in the heat session.

Results: Following the HA training program WBC had a 30%, ±45% (mean, ±95% confidence limits) greater improvement in TTE performance (p = 0.03) compared with PRC, and a 4.0%, ±5.8% greater improvement in 5 km TT performance in hot conditions (p = 0.362). WBC also reported lower levels of fatigue compared with PRC following

the HA training (6.5 ± 0.5 vs 8.5 ± 1.0 units, p <0.001, mean ± SD).

Conclusions: Immediate post-session cooling improves cycling performance in a temperate environment and reduces residual sensations of fatigue to a significantly greater extent compared with heat training with no post-exercise cooling. This post-exercise cooling strategy can be used to minimise residual perceptions of fatigue during heat training to enhance performance.

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7.2 Introduction

Heat acclimation (HA) training continues to aid performance in a hot environment. Several recent studies indicate the use of HA may improve athletic performance in cooler environments as well (Lorenzo et al., 2010; Minson & Cotter, 2016). Therefore, the potential for HA may extend beyond that of simply preparing athletes for performance in hot conditions. It may also be a means of gaining rapid physiological adaptations and performance advantage from a short, intense period of exposure to the heat. Consequently, it is important to consider optimisation and refinement strategies to ensure that athletes adapt, perform optimally and yet recover as quickly as possible so that heat exposures do not impinge on their training practices.

Current evidenced-based practice for short-term HA training appears to suggest five sessions over consecutive days for a minimum of 60 min is optimal. (Chalmers et al., 2014) These types of consecutive day training protocols can provide benefit to performance in both thermo-neutral (Buchheit, Voss, Nybo, Mohr, & Racinais, 2011) and hot conditions (Garrett et al., 2012). However, consecutive days training in hot conditions can impose significant physiological strain leading to sensations of cumulative fatigue and reductions in power output (Wingfield et al., 2016). Athletes intending to supplement their training with HA sessions should carefully consider their recovery strategies between HA sessions to ensure they are fully recovered leading into competition, while still maintaining the benefits that heat exposure brings. Therefore, the construction and practical application of HA could perhaps consider interventions to mitigate the accumulation of fatigue that can occur over the course of an intense multi-day training program.

Recovery methods following strenuous exercise include whole-body cooling such as cold water immersion (CWI), phase change garments or whole-body fanning, although it is not clear whether recovery-cooling following exercise in the heat could potentially blunt important adaptive processes. The use of rapid whole-body cooling by way of cold water immersion can facilitate recovery within 24 h between bouts of intermittent cycling (Lane & Wenger, 2004). Furthermore, Pointon and colleagues (2012) observed immediate improvements in maximal voluntary contraction (MVC) force and voluntary activation (VA) following CWI which was performed following 60 min of intense intermittent running in the heat. Similarly, Minett et al. (2014) reported improved recovery of MVC force and VA at 1 h post-exercise when CWI was applied following a 70-min intermittent running protocol performed in the heat. However, it must be noted that contradictory results were evident at 24 h post-exercise with Minett et al. (2014) reporting improved and Pointon et al. (2012) reporting attenuated MVC force following CWI treatments. Under heat stress, CWI facilitates short term recovery by rapidly reducing body temperatures, consequently ameliorating CNS mediated fatigue, and by reducing cardiovascular strain (Ihsan et al. 2016). A lack of appropriate recovery may also prevent the athlete training at a required intensity or, achieving the required load during subsequent training sessions (Hohenauer et al., 2015). Moreover, parasympathetic reactivation following CWI seems detrimental to high-intensity performances performed shortly after, but seems beneficial with regards to longer term physiological recovery and day to day training performances (Ihsan et al., 2016). Therefore, post-exercise cooling may be beneficial in aiding recovery following intense exercise in the heat. However, CWI is often not feasible and may only cover a small surface area compared with other cooling techniques if athletes are not able to fully submerse their bodies up to the neck.

A simple and often overlooked mechanism to rapidly cool the body is whole-body fans, which can reduce core temperature faster than other strategies such as phase change garments of an increase in evaporative cooling (Barwood et al., 2009). Crushed ice and ice-slushies are also a simple, effective means to reduce core temperature either pre- or post-exercise in hot conditions (Brearley, 2012; Ross et al., 2011). The cooling properties of ingested ice are such that one litre (L) of ice requires ~334 kJ to melt, and, once in a liquid form, the heat storage capacity mirrors that of a cold ingested beverage. Hence, the potential heat storage conferred by 1 L of crushed ice is ~489 kJ to melt and warm to 37 °C (normal deep body temperature), compared with ~155 kJ for cold water (0°C) to reach 37 °C (Brearley, 2012). This effect relates to the energy that solid ice absorbs to change state into liquid water without any change in temperature. The overall effect is an increased heat storage capacity of the beverage, thereby reducing core temperature more effectively when ingested (Burdon, Hoon, Johnson, Chapman, & O'Connor, 2013). Although pre-exercise ice-slushy ingestion can delay the rise in core temperature associated with prolonged exercise in the heat, these types of interventions are generally utilised to benefit acute (immediate) benefit performance, whereas recovery-cooling via ice ingestion post-exercise is a simple strategy to rapidly reduce core temperature in a short period of time (Brearley, 2012). Therefore, ingestion of ice or ice-slushies post exercise may be useful as a recovery tool during intense training such as those experienced during heat acclimation training.

The potential ergogenic effects of HA training may also have transferable benefits to exercise in more temperate conditions. This benefit would make it an attractive short-term training option for teams or athletes seeking to gain a competitive advantage by using an intensive programme. HA can improve 1 h cycling time trial (TT) performance by 5-8%, as well as improve anaerobic threshold, VO_2 max and cardiac output at 13 °C

(Lorenzo et al., 2010) and running time to exhaustion (TTE) by ~29% in a cooler environment (Scoon et al., 2007). Whereas others have reported that HA training does not improve cycling time trial performance in similar conditions (Kieser et al. 2015). Adaptations from HA include improved plasma volume expansion, cardiac and skeletal muscle efficiency, ventricular compliance and thermoregulatory adaptations such as lower resting core temperature, increased sweating and cutaneous blood flow (Tyler et al., 2016). These physiological adaptations can yield large improvements to cardiac stability, as well as moderate-to-large beneficial effects to core temperature and skin blood flow during exercise in the heat (Tyler et al., 2016), however, increases in plasma volume have been associated with both improved (Lorenzo et al., 2010), and no change in performance (Kieser et al., 2015) in a cool environment. The effectiveness of HA training as an ergogenic aid to improve performance in thermo-neutral environments remains contested and unclear (Minson & Cotter, 2016) as there are a limited amount of well controlled studies that have investigated this phenomenon. However, as heat acclimation protocols are often demanding and have been reported to induce high levels of peripheral fatigue (Wingfield et al., 2016), recovery between heat acclimation training sessions should be carefully considered., This recovery should take into account effectiveness and practicality as well as optimising opportunities for athletes to improve performance in hot and thermo-neutral environments.

The aims of this study were two-fold; first, to investigate the effectiveness of short-term HA training on improving performance in hot and thermo-neutral environments, and secondly; to investigate the usefulness of post-exercise recovery-cooling during short-term HA utilising ice-slushies and whole-body fanning. It was hypothesised that whole-body cooling following exercise in the heat would result in enhanced recovery

and improved cycling performance in a hot and thermo-neutral environment when compared with passive recovery

7.3 Methods

Study design

This study comprised 24 untrained participants who were randomly allocated into two groups in a pre-post parallel design and took place over a 12 day period. Participants were allocated to either the whole-body cooling (WBC n =12) or a passive recovery (PRC n = 12) based on their aerobic capacity and peak power output (PPO) in matched pairs. As both groups undertook the same exercise training, PRC served as an "active" control -, neither group were informed of the study outcomes for the other group. Participants undertook two tests of maximal oxygen uptake (VO_2 max) that also included a time to exhaustion protocol (TTE) once VO_2 max was attained, two heat stress tests that included a time trial performance test, and four HA training sessions (Figure 7.1). Participant characteristics are outlined in Table 7.1. All exercise was performed on a cycle ergometer (Excalibur Sport, Lode, Netherlands) within the Sports Science laboratory at the University of St Mark and St John, Plymouth, UK. Participants were instructed to refrain from other training while taking part in the study, and not to undertake any strenuous exercise within 48 h of testing sessions. Prior to taking part, participants provided written informed consent in accordance with the Declaration of Helsinki and underwent a pre-screening health questionnaire. The study protocols were approved by the University of St Mark and St John Human Research Ethics Council.
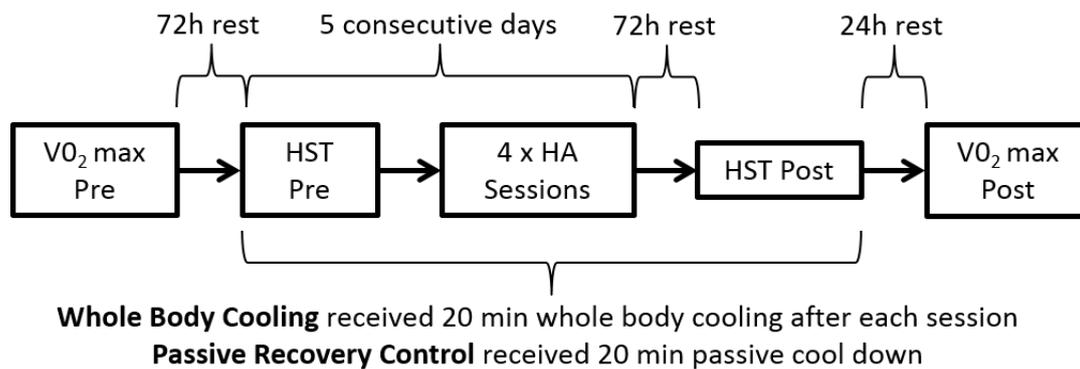
Whole Body Cooling received 20 min whole body cooling after each session
Passive Recovery Control received 20 min passive cool down

