Thermoregulatory and physiological responses to post-exercise hot water immersion and effects on endurance cycling performance

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Abstract

In 2016, repeated post-exercise head-out 40°C water immersion emerged as a practical heat acclimation strategy that improves endurance exercise performance in the heat in physically active individuals. However, it remains to be determined whether this heat acclimation strategy could improve endurance performance in the heat in well-trained endurance athletes. It would also be of interest to examine inflammatory and oxidative stress responses to this heat acclimation strategy. Addressing that question is relevant, given that the degree of hyperthermia associated with post-exercise 40°C water immersion induces inflammation and oxidative stress, and knowing that both physiological events can impact exercise performance. Another concern related to the post-exercise 40°C water immersion intervention is the risk of heat illness. Therefore, it is important to identify a thermometric method that would be suitable for monitoring body core temperature during hot water immersion sessions in 'real life'.

Study One examined the effects of repeated post-exercise head-out hot (40°C) water immersion on physiological and exercise performance outcomes in warm and hot environmental conditions in a group of well-trained non-heat acclimated male endurance athletes. **Methods:** Sixteen well-trained male cyclists completed a six-day intervention involving a daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot water immersion (HWI; n = 8) or thermoneutral water immersion (CON; n = 8) for 40 minutes. At baseline and post-intervention, participants completed two 30-minute continuous cycling tests followed by 20-km time trial tests (one at 27°C, 40% RH and the other one at 35°C, 40% RH) on two different days separated by one day of rest. **Results:** The HWI group showed a significantly decreased peak heart rate (-7.37 ± 5.21 beats·min^{-1}, p = 0.03), peak thermal sensation (-0.56 ± 0.41 arbitrary units, p < 0.01), and rating of perceived exertion (-1.00 ± 0.75 arbitrary units, p = 0.02) during the 30-minute continuous test at 27°C. The HWI group also showed a significantly decreased peak thermal sensation (-0.50 ± 0.53 arbitrary units, p = 0.03) and peak rating of perceived exertion (-1.62 ± 1.06 arbitrary units, p = 0.01) during the 30-minute continuous test at 35°C. None of these variables were altered in the CON group. Furthermore, the HWI group showed only a tendency for improved 20 km time trial performance at 27°C (p = 0.06) and 35°C (p = 0.06). **Conclusion:** The findings from

this study indicate that a six-day post-exercise hot water immersion reduces cardiovascular and perceptual strain during exercise at 27°C and reduces only perceptual strain during moderate-intensity exercise at 35°C. Although the post-exercise hot water immersion intervention did not significantly improve the 20 km time trial performance either at 27°C or 35°C, the reduction in the completion time of the latter test by 1.77% in the HWI group can be considered practically significant.

Study Two determined the acute and chronic effects of exposure to post-exercise head-out immersion in 40°C water on plasma cytokine response, oxidative stress, and antioxidant capacity. **Methods:** Fourteen well-trained male cyclists completed a six-day intervention involving a daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot water immersion (HWI; $n = 7$) or thermoneutral water immersion (CON; $n = 7$) for 40 minutes. Ten ml of a venous blood sample was taken before and after the first session to assess acute inflammatory responses, oxidative stress, and total antioxidant capacity. Resting venous blood was also sampled 48 hours after the last session to assess chronic inflammatory responses, oxidative stress, and total antioxidant capacity. The inflammatory response was assessed by measuring the following cytokines: tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), IL-6, and IL-10. Oxidative stress was determined by measuring thiobarbituric acid-reactive substances (TBARS), whereas total antioxidant capacity (TAC) was measured as a sum of all antioxidants in the plasma. **Results:** Acute post-exercise hot water immersion had no significant effects on any of the measured biomarkers (all $p > 0.05$). Acute post-exercise immersion in 34°C water significantly increased IL-6 ($p < 0.01$). Post-exercise hot water immersion over six consecutive days significantly increased resting plasma IL-1β concentration ($p < 0.01$). **Conclusion:** This study suggests that chronic heat stress imposed by post-exercise hot water immersion induces an inflammatory response but not oxidative stress in well-trained male endurance athletes.

Study Three examined whether commercially available tympanic thermometers could be a suitable substitute for the expensive ingestible telemetric pills or invasive rectal probes for monitoring core temperature during the post-exercise head-out 40°C water immersion. **Methods:** Sixteen male cyclists cycled for 40 minutes at 50% of their peak

power output in a cool environment (14°C, 40% RH), after which they were immersed in a bath of hot water for 40 minutes. Participants' tympanic and core temperatures were measured at rest, after exercise, and every 10 minutes throughout the hot water immersion session. The tympanic temperature was measured by Genius™ 2 and Braun Pro 4000 Thermoscan tympanic thermometers, whereas core temperature was measured via an ingestible telemetric pill. The latter was used as a reference against which the tympanic thermometers were validated. **Results:** No statistically significant differences in temperature readings were observed between the telemetric pill and Braun Pro 4000 Thermoscan at any time point during the hot water immersion session, and these temperature readings were significantly correlated at all time points. The overall bias in temperature reading provided by Braun Pro 4000 Thermoscan relative to the telemetric pill was within the acceptable limit (< 0.3°C). Temperatures provided by Genius™2 at the 20-, 30, and 40-minute time points during the hot water immersion period were significantly higher than those of the telemetric pill, and there were no correlations between the devices at the last two time points. The overall mean bias associated with the Genius™2 tympanic thermometer was 0.50°C. **Conclusion:** The findings from this study indicate that Braun Pro 4000 Thermoscan could be a suitable tool for monitoring core temperature during post-exercise head-out 40°C water immersion. Unfortunately, Genius™2 did not pass the validity testing and thus it is not recommended for use during hot water immersion.

I, Metodija Kjertakov, declare that the PhD thesis entitled '*Thermoregulatory and physiological responses to post-exercise hot water immersion and effects on endurance cycling performance*' is no more than 80,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

I have conducted my research in alignment with the Australian Code for the Responsible Conduct of Research and Victoria University's Higher Degree by Research Policy and Procedures. All research procedures reported in the thesis were approved by the Victoria University Human Research ethics committee, under approval numbers HRE21-057, HRE22-035.