Figure 7.1. Schematic representation for exercise testing and training timeline for the WBC and PRC groups

Table 7.1. Participant characteristics

Measure	Height (m)	Mass (kg)	Age (yrs)	VO$_2$ max (L.min^{-1})	PPO (Watts/kg)
WBC	1.78 ± 0.10	78.0 ± 6.1	21.9 ± 3.6	3.53 ± 0.59	3.2 ± 0.4
PRC	1.76 ± 0.10	76.5 ± 8.7	23.8 ± 4.4	3.55 ± 0.41	3.4 ± 0.6
P value	0.34	0.63	0.27	0.67	0.42

WBC, whole body cooling group. PRC, passive recovery control group. VO$_2$ max, maximal oxygen consumption. PPO, Peak Power Output. No significant differences between groups

Baseline Measurements

On the participant's first laboratory visit, height and body mass were measured, and familiarization ride for the VO$_2$ max cycling test was undertaken. Participants then returned to the laboratory 48 h later to undertake their first max VO$_2$ max and TTE task in temperate environment (~20 °C and 50% RH).

VO$_2$ max and Time- to-Exhaustion cycling test

The VO$_2$ max test involved progressive incremental cycling until exhaustion on an ergometer (Excalibur Sport, Lode BV, NL), beginning at an initial resistance of 100 W

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increasing by 20 W.min^{-1}. Once the participants reached their maximum workload (as observed by a plateau in oxygen uptake despite an increase in workload, heart rate within 10 b.min^{-1} of the age-predicated maximum, an RPE corresponding to max effort, and they indicated that they could not sustain a higher workload) the resistance was clamped and participants were encouraged to continue cycling until volitional exhaustion. The test was terminated if the participant was unable to maintain a pedalling rate above 80 rpm or if they chose to stop. The time for the TTE was recorded as the entire time spent pedalling at the final workload in seconds. Throughout the VO$_2$ max and TTE cycling protocol the composition of expired CO_2 and O_2 was analysed by a metabolic cart (Cortex Metalyzer 3b, Biophysik GmbH, Germany) and heart rate recorded at 5 sec intervals (Polar RS400, Polar Elektro, Finland). The peak power output reached was used to determine the cycling intensities for the subsequent heat stress tests (HST) and HA training sessions.

Heat stress test

The heat stress test was the same as used in previous work of similar design (Guy, Edwards, et al., 2016) and comprised cycling for three x 10 min sub-maximal workloads with a 3 min rest period between workloads, followed by a 5 km self-paced time trial to exhaustion (TT). Each HST was performed in an environmental chamber (Environmental Chamber, TESS, United Kingdom) at a temperature of 38 ºC and 60% RH airflow ~1.5 m.s^{-1}. Following a 5 min standardised warm-up (consisting of 3 min cycling at an RPE of 8 or 9 followed by two min of dynamic stretching), the participants completed three 10 min workloads at 50%, 60% and 70% of their peak power output corresponding to their individualised VO$_2$ max, with a 3 min rest between workloads. After the 70% workload was complete, a 5 min rest period was given before the start of

the TT. Participants were able to view rpm and informed of the distance travelled every 500 m to assist with pacing. Heart rate (RS400, Polar Elektro, Finland), and core temperature (T_c) (ttec 501-3 data logger and data logger software version 10.1, Nordex Pty Ltd, Australia; MEAS 449 1RJ rectal temperature thermistor, Measurement Specialities, United States, self-inserted 8 cm past the rectal sphincter) were sampled at 5 s intervals and skin temperature (T_{sk}) recorded every min (CD 1.0 Thermometer, Edale, United Kingdom) Fluid intake (water, *ad libitum*), and rating of perceived exertion (Borg RPE 6 – 20) (Borg, 1998) was recorded throughout the test.

Heat Acclimation Training

All participants undertook four sessions of heat acclimation training in a hot and humid environment (38 ℃ and 60% RH, airflow ~1.5 m.s^{-1}) on consecutive days. Training comprised a standardised 3 min. warm-up followed by 4 x 10 min intervals at an initial workload of 55% VO$_2$ max. As heat adaptation requires a progressively overloading thermal stimulus to induce changes (Tyler et al., 2016) the workload was increased daily by 5% to maintain intensity as the training program progressed. A 3 min rest period was given between each workload and water was consumed *ad libitum*. Heart rate was recorded at 5 s intervals and RPE and thermal comfort (TComf) recorded at the end of each interval and tympanic temperature was monitored for participant safety (Thermoscan, Braun, Germany).

Recovery

Participants who were allocated to the whole-body cooling group (WBC) undertook a 20 min post-exercise cooling protocol following each exercise and heat stress test session. Briefly, WBC participants sat on a backless chair approximately 1.2 metres from a fan that was blowing air at a rate of ~3.5 – 3.8 m.s^{-1} whilst ingesting an ice-slushy (7

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g.kg^{-1} body mass) consisting of frozen crushed ice with a carbohydrate (CHO) mix flavouring (~40 g CHO per drink, GO Energy, Science in Sport, Lancashire, United Kingdom). Cooling by whole-body fan can reduce core body temperature by 0.5 ºC over a 30 minute period (Barwood et al., 2009) and the ingestion of an ice-slushy reduces core temperature by approximately 0.6 ºC over a similar time period (Ross et al., 2011). Participants in the PRC group also sat on a backless chair for 20 min (Minett et al., 2012) whilst ingesting a room temperature drink of the same CHO and liquid content as WBC. The room temperature during the recovery period was ~20 ºC and 50% RH. This cooling strategy was selected as an alternative to cold-water immersion and was designed to minimise environmental (water access) and logistical constraints (cooling a full team), improve portability and reduce reliance on inadequate facilities when travelling (Duffield, Steinbacher, & Fairchild, 2009).

Fatigue

At the cessation of each training session participants recorded their perception of fatigue measure on a visual analogue scale and were given a score from 0-10. Participants marked their fatigue level on a 20cm line with an x. Responses were measured to the nearest cm and expressed as fatigue units. No markings were present on the scale with the exception of "0" marked as "No fatigue" and "10" marked as "Extremely fatigued".

Statistical analyses

Descriptive statistics (mean ± SD) were used to summarise the physical and performance characteristics of the intervention groups. Differences between groups were evaluated with a t-test for unpaired samples or a split plot analysis of variance for within and between-group analyses pre- to post-HA training. Where moderate or greater differences at baseline were observed an analysis of covariance was employed with

baseline scores selected as the covariate (TTE, and 5 km TT). Between group differences were further investigated with a post hoc Tukey test. Analyses were undertaken using the statistical package for social sciences (SPSS version 21, IBM, USA) with significance accepted at p<0.05. Additionally, an analysis combining traditional methods with magnitude-based inferences (effect sizes) and precision of estimation (±95% confidence limits [CL]) was employed to overcome some of the shortcomings associated with simple statistical significance testing (Hopkins & Batterham, 2016). A spreadsheet for the analysis of controlled trials was used to determine the magnitude of change between groups (Hopkins, 2006). Standardised mean changes were used to characterise the effects of whole-body cooling following exercise training in the heat and the effects on performance in hot and thermo-neutral environments. Criteria for interpreting magnitudes of effects were as follows: trivial 0–0.19, small 0.20–0.49, medium 0.50–0.79 and large 0.80 and greater (Cohen, 1992). Power analysis was conducted prior to the study and a minimum of eight participants was deemed sufficient to detect the smallest worthwhile change between means assuming the reference change in 5 km time trial performance was approximately twice the magnitude of the typical error of measurement (Garrett et al., 2011)., with a Type I error of 5% and Type II error of 20%.