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List of abbreviations

AU	Arbitrary units
BM	Body mass
CRP	C-reactive protein
CON	Control group
d	Effect size
ES	Effect size
GXT	Incremental test protocol
Hb	Haemoglobin
HSP70	Heat shock protein 70
HWI	Hot water immersion
INF-γ	Interferon-gamma
IL-1β	Interleukin 1β
IL-1ra	Interleukin 1 receptor antagonist
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12p40	Interleukin 12 p40
IL-13	Interleukin 13
IL-15	Interleukin 15
$L \cdot h^{-1}$	Liters per hour
O_2	Oxygen
PPO	Peak power output
RBC	Erythrocytes
RH	Relative humidity
TNF-α	Tumour necrosis factor
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E/\dot{V}O_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
USG	Urine specific gravity

W	Watts

Chapter one: Introduction

It is well-documented that hot environmental conditions impair exercise performance (Galloway and Maughan, 1997; Peiffer and Abbiss, 2011; Tatterson et al., 2000) and increase the risk of developing adverse health events such as heat illness (Armstrong et al., 2007; Kjertakov and Epstein, 2013). The negative effects associated with exercise in the heat are particularly pronounced in athletes who are not accustomed to exercise in such an environment (Racinais et al., 2015a). However, repeated exposure to exercise in the heat, widely known as heat acclimation, improves thermoregulation, attenuates physiological strain, and enhances exercise performance during subsequent heat exposure (Maughan and Shirreffs, 2004; Périard et al., 2015). Therefore, heat acclimation is an important training strategy for athletes residing in a cooler climate and preparing to compete in the heat.

Achieving complete heat acclimation requires usually 10 to 14 days of heat exposure (Armstrong and Maresh, 1991; Périard et al., 2015; Racinais et al., 2019b), after which the capacity to perform endurance exercises in hot conditions increases (Burk et al., 2012; Keiser et al., 2015; Lorenzo et al., 2010; Nielsen et al., 1993; Racinais et al., 2015b; Travers et al., 2020; Voltaire et al., 2002; Willmott et al., 2018). Several studies also showed that only five daily exercise sessions in the heat are sufficient to improve cycling (Racinais et al., 2015b), running (James et al., 2017), and rowing (Garret et al., 2012) time trial performance in hot conditions in previously non-heat acclimated athletes. For athletes residing in a cooler climate and preparing to compete in the heat, the use of short-term heat acclimation would be more appropriate during the tapering phase compared with traditional, longer heat acclimation protocols. Nonetheless, the findings from the studies cited above (Garret et al., 2012; James et al., 2017; Racinais et al., 2015b) apply to athletes who can either train in artificial hot conditions (e.g., environmental chamber) or afford to travel to the competition venue at least one week in advance and complete the heat acclimation process there. All other athletes will have to rely on passive heat acclimation strategies such as hot water immersion (Heathcote et al., 2018).

In 2016, Zurawlew et al., (2016) demonstrated that hot water immersion could be an effective alternative to active heat acclimation. These researchers recruited seventeen physically active, non-heat acclimatised males who completed a 6-day intervention

consisting of daily running for 40 minutes at 65% of $\dot{V}O_{2max}$ in temperate conditions (18°C, 40% humidity) followed immediately by either hot (40°C; *n* = 10) or thermoneutral (34°C; *n* = 7) water immersion up to the neck. Before and after the intervention, and on separate days, the participants completed a 40-minute continuous running test at submaximal intensity followed by a 5-km run time trial in temperate (18°C, 40% humidity) and hot (33°C, 40% humidity) conditions. The former test served to assess heat adaptations, whereas the latter was an exercise performance test. Post-intervention, a significant reduction in rectal and skin temperatures, heart rate, and rating of perceived exertion during the 40-minute continuous test in both environmental conditions was observed only in the hot water group. Similarly, only the hot water group demonstrated improvement in 5-km run time trial performance (4.9%), though the running performance was unchanged in temperate conditions. Subsequently, several other studies confirmed the ability of repeated post-exercise hot water immersion to induce heat acclimation (Ashworth et al., 2023; McIntyre et al., 2021; Waldock et al., 2021; Zurawlew et al., 2018a, 2018b, 2019), but no study attempted to examine whether this heat acclimation strategy is ergogenic for endurance performance in the heat in trained endurance athletes. Therefore, the main aim of the first study was to investigate whether post-exercise hot water immersion over six days induces heat acclimation and improves endurance performance in the heat in a group of well-trained road cyclists. The additional aim was to examine the effects of this heat acclimation strategy on physiological, perceptual, and exercise performance outcomes during exercise in a moderately warm environment. Addressing that question would reveal whether athletes can use heat acclimation to improve their exercise performance when competing during the first warm days of the spring or under unseasonably warm environmental conditions.

Another gap related to the heat acclimation strategy introduced by Zurawlew et al., (2016) is whether repeated post-exercise hot water immersion causes inflammation and/or oxidative stress. Although acute inflammation is vital in host defence and promotes tissue repair, chronic inflammation may hinder athletic performance, impair the immune system, and contribute to the development of overtraining syndrome (Cheng et al., 2020; Smith, 2003). There is evidence from the active heat acclimation literature that repeated exposure to exercise in the heat leads to a state of chronic inflammation (Hailes et al., 2011). In that study, fifteen unacclimated recreationally-

trained males underwent a five-day heat acclimation protocol consisting of daily cycling at 38°C until their rectal temperature reached 39.5°C (~40 minutes). Of the eighty inflammatory markers (cytokines) examined by the researchers (Hailes et al., 2011), resting values of eighteen were significantly increased on the fifth day compared to the first day. While the physiological mechanism(s) leading to a significant increase in some of the studied inflammatory markers is unclear, it could be assumed that the increase in core temperature to 39.5°C during the heat acclimation sessions was mainly responsible for the observed changes. Such an assumption is based on the work by Rhind et al., (2004), who showed that elevation of core temperature during exercise is a crucial determinant of cytokine release and that exercise does not induce cytokinaemia in the absence of a simultaneous rise in core temperature. Knowing that post-exercise 40°C water immersion sessions induce a degree of hyperthermia (~39.5°C) similar to that during heat acclimation sessions in the study by Hailes et al., (2011), one may suggest that the six-day post-exercise hot water immersion intervention may increase the risk of chronic inflammation. There is also evidence to suggest that this heat acclimation strategy may increase the production of free radicals and, as a result, cause oxidative stress. Indeed, several studies indicated that acute increase in core temperature to ~39°C, either by passive (Pilch et al., 2014) or active (McAnulty et al., 2005; Sureda et al., 2015) heat exposure increased indicators of oxidative stress, whereas a repeated increase in core temperature to 40°C during a ten-day heat acclimation increased resting oxidative stress (Kaldur et al., 2014). Similar to cytokines, free radicals play an important role in several physiological processes (Pham-Huy et al., 2008; Verhagen et al., 2006), but it is their overproduction and resultant oxidative stress that is concerning since chronic oxidative stress is thought to be involved in the onset of premature fatigue (Allen et al., 2008; Powers and Jackson, 2008), initiation of muscle damage (Aoi et al., 2004; Gómez-Cabrera et al., 2003), and the development of overtraining syndrome (Cheng et al., 2020; Tiidus, 1998). Given all the above, the second study aimed to examine the acute and chronic effects of exposure to post-exercise head-out immersion in 40°C water on plasma cytokine response and oxidative stress.