7.4 Results

WBC had a 30%, ±45% (mean, ±95% CL, p = 0.03) moderately greater improvement in TTE performance in the thermo-neutral condition than PRC and a 7.9%, ±8.4% (moderate effect) greater improvement in VO_2 max output following the short-term heat acclimation training. Although the WBC group also experienced a 4.0%, ±5.8% greater improvement in 5 km TT performance in hot conditions compared with PRC, this was an unclear effect (p = 0.362).

WBC reported lower levels of fatigue compared with the PRC group following the HA training (6.5 ± 0.5 vs 8.5 ± 1.0 units, p = <0.01, large effect, mean ± SD), although there was substantial uncertainty in this estimate.

In parallel with improvements in performance and aerobic capacity, WBC elicited lower self-reported perceptions of fatigue than PRC following HA training (6.5 ± 0.5 vs 8.5 ± 1.0 units, p<0.01, large effect, mean ± SD).

WBC also had substantial effects on core and skin body temperature during subsequent exercise in the heat. WBC yielded a 0.2°C, ±0.3 °C (p = 0.34) and 0.3°C, ±0.2 °C (small effects) greater reduction in mean and peak T_c (respectively) in the second heat stress test than PRC following the short-term heat acclimation training. WBC induced a 0.4°C, ±0.4 °C % (p = 0.13, small effect) greater reduction in mean skin temperature in the second heat stress test than PRC. WBC elicited a greater reduction in core temperature after each HST (ΔT_c), $HST_{1 \& 2}$ p <0.01, Table 3). There was no substantial between-group difference in the RPE in either heat stress test (HST_1 p = 0.81, HST_2 p = 0.07). While WBC experienced a significant reduction in exercising heart rate in HST_2 (-4 BPM, ±3 BPM), there was no substantial differences between groups.
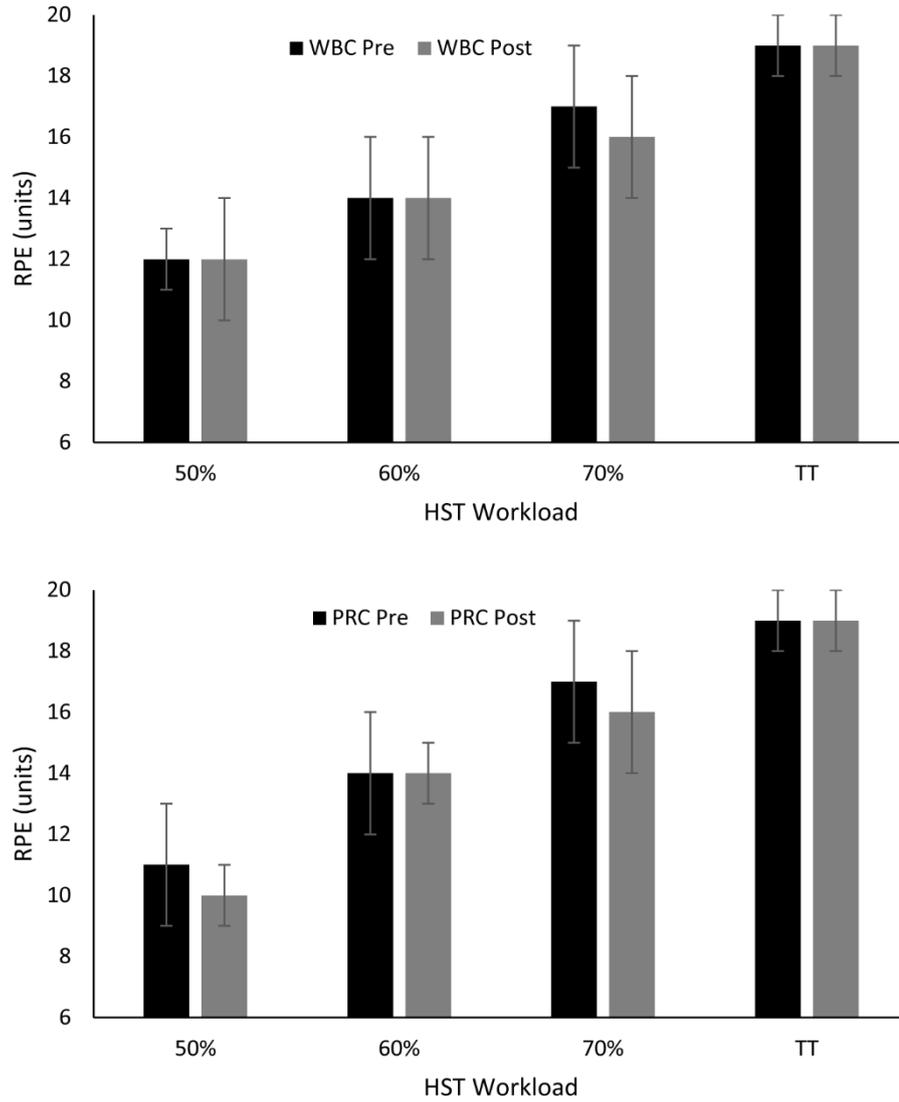
Figure 7.2. Rating of Perceived Exertion (RPE, 6-20) presented as mean ± SD for whole-body cooling (WBC) and Passive-recovery cooling groups during heat stress tests (HST,38 ºC and 60% RH.) performed pre and post five days heat acclimation training. 10 min workloads were performed at 50%, 60%, and 70% of each participants peak power output followed by a self-paced 5 km time trial (TT).

Table 7.2. Changes in maximal oxygen uptake, time to exhaustion, and time trial performance in temperate and hot conditions pre and post short-term heat acclimation training.

Measure	Pre	Post	% change	ES	Descriptor	p-value	Group*time (p value)
$\dot{V}O_2$ max (mL.kg^{-1}.min^{-1})							
WBC	45.2 ± 7.5	49.4 ± 6.0	9.3%, ±4.6%	0.62	Medium	<0.01*	0.50
PRC	46.4 ± 5.3	47.5 ± 7.4	2.4%, ±5.4%	0.17	Trivial	0.43	
TTE (s) (thermo-neutral)							
WBC	118 ± 37	265 ± 130	125%, ±47%	1.54	Large	<0.01*	0.03ᵀ
PRC	103 ± 42	161 ± 50*	56%, ±37%	1.26	Large	<0.01*	
5 km TT (s) (heat)							
WBC	1021 ± 184	931 ± 200	8.8%, ±3.2	0.47	Small	<0.01*	0.04ᵀ
PRC	1095 ± 200	1042 ± 246	4.8%, ±3.5%	0.24	Small	0.06	

Data are presented as mean ± SD and percentage change, ±95% confidence limits. WBC, whole body cooling group. PRC, passive recovery control group. VO_2 max and Time to Exhaustion (TTE) performed in thermo-neutral conditions, 5 km Time Trial (TT) performed in hot (38°C, 50% RH). The criteria to interpret the magnitude of ES were: trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater). * Significant change in pre to post values. ᵀ Significant group*time interaction.

Table 7.3. Physiological and perceptual responses to the heat stress test pre and post short-term heat acclimation training.

Measure	Pre	Post	% change	ES	Descriptor	p-value	Group*time (p value)
HR_{MEAN} (bpm)							
WBC	166 ± 14	162 ± 15	-2.4%, ±1.7%	0.28	Small	0.04*	0.62
PRC	161 ± 15	159 ± 11	-1.2%, ±2.2%	0.15	Trivial	0.67	
$T_{c\ RESTING}$ (°C)							
WBC	37.4	37.3	-0.2% ±0.3%		Trivial	0.23	0.20
PRC	37.3	37.3	-0.2% ±0.5%		Trivial	0.67	
$T_{c\ MEAN}$ (°C)							
WBC	38.3 ± 0.3	38.2 ± 0.3	-0.5%, ±0.2%	0.66	Medium	0.03*	0.34
PRC	38.1 ± 0.4	38.0 ± 0.3	-0.2%, ±0.3%	0.20	Small	0.24	
$T_{c\ \Delta}$ (°C)							
WBC	1.4 ± 0.4 ᵀ	1.4 ± 0.4	8.5%, ±17.2%	0.30	Small	0.26	0.01ᵀ
PRC	0.9 ± 0.5	0.8 ± 0.4	10.5%, ±35.7%	0.20	Small	0.39	
$T_{sk\ MEAN}$ (°C)							
WBC	34.7 ± 0.6	34.3 ± 0.7	-1.2%, ±0.7%	0.61	Medium	0.02*	0.13
PRC	34.8 ± 0.5	34.7 ± 0.5	-0.3%, ±0.5%	0.20	Small	0.51	
RPE_{MEAN} (units)							
WBC	16 ± 1	15 ± 2	6.3%, ±3.3%	0.63	Medium	0.10	0.73
PRC	15 ± 1	15 ± 1	0.0%, ±3.2%	0.00	Trivial	0.25	

Data are presented as mean ± SD and percentage change, ±95% confidence limits during exercise in each Heat Stress Test pre and post heat acclimation training. WBC, whole body cooling group. PRC, passive recovery control group. $T_{c\ \Delta}$, change in core temperature during the recovery phase following exercise in the heat. The criteria to interpret the magnitude of ES were: trivial (0–0.19), small (0.20–0.49), medium (0.50–0.79) and large (0.80 and greater). * Significant change in pre to post values. ᵀ Significant group*time interaction.

7.5 Discussion

The addition of recovery cooling utilising whole-body fans and ice-slushy ingestion following short-term heat acclimation training appears to augment exercise performance in both hot and thermo-neutral conditions to a greater extent than passive recovery. Furthermore, the recovery-cooling protocol attenuated self-reported levels of fatigue following the multi-day HA training program. Although both groups improved their time trial performance in the heat and time to exhaustion in thermo-neutral conditions (Table 7.2), the WBC group improved to a greater extent (TT ~4% and TTE ~30%) than the PRC group. This post-exercise cooling strategy can be used to minimise residual perceptions of fatigue during demanding heat acclimation protocols and simultaneously improve performance to a greater extent than through heat training alone.

The novel aspect of this study was introduction of whole-body cooling following exercise in the heat as a means to promote physiological and performance adaptations during HA training. While whole-body cooling prior to exercise can acutely improve performance in endurance-type activities such as running and cycling time trials (Ross et al., 2011; Stanley, Leveritt, & Peake, 2010), , this effect is blunted when ice is ingested during exercise in the heat (Maunder, Laursen, & Kilding, 2016). Early fatigue is often associated with exercise in the heat (Bishop et al., 2008), with temperature sensitive neural and muscle metabolic processes, for example, it has been reported that there is a reduction in efferent drive and power output during self-paced cycling tasks in the heat (Kay et al., 2001). Perceived effort during a self-paced task will also be largely influenced by the afferent feedback of skin and core temperature and heart rate (Abbiss et al. 2015). However, in this study improvements in RPE during exercise in the heat were not observed. . However, the lower reported levels of fatigue reported, combined with the greater improvement in exercise performance in a hot and thermo-neutral

environment, is encouraging. Application of mixed-method recovery-cooling may attenuate these neural processes of fatigue, and could be useful where intensity is high and full recovery between training is necessary. Future studies may wish to include self-paced exercise training tasks to further investigate the effects of the recovery intervention between each training. If lower levels of neuromuscular fatigue are present, participants receiving the cooling recovery may have the ability to undertake greater amounts of work on subsequent training days, potentially resulting in improved physiological adaptation.

Participants undertook their performance tests 72 (hot conditions) and 96 h following a 5 day long HA training program. Importantly, however, previous studies have generally focused on recovery from a single bout of exercise (Poppendieck et al., 2013), not multi-session training programs as utilised in this study. The immediate 5% improvement in endurance performance that are relevant for competitive athletes, and these benefits are realised between 24-96 h post-recovery (Poppendieck et al., 2013). However, it is important to note that the participants in this study were only moderately trained. As both groups in this study experienced a small improvement in their 5 km TT performance, the 72 hour recovery period was enough for both groups to adequately recover from the training week, although the WBC group improved by an additional 4%. The final TTE test took place 48 h after the final HST, with the WBC group improving their TTE performance in the temperate environment by a further 3% compared with the PRC group. This would suggest that the WBC group experienced greater recovery following each of the HA training sessions as well as improved recovery at the cessation of the HA training block.

The combination of internal and external cooling techniques following exercise in the heat rapidly lowered core temperature during recovery (T_c Δ ~1.5 ^{o}C reduction in 20

min, compared to a ~0.9 °C reduction for the control group, Table 7.3). The combination of ice slushy drinks and whole-body fanning resulted in greater reductions in core body temperature than has been previously reported for either intervention on its own (Barwood et al. 2009; Brearley, 2012). With ice slushies reducing core temperature by ~0.7 °C over 20 min (Brearley, 2012) and whole body fanning by 1 °C (Barwood et al. 2009). Ice-slushy drinks offer a convenient means of cooling athletes and can enhance performance by ~2% compared with a cold liquid beverage (Stanley et al., 2010). The evaporative effect of whole-body fanning allows increased dissipation of sweat from the skin and greater cooling than hand-immersion, liquid cooled, or phase change garments (Barwood et al., 2009). Whole-body fanning may have stimulated cutaneous thermoreceptors to signal the hypothalamus of the change in external temperature, redirecting blood to the core where the ingested ice cooled the warmed blood. Therefore, the combination of deep tissue (conduction) and surface skin (convection and evaporation) cooling allowed a greater transfer of heat during recovery.

Participants in both groups obtained small to moderate improvements in exercising HR, T_c, and T_{sk}. However, the WBC group exhibited reductions in exercising T_c and T_{sk}. While the WBC group experienced significantly lower core temperature during some stages of the final HST (60%, 70% and TT workloads) compared to their baseline performance, there were no between group effects observed. The WBC group also experienced improved heart rate during exercise in the 70% workload of the second HST, as well as average heart rate throughout the whole HST, however, this was not reflected in a between group effect. The classical thermal adaptations elicited during a short-term HA training block confirm earlier reports (Chalmers et al., 2014; Guy et al., 2015), and the reduced thermal and cardiovascular strain and should promote performance by allowing a greater heat storage and work capacity during the later stages of exercise

(Lorenzo et al., 2010). The mechanisms for the differential improvement in performance and physiological indices as observed in the WBC group during exercise are unclear. The whole-body fanning may have increased the efficiency of the eccrine sweat glands, and changes in the onset, volume, and mineral content of sweat signal are one of the initial signs of heat acclimation (Périard, Racinais, et al., 2015). The whole-body fanning may also have positively affected the evaporative processes of sweat accumulation on the skin, increasing the efficiency, thereby resulting in lower core and skin temperatures during exercise. This effect may promote greater recovery by increasing heat transfer through convection and evaporation from the surface of the skin that enhances performance capacity during subsequent exercise (Lorenzo & Minson, 2010), however, sweat rates and concentration at different sites (e.g. neck, thigh, chest, and back) would need to be studied to further elucidate this assertion.

HA can improve TTE in a thermo-neutral condition as both groups experienced significant improvements in their TTE following the HA training block. This supports the findings of other research that has reported improvements of 5-8% in 1 h cycling time trial tasks, as well as improved anaerobic threshold and cardiac output in similar conditions following HA training (Lorenzo et al., 2010). Furthermore, Scoon and colleagues (2006) reported that HA can improve TTE running by ~29% (Scoon et al., 2007), however, those participants were rewired to exercise for a longer duration than the participants in this study (~15 min at high intensity vs. 2 min cycling at high intensity). While adaptations from HA can include improved cardiac efficiency, lower resting core temperature, lower exercising heart rate and lower exercising core and skin temperature (Tyler et al., 2016), in this study, not all of these improvements were observed for both groups. The improvement in TTE in thermo-neutral conditions may be due to greater recovery as a result of the whole-body cooling following the final HST

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as well as less cumulated fatigue from the training week. As the participants in this study were not well-trained, the adaptations and improvements that they experienced were reasonably modest. Those with higher fitness levels are able to cope with the demands of exercising in the heat better than those that are un-trained (Morrison et al. 2014), therefore, the participants in this study may have benefited from the whole-body cooling more so than highly-trained athletes.

As the post-exercise cooling protocol reduced perceptions of fatigue, more aggressive training and recovery modes could be implemented to further drive adaptive processes Future studies should further investigate the potential blunting of inflammatory processes or muscular fatigue (e.g. maximal voluntary contractions or lower limb force development) often associated with fatiguing training programs and implementation of cold-therapies, particularly those involving exercise in hot and humid conditions. Quantifying the response of biochemical and muscle-status markers such as creatine kinase, myoglobin, and skeletal troponin (Bishop et al., 2008) could be useful. Post-exercise cooling of the muscles enhances exercise-induced mRNA expression of PGC-1α and, hence, possibly mitochondrial biogenesis (Ihsan, Watson, Choo, et al., 2014). However, a recent study has reported that post-exercise heating via water immersion following exercise in a temperate environment can improve 5-km treadmill performance by ~5% (Zuralew, et al. 2016). Zurelew and colleagues also reported improved physiological strain, for example lower final T_c and lower RPE in both hot and temperate environments. Therefore changes in circulatory dynamics and muscle metabolism as a result of post-exercise cooling seemingly contrast the blood flow demands required for muscle protein synthesis and training adaptations to occur (Minett and Costello, 2015). These contrasts in findings highlight that further work is required

to elucidate the mechanisms that can cause improvements in exercise performance during exercise in the heat with mixed-method recovery-cooling interventions.

7.6 Conclusions

Recovery-cooling using readily available equipment such as fans and crushed-ice slushies promote both meaningful physiological adaptations as well as aiding recovery during a strenuous week of short-term heat acclimation training. Furthermore, recovery-cooling protocol attenuated perceptions of fatigue that are usually present during multi-day heat training. Athletes should benefit from appropriate recovery techniques such as post-exercise cooling during demanding heat training blocks in preparation for competition.