While post-exercise head-out immersion in 40°C water provides a powerful stimulus for heat acclimation, this heating method creates a condition of uncompensable heat stress where the body cannot maintain a thermal steady state. Consequently, the core

temperature could be driven to the point of heat stroke. In fact, the medical literature has reported a case of heat stroke resulting from bathing in 40°C water (Lee et al., 2010). Although no ill health effects were reported in any of the laboratory studies that had participants submerged up to the neck in 40°C water for 30-40 minutes immediately after exercise (Ashworth et al., 2023; McIntyre et al., 2021; Waldock et al., 2021; Zurawlew et al., 2016, 2018a, 2018b, 2019), one should bear in mind that the risk of heat stroke in those studies was minimised. Indeed, all participants were in good physical health, well-hydrated, and their body core temperature was continuously monitored during immersion sessions. In a 'real life' situation, athletes may commence post-exercise hot water immersion already predisposed to heat stroke due to experiencing some of the risk factors (e.g., hypohydration, fatigue, illness) for this disorder (Epstein and Shapiro, 1995; Kjertakov and Epstein, 2013). Furthermore, visual observation of athletes may not be enough to prevent heat stroke, as the onset of this heat disorder is usually sudden and rapid, and there are no easily recognisable signs that one can use to predict its development (Roberts, 2004). Accordingly, the best way to ensure athletes' safety during hot water immersions is frequent monitoring of their body core temperature. However, the only methods (e.g., ingestible temperature pills and temperature probes) by which body core temperature can be accurately measured during hot water immersion sessions require expensive equipment and are invasive, and thus are most often restricted to laboratory settings. Tympanic thermometers overcome those limitations, are easy to use, and some brands (Genius and Braun) of these devices have been validated for monitoring body core temperature during exercise in the heat (Otani et al., 2010; Fenemor et al., 2020; Morán-Navarro et al., 2019) and in clinical settings (Bock et al., 2005; Kocoglu et al., 2002; van Staaij et al., 2003). Yet, since the applicability of those findings is likely limited to the studied interventions, the usefulness of tympanic thermometers to monitor core temperature during post-exercise head-out 40°C water immersion needs to be confirmed before these devices are accepted as viable screening tools for hyperthermia during the latter scenario. Therefore, the third study aimed to examine the validity of the GeniusTM 2 and Braun Pro 4000 Thermoscan tympanic thermometers to predict core temperature during post-exercise hot water immersion.

Chapter two: Literature review

2.1. Cycling in the heat

Compared with exercise in thermoneutral climates, exercise in the heat is associated with a significantly higher increase in heart rate, body temperature, glycogen utilisation, and ratings of perceived exertion (Logan-Sprenger et al., 2012; Logan-Sprenger et al., 2013). Thus, it is not surprising that compared with thermoneutral climates, endurance exercise performance in the heat is impaired (Périard and Racinais, 2019). The following several studies (Galloway and Maughan, 1997; Peiffer and Abbiss, 2011; Racinais et al., 2015b; Tatterson et al., 2000) clearly show that the physiological functioning and endurance cycling performance of road cyclists are closely related to environmental conditions.

In the study by Galloway and Maughan (1997), eight healthy males completed four rides to exhaustion at 70% of their $\dot{V}O_{2max}$ in an environmental chamber at air temperatures of 3°C, 10°C, 20°C, and 30°C with relative humidity (RH) of 70%. The rides were performed on different days, separated by one or two weeks. During each ride, the researchers recorded participants' ratings of perceived exertion, skin and rectal temperature, and heart rate. Change in body mass in response to the rides was also measured to calculate sweat rate. The air temperature had a significant impact on time to exhaustion, with the exercise duration being longest at 10°C (93.5 ± 6.2 minutes) and shortest at 30°C (51.6 ± 3.7 minutes). No significant difference in the time to exhaustion was noted between the rides at 3°C (81.4 ± 9.6 minutes) and 10°C (81.2 ± 5.7 minutes), but there was a significant difference when these two rides were compared with the other two rides. The air temperature also had a significant influence on the other measured variables. The rectal temperature was highest during the ride at 30°C and lowest during the ride at 3°C, and it was significantly higher during the ride at 20°C compared with the other two colder rides. The skin temperature was significantly different between the rides, and the highest value was recorded during the ride at 30°C. The heart rate and rating of perceived exertion were significantly higher during the ride at 30°C compared to the other three rides. The sweat rates during the rides at 3°C, 10°C, 20°C, and 30°C were 0.55 $L \cdot h^{-1}$, 0.65 $L \cdot h^{-1}$, 0.78 $L \cdot h^{-1}$, and 1.15 $L \cdot h^{-1}$, respectively, and were significantly different from each other.

In a subsequent study, Tatterson et al., (2000) had eleven members of the Australian Road National Cycling Squad performing two 30-minute cycling time trials in an environmental chamber at 32°C and 23°C on different days separated by two days. During the trials, rectal and skin temperatures, heart rate, ratings of perceived exertion, power output, and sweat rate (*via* the change in body mass) were recorded. The average power output was significantly higher during the trial at 23°C than during the trial at 32°C (345 ± 9 watts versus 323 ± 8 watts). The rectal temperature was similar between the trials, but the skin temperature was significantly higher during the trial in the heat compared with the trial in the temperate environment. A significantly higher value of heart rate was noted during the trial at 32°C compared with the trial at 23°C, although the peak heart rates were not significantly different at the end of the trials. Similarly, the ratings of perceived exertion were significantly higher throughout the trial in the heat, but the values noted at the end of both trials were not significantly different. Participants lost significantly more sweat during the trial in the heat compared with the trial in the temperate environment (2.25 ± 0.4 $L \cdot h^{-1}$ versus 1.88 ± 0.10 $L \cdot h^{-1}$).

Peiffer and Abbiss (2011) recruited nine trained male cyclists to perform four 40 km cycling time trials in an environmental chamber at 17°C, 22°C, 27°C, and 32°C (relative humidity of 40% in all trials). The time trials were separated by five to fourteen days. Rectal temperature, heart rate, ratings of perceived exertion, thermal sensation, and power output were recorded during the time trials. The time taken to complete the 40 km time trial was significantly shorter in the 17°C (58.8 ± 2.0 minutes), 22°C (59.0 ± 2.3 minutes), and 27°C (59.1 ± 2.3 minutes) conditions compared with the 32°C (60.7 ± 2.9 minutes) condition. Furthermore, significantly higher average power output was sustained during the time trial at 17°C (329 ± 31 watts), 22°C (324 ± 34 watts), and 27°C (322 ± 32 watts) conditions compared with 32°C (309 ± 35 watts) condition. A significantly higher rectal temperature was reported during the last 10 km of the time trial at 32°C compared with the time trials at 17°C and 22°C. No other significant differences in the rectal temperature were noted between the trials. The only significant difference in the heart rate was observed between 32°C (168 ± 10 $beats \cdot min^{-1}$) and 17°C conditions (164 ± 7 $beats \cdot min^{-1}$). Participants reported a significantly higher value of thermal sensation during the trial in the heat (5.9 ± 0.7 arbitrary units [AU]) compared with the trials at 17°C (3.7 ± 1.0 AU), 22°C (4.5 ± 0.8 AU), and 27°C (4.8 ± 0.9 AU). They also reported a significantly higher rating of

perceived exertion score for the trial at 32°C (17.1 ± 1.5 AU) compared with the trial at 17°C (15.8 ± 1.5 AU) and 27 °C (16.1 ± 1.3 AU).