(1) Introduction

(2) Review of STHA and MTHA training programs

(3) Review of inflammatory mediators in reponse to exercise in the heat

(4) Reliability of serum biomarkers associated with heat stress and inflammation

(5) Comparisons of physiological responses to exercise in the heat between residents of the tropical and temperate zones

(6) The effects of STHA and supplementary "top up" training on physical performance and inflammation in the heat

(7) The effects of post-exercise cooling during STHA training on performance in hot and thermo-neutral environments

(8) Discussion and synthesis, future directions, and conclusions

8. Discussion and synthesis, future directions, and conclusions

8.1 Overview

This chapter discusses and synthesises the findings of this thesis, proposes future directions of research and presents the conclusions and practical applications.

8.2 Discussion and Synthesis

The findings of this thesis suggests that undertaking short-term heat acclimation (STHA) training can provide moderate to large improvements in cycling performance in moderately fit individuals, which supports Hypothesis Four. These performance benefits are further enhanced by utilising post-exercise cooling immediately following HA training, in support of Hypothesis Five. Furthermore, the immune responses to strenuous cycling tasks in the heat are similar between recreationally active males who reside in tropical and temperate zones, these findings did not support Hypothesis Two. Strenuous exercise that lasts for ~60 min in a hot and humid environment does not result in endotoxaemia or severe alterations to immune function, in support of Hypothesis Three. Importantly, however, the blood biomarkers selected in these studies can exhibit a large degree of inter-sample measurement ("noise") in comparison to their anticipated physiological response ("signal"), these findings support Hypothesis One. Therefore, careful consideration should be given to their case-specific usefulness in experimental and practical settings where the normal resting biological variation has not been quantified, or where evidence indicates excessive noise. These outcomes also support the notion that heat acclimation training is beneficial to exercise performance in thermo-neutral environments in moderately trained young adult males.

The majority of HA research has focused on performance outcomes following STHA and medium-term heat acclimation training programs (Garrett et al., 2011; Lorenzo et al., 2010) with minimal emphasis on the inflammatory or immune effects. Importantly, no study has investigated whether acclimatisation status (i.e. geographical residency) influences the inflammatory response to exercise in the heat. Furthermore, no studies have investigated the use of whole-body cooling to facilitate recovery during STHA training. Most studies incorporating cold-therapies and exercise in the heat have primarily investigated their acute effects on recovery from single bouts of exercise, or as a means to pre-cool athletes prior to or during exercise in the heat (Barwood et al., 2009; Maunder et al., 2016; Ross et al., 2011). The outcomes of this thesis can be used to design and implement heat acclimation training programs that provide meaningful physiological and performance benefits.

Chapter Six detailed the effects of STHA training followed by three additional "top up" sessions every three days, and the associated immune, performance, and physiological responses to this type of training program. Short-term heat training with the addition of supplementary top-up training sessions over 18 days enhanced time-trial performance by ~9% in recreationally-active healthy adults, although thermo-neutral exercise training alone was a sufficient stimulus for performance gains of ~6% over seven days, which was similar to the gains of the heat training group over this time period. Nevertheless, training in either the hot or neutral conditions proved beneficial to performance compared with a control (non-exercise) condition. Importantly, none of the experimental groups exhibited substantial acute or cumulative changes in lipopolysaccharide (LPS), immunoglobulin M (IgM), or interleukin-6 (IL-6), indicating the training and heat load did not stimulate a negative immune response. It is possible that a more intense heat training protocol may lead to greater physical and immune

responses. However, the outcomes of the post-exercise cooling study (Chapter Seven) indicate whole-body cooling following exercise in the heat reduces perceptions of fatigue, while allowing greater training loads to be undertaken (progressive overload of intensity each day). Therefore, it is likely that a combination of greater heat loads and post-exercise cooling would not result in undesirable immune responses, however, further work is needed to fully evaluate this assumption.

Recovery-cooling following STHA training elicited meaningful physiological adaptations (lowered heart rate and core and skin temperature during exercise), performance outcomes (improved time trial and time to exhaustion capabilities), and reduced perceptions of fatigue. In comparison, those participants who undertook a similar heat acclimation training protocol (Chapter Six), did not experience any beneficial physiological adaptations. This is despite participants in Chapter Six receiving an additional day of heat acclimation training (four vs five days). The participants completing STHA training with post-exercise cooling (or passive recovery) undertook cycling training that utilised a progressive overload with 5% increments in intensity per day, whereas participants in Chapter Six exercised at a constant workload throughout the acclimation period (~55% of VO$_2$ max). Although participants were exercising at higher intensities during the later stages of the HA program, it is likely that the recovery-cooling protocol attenuated central and peripheral fatigue.

Although the use of isothermic (target core temperature) vs. fixed intensities can provide more rapid adaptations over short- and medium-term timescales (Gibson et al., 2015), the use of isothermic equipment was beyond the capabilities of the available equipment (i.e. real time rectal temperature monitoring). Therefore, prescribing fixed intensity (with intensity progression of ~5% per day) may be more appropriate for

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moderately fit and recreationally active adults in non-laboratory settings. Athletes should benefit from appropriate recovery techniques while undertaking demanding heat training blocks, particularly around times of competition, while the addition of top-up sessions should also promote retention of these adaptations over a longer time period.

Elite male and female endurance athletes appear to be detrimentally affected by hot conditions (>25ºC) by up to 3% in events such as the marathon and 10,000 m running. Highly trained cyclists have also been reported to suffer a mean power decrement of ~-16% on the first day of heat exposure relative to performance in a cool environment. These decrements can be reduced to -8% after 1 week of training in the heat and to -3% after 2 weeks (Racinais, Périard, Karlsen, & Nybo, 2015). As elite athletes already possess some physiological adaptations associated with heat acclimation (Garrett et al., 2012) it is surprising to observe such a large decrease in performance at this level. However, a recent study has reported that only ~15% of elite track and field athletes partake in specific HA training leading up to competition in a hot environment (Périard et al., 2016). Therefore, it appears that even highly-trained athletes would benefit from HA training to improve performance. Although the performance of highly-trained athletes can be improved by heat acclimatisation, these improvements may be related to better pacing strategies that alter the downstream thermoregulatory responses, rather than meaningful physiological adaptations (Racinais et al., 2015). It is, therefore, likely that elite athletes would benefit from longer duration protocols (>14 days), allowing them to experiment with fluid, nutritional, and pacing strategies while maintaining appropriate volume and intensity of training.

Another issue for organisations and teams is the degree of underlying acclimatisation to heat related to geographical residency. However, this issue may pertain to

recreationally and moderately trained individuals, rather than elite athletes. The responses to ~60 min of cycling in the heat showed differences in the perceptual responses to exercise and modest physiological differences between residents of the tropical and temperate zone (Chapter Five). These differences include increased post-exercise concentrations of IL-6 and increased pre-exercise concentrations of LPS and IgM in the tropical residents. However, the tropically acclimatised participants tolerated the heat with significantly lower RPE and trends for lower peak core temperature and greater fluid loss than residents of the temperate climate. Repeated exposures one week apart yielded minor physiological advantages for the tropically acclimatised participants (e.g. moderate reductions in exercising heart rate compared with participants residing in temperate climates), however, no substantial performance benefits were obtained. Consequently, the heat stress test task did not appear to impose a substantial immune threat to recreational athletes who reside in tropical or temperate climates given that the concentrations of these biomarkers did not rise above clinically-relevant levels (Gonzalez-Quintela et al., 2008). However, it is unknown if there would be a differential effect following longer duration exercise and heat exposure (e.g. >90 min), or multiple exposures per day. Regardless of background heat acclimatisation status, recreational athletes would likely benefit from heat acclimation training strategies to improve exercise performance in the heat.

An additional outcome of this thesis was that STHA training can also benefit performance in a thermo-neutral environment (~20 ^{o}C and 50% RH). It is proposed that adaptations from HA such as plasma volume expansion, improved cardiac and skeletal muscle efficiency, ventricular compliance, lower resting core temperature, increased sweating and cutaneous blood flow would also benefit exercise in cooler environments as well as the heat (Minson & Cotter, 2016). We observed that STHA with progressive

overload improved time to exhaustion in a cycling task, and these improvements were greater in the group that received post-exercise cooling following their final heat stress test (Chapter Seven). Heat exposure/load could, therefore, be considered an additional stimulus for training environments where the need for physiological stress is high to drive adaptation. This approach should also enable coaches and athletes to reduce mechanical load due to lower external work intensities required to elicit the desired internal work rate (e.g. cardiac frequency, perceived exertion).