The study of Racinais et al., (2015b) has a greater ecological value than the former three studies because it was conducted outdoors. In fact, the researchers tested nine competitive male cyclists during two different 43.4 km time trials performed outdoors at similar terrain and wind speed, where the first one was conducted in Denmark at 8°C (wind speed of 6.0 meters per second) and the second one in Qatar at 36°C (wind speed of 6.8 meters per second). Data collected during the time trials included power output and heart rate, whereas rectal temperature was measured at the end of the trials. Consistent with the findings of the previous three studies, power output was significantly higher during the time trial at 8°C compared with the time trial at 36°C (304 ± 9 watts versus 256 ± 19 watts). As a result, the time to complete the time trial in the colder condition (66.13 ± 3.26 minutes) was significantly shorter than the time trial in the heat (77.17 ± 6.26 minutes). Although not significant, the increase in the heart rate during the time trial at 36°C was seven beats·min^{-1} greater compared with the heart rate during the time trial at 8°C. Finally, a significantly higher rectal temperature was measured at the end of the time trial in the heat (40.2 ± 0.4°C) versus the one at 8°C (38.5 ± 0.6°C).

In addition to the detrimental effects on exercise performance, hot environmental conditions can lead to the development of heat illness, including heat edema, heat cramps, heat syncope, heat exhaustion, and heat stroke (Barrow and Clark, 1998; Coris et al., 2004; Kjertakov and Epstein, 2013). *Heat edema* is the mildest form of heat illness, which appears as a swelling of hands and feet, usually in non-acclimated people. *Heat cramps* are involuntary, painful, spasmodic contractions of muscles, which tend to occur in settings of heavy sweating coupled with inadequate water and sodium intake (Bergeron, 1996). *Heat syncope* is fainting that is typically associated with prolonged standing. *Heat exhaustion* is the most common form of heat illness, defined as an inability to continue physical activity in the heat (Knochel, 1989). It is believed to be caused by the inability of the body to sustain the necessary rate of cardiac output to simultaneously meet the demands of blood flow for skin, vital organs, and exercising muscles. Dehydration-induced plasma volume depletion is thought to be the main factor for the development of this illness (Knochel, 1989). *Heat stroke,*

defined by a body core temperature above 40ºC and central nervous system dysfunction, occurs when the body's thermoregulation system fails to remove the excess heat accumulated from the external environment and metabolic processes (Knochel, 1989). The last heat illness is a true medical emergency that can result in significant morbidity and mortality if not recognised and promptly treated. In fact, heat stroke has been reported to be the second leading cause of death among athletes after cardiovascular events (Muniz-Pardos, 2019). Although American-type football players and long-distance runners are the most common victims of heat stroke (Kjertakov and Epstein, 2013), any athlete exercising in the heat is at risk of developing extremely dangerous hyperthermia.

In the context of extreme hyperthermia, it might be interesting to note that body temperatures considerably above that associated with the onset of heat stroke have been reported in asymptomatic road cyclists. Racinais et al., (2019a) measured body core temperature using gastrointestinal pills in forty elite male and female cyclists during the 2016 UCI Road World Championship held in the heat (37ºC) and observed that a few female riders experienced an increase in core temperature above 41ºC during the 40 km team time trial race. The highest measured core temperature was 41.5ºC. However, it is likely that the researchers failed to record the 'real' peak core temperatures in their participants due to the fact that the gastrointestinal pills (e-Celsius^{TM}) they used underestimated rectal temperature by 0.34ºC during cycling in the heat (Travers et al., 2016). The latter method is considered the 'gold standard' for measuring body core temperature (Casa et al., 2007; Gant et al., 2006; Ganio et al., 2009; Hosokawa et al., 2016). Measuring body temperature *via* the rectal route in a marathon study, Maron et al., (1975) observed a value of body temperature of 41.9ºC in one of their participants who was free from any symptoms of heat illness. Considering that people have died from heat stroke (Chao et al., 1981; Kim et al., 2006; Rav-Acha et al., 2004; Sohal et al., 1968; Zhou et al., 2006) associated with the degree of hyperthermia lower than that reported by Racinais et al., (2019a) and Maron et al., (1975), it is clear that difference in tolerance to extreme hyperthermia exists between individuals. A question that remains open is whether the athletes who developed extreme hyperthermia in those two studies (Racinais et al., 2019a; Maron et al., 1975) were able to perform at their best.

Given the negative physiological effects and health risks associated with exercise in the heat, it is not surprising that massive research effort has been spent on developing effective heat mitigation strategies. Heat acclimation, fluid ingestion and pre-exercise cooling have repeatedly been shown to improve thermoregulation and endurance capacity during exercise in the heat. A recent meta-analysis demonstrated that heat acclimation is a more effective heat mitigation strategy than the latter two strategies (Alhadad et al., 2019). This section will focus on heat acclimation. Other heat-mitigation strategies are covered elsewhere (Armstrong et al., 2007; Racinais et al., 2015a).

2.2. Heat acclimation

The ability of repeated exposure to exercise in a hot environment to induce physiological adaptations that improve thermoregulation, attenuate physiological strain and enhance endurance capacity during subsequent heat exposure has been known since the early 1940s (Robinson et al., 1943). The physiological adaptations include, but are not limited to, improved cardiovascular stability (Périard et al., 2016), reduced resting and exercising body core temperature (Taylor, 2014), increased skin blood flow and sweating (Périard et al., 2015), and improved skeletal muscle metabolism (Febbraio et al., 1994; Kirwan et al., 1987). The process of adaptation to heat can be induced in naturally hot environments (i.e., acclimatisation) and in artificial hot environments (i.e., acclimation). Both strategies will be referred to as heat acclimation throughout the text.

There are four different heat-exercise protocols by which heat acclimation can be achieved, including (1) self-paced exercise; (2) fixed-intensity exercise; (3) controlled heart rate, where a given level of cardiovascular stress is maintained; and (4) controlled hyperthermia, where a target body core temperature is maintained (Pryor et al., 2019; Racinais et al., 2019b). Additionally, passive heat exposure can also induce adaptations to heat (Heathcote et al., 2018). Irrespective of the method used, achieving effective heat acclimation requires maintenance of core temperature at ~38.5°C throughout the heat acclimation sessions (Pryor et al., 2019). It should be noted that there appear to be no additional adaptational benefits when the core temperature is raised to 39°C during the heat acclimation sessions (Gibson et al., 2015).

2.2.1. Induction of heat acclimation

In general, heat acclimation is a rapid process that begins on the first day of heat exposure. During the initial exposure to heat stress, the physiological strain is high, as demonstrated by an elevated heart rate and body temperature. However, the physiological strain caused by heat stress progressively decreases each day of the heat acclimation period (Périard et al., 2015). The timeline of physiological adaptations to heat is classified into short-term (< 7 days), medium-term (8 to 14 days), and long-term (> 14 days) (Périard et al., 2015; Pryor et al., 2018; Tyler et al., 2016). Importantly, 75-80% of adaptations that influence exercise performance occur in the first four to seven days of the heat acclimation process (Pandolf, 1998). Cardiovascular changes are the earliest adaptive response to chronic heat exposure, and they are characterised by reduced resting and exercising heart rate and hypervolemia (Armstrong and Maresh, 1991; Périard et al., 2015). Expansion of plasma volume leads to increased stroke volume, which is thought to be the primary mechanism for reduced heart rate. These improvements reduce the cardiovascular strain experienced by an athlete for any given exercise intensity. In addition, plasma volume expansion provides thermoregulatory benefits such as increased cutaneous blood flow and improved sweat response (Convertino, 1991; Guy et al., 2015). Plasma volume has been reported to increase in a range between 3% to 27% within the first five days of the onset of a heat acclimation protocol (Périard et al., 2016; Racinais et al., 2019b). The magnitude of the increase in plasma volume depends on the heat acclimation protocol and the participant's fitness status (Périard et al., 2015). One study also showed that dehydration can lead to a greater plasma volume expansion during a heat acclimation intervention compared with euhydration (Garrett et al., 2014), but subsequent studies were unable to confirm that finding (Pethick et al., 2019; Neal et al., 2016; Schleh et al., 2018; Travers et al., 2020). In addition to the physiological changes associated with the first phase of the heat acclimation process, perceptually, the thermal sensation is reduced while the thermal comfort is improved (Costa et al., 2014; Garret et al., 2019; Willmott et al., 2017).

The reduction of skin and body core temperature at rest and during exercise occurs following five to eight days of the onset of the heat acclimation process (Armstrong and Maresh, 1991; Périard et al., 2015). Reduced skin temperature is known to result in behavioural alteration during exercise via reduced thermal perception, which could

impact pacing strategies and endurance performance (Schlader et al., 2011). Heat acclimation can reduce skin temperature in the range from 0.2 to 0.8°C (Garret et al., 2019; James et al., 2017; Zurawlew et al., 2018a). The reported reduction of body temperature in response to heat acclimation is between 0.1 – 1.0°C, depending upon the protocol and length of the heat acclimation (Garrett et al., 2009; Garrett et al., 2014; Gibson et al., 2015; Gill and Sleivert, 2001; James et al., 2017; Lorenzo et al., 2010; Weller et al., 2007). This adaptation allows exercising athletes to experience a greater magnitude of body temperature increase before the threshold associated with the performance decrement is surpassed (Nielsen et al., 1993). However, the exact mechanisms behind the heat acclimation-induced decrease in body temperature remain to be elucidated (Taylor, 2014).

An enhanced sweating response is observed after nine days of exercising in the heat (Armstrong and Maresh, 1991; Périard et al., 2015). This adaptation is characterised by a reduced body temperature threshold for the onset of sweating, increased skin blood flow and increased sweat rate. All these changes allow an athlete to thermoregulate more effectively during exercise in the heat. However, increased capacity for evaporative heat loss also results in greater body water loss and thus increased risk of severe dehydration and associated ill effects such as impaired endurance performance (Sawka et al. 2007) and increased risk of heat illness (Kjertakov and Epstein, 2013). A well-trained, heat-acclimated endurance athlete may experience sweat losses of more than 3 $L \cdot h^{-1}$ during intensive exercise in the heat (Armstrong et al., 1986). In addition to increased sweating with the heat acclimation, sweat becomes more dilute, resulting in less loss of electrolytes (Allan and Wilson, 1971; Chinevere et al., 2008; Kirby and Convertino, 1986) and improved maintenance of fluid-electrolyte homeostasis when an athlete is thermally challenged. In fact, preventing deviation of plasma sodium outside the range of normonatremia (135-145 $mmol \cdot L^{-1}$) during exercise is crucial for both exercise performance and health (Hew-Butler et al., 2015). Another adaptation reported to occur after nine days of heat acclimation is reduced blood and muscle lactate concentrations during submaximal exercise (Young et al., 1985). Complete cardiovascular and thermoregulatory adaptations to heat are usually achieved within ten to fourteen days of heat exposure (Armstrong and Maresh, 1991; Périard et al., 2015; Racinais et al., 2019b), after which the capacity to perform endurance exercises in hot conditions increases (Burk et al.,

2012; Keiser et al., 2015; Lorenzo et al., 2010; Nielsen et al., 1993; Racinais et al., 2015b; Travers et al., 2020; Willmott et al., 2018). Some studies also support the use of moderate-term (8-14 days) heat acclimation protocols to improve endurance performance in temperate conditions (Lorenzo et al., 2010; Maunder et al., 2021; Rendell et al., 2017; Scoon et al., 2007). The former topic is covered in the next section, whereas the latter one is covered in section 2.2.5.

While the cellular mechanisms that drive heat adaptations are not fully elucidated, a study by Kuennen et al., (2011) suggests that heat shock protein 70 (HSP70) plays a vital role in the process of heat acclimation. In that study, eight physically active ($\dot{V}O_{2max}$ = 55.6 ± 2.3 ml·kg^{-1}·min^{-1}) non-heat acclimated men completed two seven-day heat acclimation interventions, with a washout period of 95 ± 9 days. One heat acclimation intervention was under the influence of quercetin (HSP70 response inhibitor) supplementation (2000 mg/day), whereas the other one was under the influence of a placebo supplement (control condition). Before and after the heat acclimation interventions, which consisted of daily exercising on a treadmill for 140 minutes in a heated (46°C, 20% RH) environment chamber, participants completed a 45-minute continuous test in the heat (46°C, 20% RH) to assess heat adaptations. The latter included measuring rectal and skin temperatures, heart rate, and sweat rate. In addition, before and after the first and the seventh heat acclimation session, the researchers collected venous blood samples, from which peripheral blood mononuclear cells were isolated and analysed for HSP70 protein content. No increase in HSP70 was observed after the first heat acclimation session in either condition. HSP70 content was significantly increased after the seventh heat acclimation session in the control condition, but it was unchanged in the quercetin condition. Rectal and skin temperatures and heart rate were significantly reduced during the continuous test performed following seven days of heat acclimation under placebo supplementation. The sweat rate was significantly increased, and plasma volume increased by 16 ± 4%. Interestingly, neither rectal nor skin temperature was reduced during the continuous test following heat acclimation under quercetin supplementation. Although a significant decrease in heart rate was observed during the test, the decrease was not evident until twenty minutes of exercise had elapsed. In contrast, the decrease in heart rate during the post-heat acclimation testing in the control condition was detectable after only five minutes from the start of the test. The magnitudes of increase in sweat rate