While blood biomarkers are potentially useful for quantifying physiological responses to exercise, the reliability of some markers was unclear. For example, the response of IL-6 and C-reactive protein (CRP) following exercise in the heat appears to be greater than the normal resting variability, making these biomarkers potentially useful. However, the short-term variability of other biomarkers such as extracellular heat shock protein 72 (eHSP72), LPS, and IgM can overshadow the observed change following ~60 min of exercise in a hot environment, resulting in unclear effects. The within-subject analysis also indicated that individuals consistently regulate the concentration of these biomarkers within normal homeostatic limits when measured seven days apart. However, the relatively high between-subject variation suggests that it is not possible to establish a standardised concentration (or reference value) of each biomarker suitable for all individuals. This reliability was further investigated in Chapter Five, with the variation of blood biomarkers at rest reported for residents of tropical and temperate climates. While tropical residents had higher variability in LPS (coefficient of variation [CV] 71% vs 33%) and IgM (CV 46% vs 21%) after exercise than temperate residents, the latter group exhibited higher variability of resting concentrations of IL-6 compared with tropical residents (CV 85% vs. 36%). These differences in variability did not appear to alter the exercise-induced response of these markers. It appears that while

differences in resting and post-exercise concentrations of these blood biomarkers are likely, their usefulness in determining an acute immune response following ~60 min of exercise in the heat is limited. Following a greater heat and/or exercise stimulus these markers may be beneficial, however, the reliability of these markers following longer duration exercise is yet to be determined.

Other considerations that may have been strengthened the outcomes of this thesis include the measurement of body surface area investigations in the heat gain/heat loss of the participants. Furthermore, electrolyte concentrations (Na+) changes were also considered to track these changes in relation the participant's acclimation, however pilot testing of these measures yielded unreliable data.

While submaximal CV responses (and other physiological variables) were measured during the heat stress test within each study at 50%, 60%, and 70% of peak power output for each participant, longer duration protocols may have shown greater differences in adaptations. A shorter protocol was used for both the submaximal and performance portions of each HST due to the training status of the participants. Pilot testing determined that longer tasks (e.g. 10km TT) were too taxing and participants were unable to pace them effectively. This inability was mostly likely related to the limited cycling experience.

8.3 Summary

STHA training combined with rapid post-exercise cooling immediately following heat exposure provides meaningful improvements in exercise performance in a hot and humid, and a temperate environment. The addition of supplementary "top up" sessions further enhances improvements in performance following the initial acclimation period for recreationally active young men. Healthy recreationally active males can exercise

and train for up to 60 min while exposed to high heat loads (35-38 ºC and 60-70% RH) without unsafe translocation of LPS or significant reductions in IgM. The risk of exercise-induced endotoxaemia during this duration and intensity of exercise in this group is low, even though lesser trained individuals are purportedly more susceptible to heat stress and inflammation. Although fatiguing, STHA training can be considered a viable intervention to increase performance in hot or thermo-neutral environments, even for residents of the tropical zone. However, performance benefits are not always dependent on changes in physiological variables such as lowered heart rate or core temperature. Care should be taken when reporting observed changes in blood biomarker concentrations to ensure the normal variability (at rest) is not greater than changes expected with exercise.

8.4 Practical applications

HA training for elite and well trained athletes

- Elite and highly trained athletes preparing for competitions conducted in hot environments need to consider the duration and demands of their sport, and whether heat acclimation training yields a substantial benefit to their performance. Endurance athletes at the elite level likely require a much longer acclimation period (>14 days) to realise substantial performance benefits given they typically possess many of the adaptations associated with heat acclimation. Sprint athletes, however, may benefit from higher ambient temperatures and, in preparing for competitions in hot environments, could minimise their time acclimating to ensure they benefit from the high ambient temperature.

HA training for recreational athletes

- Recreational athletes with no previous history of heat stress or illness can undertake STHA training (4 or 5 consecutive sessions) to benefit exercise performance in hot conditions. These adaptations and performance outcomes can be increased by undertaking supplementary top-up heat training sessions every third day to ensure the preservation of their adaptations. Athletes who are recreationally trained should consider STHA training over five consecutive days, utilising a progressive increase (overload) of external workload of ~5% per day.

Recovery during HA training

- As the protocols required to elicit positive adaptations during heat acclimation are potentially fatiguing, athletes could utilise whole body cooling to promote recovery between training sessions. Crushed iced slushies and portable fans are cost effective and practical cooling methods that athletes can readily access. Recreational athletes can also improve their cycling performance in both hot and thermo-neutral conditions by undertaking heat acclimation training combined with rapid post-exercise cooling.

Optimisation of HA training

- The increased thermal load presented by exercising in a hot and humid environment could be used to offset reductions in mechanical load in circumstances where this would be beneficial to an athlete. Athletes preparing for competition often reduce volume and maintain intensity to taper for an event. Therefore, using thermal stress in place of mechanical stress enables the athlete to experience increased cardiac output and perception of effort.

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- The outcomes of these studies suggests that ~60 min of exercise (4 x 10 min blocks) at an intensity corresponding to ~55% of VO_2 max improves cycling time trial and time-to-exhaustion performance, without resulting in clinically meaningful levels of inflammation or heat stress.

Occupational applications

- Other individuals such as first responders and outdoor workers may also benefit from rapid whole-body cooling following strenuous exercise and/or work in the heat. This rapid cooling may facilitate recovery from mild heat stress, as well as promote thermoregulatory adaptations during periods of heat acclimation/acclimatisation.

8.5 Recommendations for future research

Topics that warrant further investigation based on this research include:

Elite athlete adaptations

- Chapter Three highlighted some instances where elite athlete performance is limited by the heat. The investigation of higher intensity training programs may be more beneficial for elite athletes to gain meaningful physiological and performance adaptations.

Biomarker modelling

- Chapter Four quantified the biological variability of blood biomarkers associated with heat stress, inflammation, and endotoxaemia. The study of a wide panel of biomarkers can often create larger inter-sample variability than the expected biological response, limiting the confidence in interpreting data on the readiness of an athlete to undertake training or competition. Heat loss and heat gain equations coupled with thermoregulatory parameters (e.g. heart rate, core and skin temperature) could be considered alongside carefully selected biomarkers (by quantifying variability) to determine individual responses to training and readiness for competition.

Intensity and duration of training

- The training intensities in this study were selected to elicit meaningful physiological and performance outcomes, while maintaining a balance between load, overload, and inflammation. As post-exercise cooling reduced post-exercise fatigue and improved physiological and performance outcomes, it may

be possible to increase the training and heat load during HA training without inducing endotoxaemia or severe immune responses.

Post-exercise cooling mechanisms

- The usefulness of post-exercise cooling should be further investigated to determine the mechanism of action by comparing different duration and intensities of heat training, coupled with cold-therapies as a recovery tool. Further elucidation of markers of acclimation such as resting eHSP72, the expression of PGC1α, and markers of muscle damage is warranted.

Pre- and post-exercise cooling interventions

- Ingestion of crushed ice and slushies has been widely used as an ergogenic aid prior to and during exercise to limit the rise in core temperature. However, the combined application of pre- and post-exercise cooling is yet to be investigated. The pre-exercise cooling may limit core temperature increases during exercise, thereby limiting the occurrence of important physiological adaptations to heat stress. The results of this thesis have demonstrated that post-exercise cooling improves adaptations during STHA training. Therefore, investigation of a combined cooling approach could provide meaningful insights whether prolonged elevation of core temperature is required to elicit meaningful adaptations to the heat.

Perception of effort and pacing

- The ability to effectively regulate pace and racing strategy in the heat is an important consideration for athletes participating in endurance events around the globe. As residents in tropical climates appear to perceive cycling tasks in the

heat as easier compared with residents of the temperate zone, acclimation programs may investigate the changes in perceptual efforts during exercise in the heat. Workloads of differing intensity and duration should be compared with matched efforts in a temperate environments to evaluate afferent feedback from the regulatory and sensory systems. This approach would facilitate greater understanding of the work and effort requirements as well as the perceptions of exercise in a hot and humid environment.

Outdoor workers and first responders

- While this research focused on the inflammatory and immune responses following controlled exercise in the heat, it is often the case that outdoor workers and emergency responders may not have control over the intensity and duration of their work efforts. For example, rural firefighters may experience uncompensable heat loads over multi-day deployments, with little opportunity for recovery. Future research could investigate the physiological preparedness of these cohorts as well as interventions to mitigate the effects of immune stress and inflammation. While they have the ability to down-regulate their efforts at any-stage, the current demands of their job may not permit them to do so.

8.6 Delimitations

The studies within this thesis are delimited as follows:

1. Due to the participant population groups in this thesis being limited to healthy recreationally active males from local sporting clubs and university students, the extrapolation of the findings to other population groups such as females and older

adults may not be directly applicable due to differences in heat tolerance and biomarker responses.

2. A wider range of biomarkers such as extra- and intra-cellular heat shock proteins or PGC1α would have been useful in determining physiological adaptations occurring during the HA training. However cost and the ability to sample muscle biopsies was a limiting factor relating to the inclusion of these markers.