and plasma volume in the quercetin condition were similar to that in the control condition. Impaired adaptational responses and blunted HSP70 response to heat acclimation under quercetin supplementation, which was not the case under placebo supplementation, imply that HSP70 plays a vital role in the process of heat acclimation.

2.2.2. Effects of medium-term heat acclimation on endurance cycling performance in the heat

While hundreds of studies have been devoted to the effects of moderate-term (8-14 days) heat acclimation in various populations, for the purpose of this thesis, studies focusing on cyclists (Choo et al., 2020; Lorenzo et al., 2010; Keiser at al., 2015; Racinais et al., 2015b; Schmit et al., 2017; Travers et al., 2020) will be presented in this section.

Chronologically, the study by Lorenzo et al., (2010) is the first that assessed the effects of moderate-term heat acclimation on endurance performance in cyclists. Twelve participants ($\dot{V}O_{2max}$ = 66.9 ± 2.1 ml·kg^{-1}·min^{-1}) completed a cycling GXT test and one-hour time trial test in an environmental chamber at 38°C and 30% RH before and after ten days of heat acclimation which consisted of daily cycling for 90 minutes at 40°C and 30% RH. Eight matched ($\dot{V}O_{2max}$ = 66.8 ± 1.7 ml·kg^{-1}·min^{-1}) participants served as a control group that underwent the same testing procedures but trained at 13°C. The main measured variables were lactate threshold and $\dot{V}O_{2max}$ (both determined during the GXT), power output and exercise performance during the time trial, heart rate and rectal temperature at the end of the heat acclimation sessions, and plasma volume at rest before and after the heat acclimation. The time trial performance was significantly improved following heat acclimation by 8% (718.7 ± 42.3 versus 776.2 ± 50.9 kJ. The power output at lactate threshold and $\dot{V}O_{2max}$ were significantly increased following heat acclimation by 5% (3.45 ± 0.80 watts/kg versus 3.60 ± 0.79) and 8% (55.1 ± 2.5 versus 59.6 ± 2.0 ml·kg^{-1}·min^{-1}). The heart rate and rectal temperature at the end of the tenth heat acclimation session (150 ± 3 beats·min^{-1}, 38.8 ± 0.1°C, respectively) were significantly lower than that of the first session (165 ± 2 beats·min^{-1}, 39.3 ± 0.1°C, respectively). Post-heat acclimation, plasma volume increased by 6.5 ± 1.2. In contrast, the control group experienced no changes in any of the above variables.

Subsequently, two heat acclimation papers were released simultaneously (Keiser et al., 2015; Racinais et al., 2015b). Keiser et al., (2015) applied a cross-over design,

where seven well-trained ($\dot{V}O_{2max}$ = 61.2 ± 4.4 ml·kg^{-1}·min^{-1}) male cyclists completed two training blocks of ten days in an environmental chamber at either 18°C, 30% RH or 38°C, 30% RH, separated by three months. The duration of the training sessions was 90 minutes, and they were performed at 50% of the maximal heart rate, determined during a cycling GXT. Before and after both interventions, the cyclists completed a GXT and a 30-minute time trial test at 38°C, 30% RH. Additionally, measured variables included plasma volume, sweat rate, and sweat sodium concentration. Heat acclimation increased the average power output during the time trial by 10.4 ± 3.1% (208 ± 12 versus 228 ± 11 watts, ES = 0.63). Heat acclimation also increased peak power output and $\dot{V}O_{2max}$ by 7.9 ± 1.7% (311 ± 13 versus 335 ± 12 watts, ES = 0.74) and by 9.6 ± 2.1% (4.3 ± 0.2 versus 4.8 ± 9.2 ml·kg^{-1}·min^{-1}, ES > 0.80), respectively. Furthermore, heat acclimation increased plasma volume by 6 ± 2% (ES = 0.53). A 26 ± 6% (ES > 0.80) increase in sweat rate was noted between the first (1.44 ± 0.10 L·h^{-1}) and the tenth (1.74 ± 0.11 L·h^{-1}) heat acclimation session. Sweat sodium concentration decreased by 19 ± 7% (ES > 0.80) from the first (167 ± 22 mmol·L^{-1}) to the tenth (127 ± 10 mmol·L^{-1}) heat acclimation session. None of the variables were altered in the control group.

In the study by Racinais et al., (2015b), nine well-trained ($\dot{V}O_{2max}$ = 4.8 ± 0.2 L·min^{-1}) non-acclimated male cyclists travelled from Denmark to Qatar to complete a two-week heat acclimation training camp. The average air temperature and relative humidity during the training camp were 34 ± 3°C and 18 ± 5%, respectively, and the cyclists trained on an average of 13 ± 1 hour per week. On days one, six, and fourteen during the training camp, the cyclists performed a 43 km cycling time trial test. This same time trial test was performed in Denmark in a cold environment (8°C) before arriving in Qatar. All the training and testing were conducted outdoors, and the time trials were completed at similar terrain and wind speeds. During the time trials, the researchers recorded performance time and heart rate, and they also measured the cyclists' rectal temperatures at the end of the trials. The time to complete the time trial in Denmark (66.13 ± 3.26 minutes) and the last time trial in Qatar (65.37 ± 3.44 minutes) was similar but it was significantly shorter than the first time trial in Qatar (77.17 ± 6.26 minutes). Compared with the first time trial, the increase in heart rate was 7 beats·min^{-1} and 5 beats·min^{-1} higher during the second and the last time trial, respectively, though the differences were not statistically significant. On the other hand, the

difference in the end-time trial rectal temperature between the first (38.5 ± 0.6°C) and the other two time trials (40.2 ± 0.4°C and 40.1 ± 0.4°C) reached statistical significance. Results from the comparison between the data set referring to the time trials completed on the first and the sixth day in Qatar are presented in the next section of the thesis, as those results fall under the short-term heat acclimation literature.

Although the study by Schmit et al., (2017) was conducted on triathletes, their findings are relevant to cyclists because the researchers used cycling as a mode of exercise to assess the physiological and performance effects of the heat acclimation intervention. Thirteen well-trained non-acclimated male triathletes ($\dot{V}O_{2max}$ = 64.9 ± 6.9 ml·kg^{-1}·min^{-1}) completed a 30-minute continuous cycling test at 50% of peak power output (i.e., heat stress test) and a 20 km time trial test, both in the heat, on two consecutive days, before and after an eight-day heat acclimation. The measured variables during the heat stress test included heart rate, rectal temperature, sweat rate, sweat sodium, and thermal comfort. Similar to the previous study (Racinais et al., 2015b), the triathletes heat acclimated in a naturally hot environment (30 ± 5°C, 74 ± 15% RH) by performing 842 ± 216 minutes of training. However, the testing took place in an environmental chamber at 35°C and 50% RH, and data were analysed using a magnitude-based inference approach. Accordingly, the researchers described the improvement in the post-acclimation time trial performance of 3.1 ± 1.7% as *almost certain*. Comparing the change in average power output (11.7 ± 4.1%) between the pre-and post-heat acclimation time trial, the researchers concluded that heat training had an *almost certain very large to extremely large* improvement in power output. The change in plasma volume of 11.8 ± 6.9% was described as *almost certain*. The decrease in rectal temperature by 0.2 ± 0.3°C and an increase in sweat rate by 0.26 ± 0.25 L·h^{-1} during the post- compared to pre-acclimation heat stress test were described as *almost certain*. The observed decrease in the sweat sodium concentration of 221 ± 200 mmol·L^{-1} during the post-acclimation heat stress test was described as *likely*. The change in the last measured variable (thermal comfort) during the heat stress test was described as *unclear*.

Recently, two more papers were published a few months apart (Choo et al., 2020; Travers et al., 2020). The researchers in the former study recruited twenty recreational male cyclists/triathletes to study the effects of pre-cooling on the physiological and

performance adaptations to heat acclimation. The participants completed ten consecutive days (60 minutes per day) of exercising in an environmental chamber with air temperature and RH set at 35°C, 50%, respectively, preceded by either 30 minutes of mid-sternal water immersion at 22°C (*n* =10) or no cooling (control, *n* =10). Before and after the interventions, the participants completed a 25-minute heat stress test (to assess heat adaptations), followed immediately by a 20-km time trial test at 35°C, 50% RH. Post-heat acclimation, a significant decrease in rectal temperature was observed at rest (–0.3°C) and during (–0.2°C) the heat stress in the pre-cooling group, but not in the control group. Neither group showed a change in resting heart rate following heat acclimation, whereas heart rate during the post-acclimation heat stress test was reduced by 5 beats·min^{-1} and 6 beats·min^{-1} in the control and pre-cooling groups, respectively. The sweat rate was unchanged in either group, but heat acclimation reduced rectal temperature at the onset of sweating in the pre-cooling group. Heat acclimation did not alter either the ratings of perceived exertion or thermal comfort during the post-intervention heat stress test. Post-acclimation, the time to complete the time trial was reduced in the pre-cooling group (ES = 0.93), but not in the control group (ES = 0.52). However, there was no significant difference in the absolute change in the timer trial performance between the groups.

Travers et al., (2020) recruited eight trained ($\dot{V}O_{2max}$ = 55 ± 7 ml·kg^{-1}·min^{-1}) male cyclists/triathletes to assess the effects of hydration status on physiological responses to heat acclimation. In a cross-over design, the participants completed two ten-day heat acclimation interventions, one in a euhydrated and one in a hypohydrated state, with a washout period of 10 ± 3 weeks. Heat acclimation was induced in an environmental chamber (40°C, 40% RH) where the participants rode for 90 minutes per day at a heart rate corresponding to 65% of $\dot{V}O_{2max}$. Hydration status was manipulated by having the participants drink different amounts of fluid during the heat acclimation sessions to either remain well-hydrated (euhydrated group) or induce a body water deficit equivalent to 2.85% (hypohydrated group) by the end of the sessions. The effects of heat acclimation on endurance exercise performance were assessed by a 30-minute time trial test. Neither intervention altered resting or exercising rectal temperature or heart rate when responses of these variables were compared between the first and the last heat acclimation session. In the euhydrated group, skin temperature decreased by 0.63 ± 0.50°C from the first to the last training

session, and there was a tendency for sweat rate to increase from the first (1.55 ± 0.17 $L \cdot h^{-1}$) to the last (1.74 ± 0.21 $L \cdot h^{-1}$) training session. In contrast, heat acclimation had no impact on either skin temperature or sweat rate in the hypohydrated group. Sweat loss produced during the last training session was significantly greater in the euhydrated than in the hypohydrated group. No heat acclimation intervention had a significant impact on either the ratings of perceived exertion or thermal comfort. Finally, average power output during the post-heat acclimation time trial was significantly increased (by 9%) in the euhydrated but not in the hypohydrated group.

Based on the evidence presented in this section, a medium-term heat acclimation intervention consisting of daily training in the heat for at least 90 minutes in a euhydrated state should induce significant heat adaptations and improve the endurance performance of male cyclists in the heat in the range of up to 15%. However, implementing ten to fourteen heat acclimation sessions in an already established training schedule can be challenging. For those reasons, short-term heat acclimation protocols would be more appealing for competitive cyclists because they would cause less disruption to the training process and are less costly.

2.2.3. Effects of short-term heat acclimation on endurance cycling performance in the heat

The findings that many of the important adaptations to heat emerge in the first 4-7 days of heat exposure prompted researchers to develop short-term (≤ 7 days) heat acclimation protocols.

Garret et al., (2009) were perhaps the first researchers to report the beneficial effects of short-term heat acclimation on exercise performance in the heat. In that study, ten non-acclimated moderately trained ($\dot{V}O_{2max}$ = 4.26 ± 0.37 $L \cdot min^{-1}$) males underwent a five-day heat acclimation programme during which they exercised daily for 90 minutes in an environmental chamber (40°C, 60% RH) at a fixed body core temperature of 38.5°C. Prior to and after the heat acclimation period, a heat stress test was conducted by having the participants cycle for 90 minutes at 40% of peak power output at 35°C. In addition, the participants were subjected to an incremental test to exhaustion 10 minutes after completing the heat stress test. The outcome variables during the heat stress test were resting and exercising heart rate and rectal temperature, and resting plasma volume. On days one and five of the heat acclimation intervention, blood

samples were taken before the sessions and sweat sodium concentration was measured during the sessions. Heat acclimation did not lower resting rectal temperature or heart rate. However, rectal temperature and heart rate at the end of the second heat stress test were reduced by 0.3°C and 13 beats·min^{-1}, respectively, compared with the values of these variables obtained at the end of the first test. No significant change in sweat sodium concentration or resting plasma volume was seen with heat acclimation. Heat acclimation increased exercise performance by 14%.