3. Given the design of these studies and availability of participants, it was not possible to capture data at additional time points, for example, two, four, or 24 h post exercise and 14 or 28 days after the cessation of the training and testing programs. The timescale and decay of the adaptations observed in these studies may have provided further insights on the design of HA training protocols such as "top up" frequency and preservation of adaptations Therefore, the results may not reflect or be extrapolated to time points outside of what was collected.

4. As the performance measures are limited to cycling performance variables, these findings may differ in other exercise modalities that cause greater eccentric loading such as running or downhill walking.

5. Other markers of heat acclimation may be considered such as sweat onset, electrolyte concentrations and plasma volume changes. Initial pilot work attempted to investigate changes in electrolyte (Na+ concentrations and plasma volume changes), however these measures yielded unreliable data.

8.7 Limitations

The findings of the thesis were limited as follows:

1. The use of different cycle ergometers in Chapter Five did not allow a direct comparison of cycling performance between the residents of the tropical and

temperate environments. As residents of different climates are inevitably based in different locations it is not always possible to perfectly match the experimental equipment used for testing all variables, however every effort was made to match the standardisation processes as much as practically possible.

2. Participants in all studies were requested to limit their physical activity prior to all testing sessions. However, it is plausible that some participants may have undertaken activities that could have affected the results of these analyses (e.g. physical activity or working outdoors in a physically demanding job), although this is a possibility in almost all exercise-based studies.

3. Participants may not have given a true "maximal" effort in their initial VO_2 max test, thereby affecting the intensities in the subsequent heat stress tests and training sessions. However, every effort was made to encourage and motivate participants to maintain pedalling rate and continue in the later stages of the VO_2 max test. Standard primary and secondary satisfaction criteria was used to assist this process (Midgley et al., 2008).

4. Sample size was limited to accessibility of participants who met the recruitment criteria and were able to commit to the timeframe required to complete the study protocols. However, sample size calculations were conducted to determine and justify the sample sizes.

5. Standard technical and biological variability. Every effort was made to control for such fluctuations by calibrating equipment, performing reliability work on protocols, requiring participants to wear the same clothing and maintain similar dietary and fluid intakes and conducting tests at the same time of day.

6. Plasma volume analyses did not yield reliable results for Hct and Hb, therefore responses of large analytes such as IgM that cannot escape the vascular space were not able to be corrected for changes in plasma volume.

8.8 Conclusions

- Residents of the tropical zone perceive matched sub-maximal exercise in the heat as easier in comparison to their temperate counterparts, however these perceptions do not appear to influence performance.

- Background heat acclimatisation status may influence resting concentrations of IgM and LPS following exercise in the heat, however both populations (tropical and temperate) appear to regulate these biomarkers within safe homeostatic limits.

- Recreational athletes can benefit from short-term heat acclimation training at a fixed intensity at ~55% of VO_2 max for 60 $min.day^{-1}$ to improve exercise performance in the heat, although progressive increases in work intensity of ~5% each day are recommended to elicit greater performance and physiological adaptations.

- Additional "top up" training every three days further improves cycling time trial performance in hot conditions compared to short-term heat training only.

- Short- and medium-term heat acclimation training consisting of ~60 min of heat exposure exercising at ~55% of VO_2 max does not appear to pose a substantial threat to the immune system or invoke endotoxaemia in healthy, recreationally active males.

- Short-term heat acclimation training is enhanced with immediate post-exercise cooling utilising an ice-slushy (7 $g.kg.bw^{-1}$) and whole body fanning (3.6 $m.s^{-1}$)

to improve performance, enhance physiological adaptations and ameliorate accumulated fatigue that can occur from a high frequency heat acclimation program.

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10. Appendices

10.1 Conference Abstracts

Immediate post-exercise cooling following heat acclimation training improves cycling performance.

Guy, J. H., Edwards, A. M., Miller, C. M., Deakin, G. B., & D. B. (2016, September). SSEPAT conference proceedings (Townsville, Australia). Oral presentation.

Introduction

While heat acclimation (HA) training is an effective means to promote rapid physiological and performance adaptations in a short-period of time[2], these interventions often increase fatigue[3]. The purpose of this study was to quantify the effect of immediate whole-body cooling following HA training to optimise recovery and performance across an intense short-duration (7 day) protocol.

Methods

Twenty four moderately trained males (age 23.8 ± 4.4 years, stature 1.76 ± 0.1 m, body mass 76.5 ± 8.7 kg, VO_2 max 46.4 ± 5.3 mL.kg^{-1}.min^{-1}; mean $\pm$ SD) were allocated to either Cool (n=12) or Passive (n=12) training groups and undertook four daily sessions of HA training. Before and after HA training participants undertook a time-to-exhaustion (TTE) test in thermo-neutral conditions (20 °C, 50% relative humidity) and a heat stress test (HST) that included a 5 km time trial in hot conditions (38°C, 60% RH) on a cycle ergometer[1]. Participants in Cool received a 20 min post-exercise cooling intervention comprising whole-body fanning (~3.6 m.s^{-1}) and ingestion of a 500 mL ice-slushy immediately after each training session.

Results

Cool had a 30%, $\pm$45% (mean, $\pm$95% confidence limits, p =0.03) moderately greater improvement in TTE performance in thermo-neutral conditions and a small 4.0%, $\pm$5.87% (p = 0.04) improvement in 5 km TT performance in hot conditions compared to Passive. Cool also reported lower levels of fatigue than Passive after the HA training (6.5 ± 0.5 vs 8.5 ± 1.0 RPE units, p = 0.01, mean $\pm$ SD).

Conclusion

Immediate post-session cooling after short-term heat acclimation training improves cycling performance in hot and thermo-neutral conditions and reduces sensations of fatigue. Mixed cooling methods utilising whole-body fanning and ice-slushies are a simple and efficient recovery intervention.

References

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Short-term versus medium-term heat acclimation in tropically acclimated males: performance and inflammation.

Guy, J. H., Edwards, A. M., Deakin, G. B., Miller, C. M., & Pyne, D. B. BASES Conference 2015 (Burton upon Trent, United Kingdom). Oral presentation.

Winner of BASES 2015 Best Oral Presentation (Student).

Introduction

Although short-term (<7 exposures) heat acclimation protocols can yield some positive performance benefits, it appears that longer term (8-14 exposures) protocols are more beneficial for endurance athletes. However, what is unclear is whether short, often demanding programmes also evoke acute stress that could overload anti-inflammatory pathways. The aim of this study was to determine whether undertaking heat training causes a significant change in blood biomarkers associated with heat stress and inflammation.

Method

With institutional ethical approval 16 male participants were randomly allocated to either a heat training group (EXP, n = 8; training at 35°C, 70% RH) or a control group (CON, n = 8; training at 20°C, 45% RH). All participants performed seven training sessions and three heat stress tests (HST) over 18 days, involving an intense first week of six sessions in seven days, followed by three top-up sessions over nine days. Exercise training sessions comprised 4 x 10 min stationary cycling at 55% of VO_2 max in either EXP or CON environments. The HST required participants to complete three sub-maximal workloads of 10 min duration (50%, 60% and 70% VO_2 max) on a cycle ergometer followed by a 5 km time trial (35°C, 70% RH). Serum blood samples were collected pre and post each HST and analysed for the concentrations of interleukin-6, immunoglobulin M and lipopolysaccharide.

Results

EXP and CON groups had a significant improvement in time trial performance (s) between HST1 (baseline) and HST2 (7 days), (EXP, 590 s ± 48 s mean ± 95%CI, 556 s ± 39 s, p = 0.04, E.S = -0.65; CON, 613 s ± 37 s, 575 s ± 35 s, p = 0.02, ES = 0.88), however, EXP were also faster in HST3 (18 days) vs HST1 and HST2 (541 s ± 35 s, p = 0.02, E.S = -0.98). There was no significant pre to post time or group differences for immunoglobulin M or lipopolysaccharide.

Conclusion

Although short-term heat training can enhance 5 km cycling time trial performance, this effect is no greater than matched exercise training in temperate conditions. However, the addition of three top-up heat training sessions between days 7 – 18 was sufficient to infer further performance adaption for the heat training group alone. Elevations in pro- and anti-inflammatory cytokines (i.e. interleukin-6) were insufficient to trigger further systemic inflammation. The findings of this study suggest intensive short-term (<7 days) heat training protocols should be supplemented by periodic post-programme top-up sessions.

Reliability of serum biomarkers associated with heat stress, inflammation and immunosuppression in healthy, tropically acclimatised, active individuals.

Guy, J. H., Edwards, A. M., Miller, C. M., Deakin, G. B., & Pyne, D. B. (2014, July). ECSS conference proceedings (Amsterdam, Netherlands). Oral presentation.

Introduction

Cytokines, lipopolysaccharides, and shock proteins play an important role in regulating the levels of stress, inflammation and heat acclimation during exercise in hot environments. The aim of this study was to quantify the within-subject variability of serum biomarkers that have been commonly used to examine immune function and inflammation following heat exposure in healthy active males.

Methods

Twelve recreationally active healthy males (age; 24 ± 4.0 years, VO_2 max; 50.8 ± 6.4 mL.kg^{-1}.min^{-1}, height; 1.78 ± 0.05 m, weight; 74.1 ± 8.9 kg, mean $\pm$ SD) participated in this study. Over a 14 day period subjects abstained from high intensity exercise and reported to the laboratory on three occasions at a similar time of day on day 0, day 7, and day 14. On each visit, subjects provided an 8mL serum blood sample. Commercially available ELISA bench top kits were used to analyse the samples for the serum concentrations of C-reactive protein (CRP), Interleukin-6 (IL-6), Heat Shock Protein 72 (HSP72), and Lipopolysaccharides (LPS). Data are presented as mean within-subject coefficients of variation $\pm$ SD (CV) with units of measurements for each variable expressed as a minimum-maximum range.

Results

The biomarkers with the least variation across the 14 day study period were LPS ($7.66 \pm 6.41\%$; 0.16-0.27 EU.ml^{-1}) and IL-6 (CV of $13.7 \pm 18.6\%$; 0.47-4.21pg.ml^{-1}). HSP72 levels were consistently beneath the detectable range, demonstrating very low concentrations of this protein at rest in the human body. The most variable marker was CRP with a CV of $37.7 \pm 26.9\%$ (0.70-29.0 mg.mL^{-1}).

Discussion

These data indicate LPS, HSP72, and IL-6 are relatively stable biomarkers of inflammatory status in the absence of a heat stress or exercise interventions across a 14-day period. Although CRP produced a substantially larger within-subject CV (~38%), CRP values can increase up to 10,000-fold following an acute stress response (Pepys & Hirschfield, 2003). Consequently, evaluation of CRP should not necessarily be discounted as a biomarker of acute stress if the magnitude of change following an intervention is similar to, or larger than, the day to day biological variability. Further research examining the efficacy of these biomarkers following heat and/or exercise stress is required to clarify their use in clinical and research settings.

References

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Exercise in the heat in comparison to temperate conditions: Can acclimation improve athletic performance?

Guy, J. H., Deakin, G. B., Edwards, A. M., Miller, C. M., &Pyne, D. B. (2013, November). SSEPAT conference proceedings (Cairns, Australia). Oral presentation.

Introduction

Environmental conditions outside of the thermo-neutral zone are often reputed to compromise athletic performance. Although evidence suggests performance may be improved by undergoing acclimation prior to competition in the heat[2], the extent of impairment has surprisingly not yet been thoroughly investigated. The purpose of this study was to determine the degree of impairment in performance of endurance athletes in challenging environments, and the effectiveness of existing acclimation training protocols.

Methods

IAAF world championships (1999-2011) data was analysed to determine the effect of temperature on performance during athletic track events. Additionally, data from studies on short- ($\leq$ 7 days) and medium-8-14 days) term heat acclimation training (STHA and MTHA) was extracted to determine the effect of acclimation on: time to exhaustion, athletic performance, final exercising heart rate, core temperature, and plasma volume. Standardised mean differences were interpreted using Cohen's d (small: <0.2, medium: 0.2-0.8, or large: >0.8)[1], and data is expressed as mean change ±SE.

Results

Marathon races held in hot ($\geq$ 25°C) were 3.1±0.2% (males) and 2.7±0.1% (females) slower compared with cool conditions (<25°C). In contrast male sprinters were faster in both 100 m (1.3±.3%) and 200 m (1.3±.2%) races when events were held in hot conditions. The effect of STHA and MTHA studies on performance and physiological measures are shown in Table 1.

Table 1. Percentage change and effect of short- and medium- term acclimation training

Acclimation Period	TTE	Athletic Performance	Heart Rate	Core Temperature	Plasma Volume*
STHA (≤ 7 days)	Medium ↑ 23.4±9.2%	Medium ↑ 0.8±0.2%	Large ↓ -3.9±1.6%	Medium ↓ -0.4±0.2%	↑ 2.3±2.1%.
MTHA (8-14 days)	Large ↑ 17.0±10.2%	Medium ↑ 2.2±1.3%	Large ↓ -7.6±1.1%	Large ↓ -0.7±0.1%	↑ 9.5±1.4%.

TTE: Time to exhaustion. STHA: Short-term heat acclimation. MTHA: Medium-term heat acclimation. ↑: Increase ↓: Decrease. Effect sizes are reported as: small (<0.2), medium (0.2-0.8), or large (>0.8). Percentage change is expressed ±SE.* Effect size not applied as the selected studies did not report pre-post values.

Conclusion

Endurance athletes are susceptible to performance impairment when competing in distance events that take place in hot ($\geq$ 25°C) and humid conditions. It appears that worthwhile acclimation can be achieved via both short-term ($\leq$ 7 days) and moderate term heat acclimation programs. The larger improvement with MTHA indicates athletes should contemplate longer heat acclimation programs where possible.

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10.2   Ethical approval

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10.3   Permission for print

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10.4 Associated Publications

Throughout my PhD candidature I was also involved in the publication of several other research papers that investigated the cardiorespiratory and brain regulation of exercise in thermally challenging environments as well as the immune and inflammatory response of firefighters in response to heat stress. Additionally, I co-authored a brief review on managing heat and immune stress in athletes with evidence-based strategies.

10.4.1 Brain and cardiorespiratory responses to exercise in hot and thermo-neutral conditions

Reference

Abstract

The aim of this study was to test whether or not concurrent evaluations of brain (electroencephalography [EEG]) and cardiorespiratory responses to exercise are influenced by environmental conditions. 10 adult male participants performed a standardized incremental exercise test to exhaustion on a cycle ergometer in an environment controlled laboratory on 2 separate occasions, in a randomized order; one in a hot condition (34.5°C) and one in a thermo-neutral condition (20°C). EEG, heart rate and expired air were collected throughout. EEG data were decontaminated for artefacts, log-transformed and expressed as aggregated alpha and beta power responses across electrodes reflecting the frontal cortex of the brain. Performance outcomes showed there was no difference in VO$_2$ peak across hot (42.5 mL.kg^{-1}.min^{-1}) and neutral (42.8 mL.kg^{-1}.min^{-1}) conditions, although ventilatory threshold (VT) occurred at a lower threshold (68%) in hot compared to neutral condition (74%) ($p<0.05$). EEG alpha and beta wave responses both demonstrated significant increases from baseline to VT ($p<0.01$). EEG beta-band activity was significantly elevated in the heat compared to the neutral condition. In conclusion, elevated EEG beta-band activity in response to incremental exercise in the heat suggests that beta-band activation and cortical awareness increases as exercise becomes increasingly intense.

10.4.2 Immune and inflammatory responses of Australian firefighters after repeated exposures to the heat

Abstract

When firefighters work in hot conditions, altered immune and inflammatory responses may increase the risk of a cardiac event. The present study aimed to establish the time course of such responses. Forty-two urban firefighters completed a repeat work protocol in a heat chamber ($100 \pm 5°C$). Changes to leukocytes, platelets, TNFα, IL-6, IL-10, LPS and CRP were evaluated immediately post-work and also after 1 and 24 h of rest. Increases in core temperatures were associated with significant increases in leukocytes, platelets and TNFα directly following work. Further, platelets continued to increase at 1 h ($+31.2 \pm 31.3 \times 10^9$ l, $p<0.01$) and remained elevated at 24 h ($+15.9 \pm 19.6 \times 10^9$ l, $p<0.01$). Sustained increases in leukocytes and platelets may increase the risk of cardiac events in firefighters when performing repeat work tasks in the heat. This is particularly relevant during multi-day deployments following natural disasters.

10.4.3 Managing heat and immune stress in athletes with evidence-based strategies

Abstract

Heat and immune stress can affect athletes in a wide range of sports and environmental conditions. The classical thermoregulatory model of heat stress has been well characterised, as has a wide range of practical strategies largely centered on cooling and heat acclimation training. In the last decade evidence has emerged of an inflammatory pathway that can also contribute to heat stress. Studies are now addressing the complex and dynamic interplay between hyperthermia, the coagulation cascade and a systemic inflammatory response occurring after transient damage to the gastrointestinal tract. Damage to the intestinal mucosal membrane increases permeability resulting in leakage of endotoxins into the circulation. Practical strategies that target both thermoregulatory and inflammatory causes of heat stress include pre-cooling, short-term heat acclimation training, nutritional countermeasures including hydration, energy replacement and probiotic supplementation, pacing strategies during events, and post-event cooling measures. Cooperation between international, national and local sporting organisations is required to ensure that heat management policies and strategies are implemented effectively to promote the well-being and performance of athletes.