The finding that a five-day heat acclimation improves performance during an incremental test to exhaustion in the heat in previously unacclimated individuals was subsequently confirmed by Chen et al., (2013). These researchers allocated fourteen male badminton players ($\dot{V}O_{2max}$ = 53.0 ± 5.3 ml·kg^{-1}·min^{-1}) into two different groups that trained for five days in ether hot (38.4 ± 0.4°C, 52.0 ± 4.6% RH, *n* =10) or thermoneutral (24.1 ± 0.3°C, 51.5 ± 4.5% RH, *n* =10) environment. The training sessions were conducted on a cycling ergometer, and their duration (25-45 minutes) and intensity (10% below to 10% above the ventilatory threshold) increased progressively throughout the intervention. A cycling GXT was completed before and after the training interventions. During the post-intervention testing, the heat acclimation group showed a 6.6% increase in performance time, while the performance time was unchanged in the control group. Furthermore, the heat acclimation group showed reduced heart rate (10% at 120 watts, 7.7% at 180 watts, and 5.1% at 240 watts) during the second GXT.

In their second study, Garret et al., (2012) demonstrated the efficacy of their heat acclimation method to improve 2-km rowing time trial performance at 35°C in eight highly-trained ($\dot{V}O_{2max}$ = 4.9 ± 0.2 L·min^{-1}), unacclimated male rowers. Similar to their first study, the exercise performance test was preceded by a heat stress test consisting of 20 minutes of rowing. The outcome of the measured physiological variables during the heat stress test in response to heat acclimation mirrored that of the first study. Indeed, heat acclimation had no impact on the resting rectal temperature or heart rate, but both rectal temperature and heart rate were significantly reduced at the end of the second heat stress test (–0.3°C and –14 beats·min^{-1}, respectively). Sweat sodium concentration was unchanged, but in contrast to the first study, the percentage change in resting plasma volume increased by 4.5 ± 1.6% from day one to day five of the heat

acclimation intervention. The 2-km rowing time trial was improved by 1.5% following heat acclimation, which led the authors to conclude that their heat acclimation method can induce gains in exercise performance in highly-trained athletes. However, given that the performance test used in the study has a coefficient of variation higher than 1.5% (Schabort et al., 1999), one might question the authors' conclusion.

The findings of Garret et al., (2009; 2012) motivated several other groups to study the effects of short-term heat acclimation on endurance cycling performance in the heat (Guy et al., 2016; Osborne et al., 2021; Pethick et al., 2019; Wingfield et al., 2016).

Wingfield et al., (2016) studied the effects of exercise intensity and duration during a five-day heat acclimation on 20-km cycling time trial performance in twenty unacclimated recreationally trained ($\dot{V}O_{2peak}$ = 41.9 ± 3.7 $ml \cdot kg^{-1} \cdot min^{-1}$) males. Participants were divided into a group (*n* =10) that exercised for 30 minutes per day at an intensity that varied between 40% and 70% of peak power output every three minutes and a group (*n* =10) that exercised for 90 minutes per day at 40% of peak power output. The training and testing were conducted in a temperature-controlled room maintained at 32.0 ± 1.6°C. The group that trained for 90 minutes per day completed the post-acclimation time trial significantly faster (–5.9 ± 7.0%) than the baseline time trial, which was not the case with the other group. The improvement in the time trial performance in the former group is lower than that (11%) reported in the study by Racinais et al., (2015b), cited in the previous section, during the 43-km time trial following five days of natural heat acclimation. However, direct comparison between the studies is difficult due to the inconsistency in their methodologies (i.e., duration of the time trials, physical fitness of the cyclists, and duration of the heat acclimation sessions). It should be mentioned, though, that the last time trial in Racinais's study was completed in an even shorter time than the one after 5 days of training in the heat, suggesting that moderate-term heat acclimation is superior in improving endurance cycling performance compared with short-term heat acclimation. Their findings could be explained by the evidence that moderate-term heat acclimation induces more complete heat adaptations compared with short-term heat acclimation (Moss et al., 2020; Willmott et al., 2018).

Guy et al., (2016) showed that short-term heat acclimation could also be beneficial for cycling distances considerably shorter than in the previous two studies (Racinais et

al., 2015b; Wingfield et al., 2016). In that study, twenty-four unacclimated moderately trained ($\dot{V}O_{2max}$ = 45.0 ± 5.0 ml·kg^{-1}·min^{-1}) males were allocated into three different groups that either exercised in 35°C, 70% RH (*n* =8), exercised in 20°C, 45% RH (*n* = 8) or did not exercise at all (control group, *n* =8). The former two groups completed seven 40-minute daily cycling sessions at 55% $\dot{V}O_{2max}$. At baseline, after the fourth, and after the seventh training session, the participants completed a heat stress test in an environmental chamber (35°C, 70% RH) consisting of three ten-minute workloads at 50, 60, and 70% of peak power output followed by a 5-km time trial. The control group completed the heat stress testing at the same time as the other two groups. Although both training groups demonstrated similar improvement (~6%) in the second time trial compared to the baseline time trial, only the heat acclimation group completed the third time trial significantly faster (3%) than the second one. Furthermore, a significant reduction in rectal temperature was reported for the heat acclimation group from the first to the second heat stress test during the 60% workload (–0.22 ± 0.14°C) and from the first to the third heat stress test during the 50% (–0.18 ± 0.10°C), 60% (–0.23 ± 0.18°C), and 70% (–0.34 ± 0.27°C) workload. No reduction in rectal temperature was observed in the other two groups. The heat acclimation group also showed improved thermal tolerance from the first to the third heat stress test (3.0 ± 0.5 AU versus 2.0 ± 1.0 AU). Finally, no within or between groups differences in heart rate were detected during the heat stress tests.

In contrast to the positive findings of Guy et al., (2016), Racinais et al., (2015b), and Wingfield et al., (2016), Pethick et al., (2019) and Osborne et al., (2021) failed to find improvement in cycling time trial performance in the heat following short-term heat acclimation. The aim of the study by Pethick et al., (2019) was to investigate the impact of hydration status during a five-day heat acclimation intervention on plasma volume and a 20-km time trail performance. For the purpose of the study, thirty male and three female sub-elite endurance-trained (minimum $\dot{V}O_{2max}$ of 50 ml·kg^{-1}·min^{-1}) participants were allocated into three different groups that either completed the heat acclimation sessions euhydrated (*n* = 10), completed the heat acclimation sessions hypohydrated (*n* = 14) or completed five training sessions at 22°C euhydrated (control condition, *n* = 8). Heat acclimation was induced in an environmental chamber at 35°C following the protocol by Garret et al., (2009), whereas the hydration status was manipulated as previously described in the study by Travers et al., (2020). Resting plasma volume

and rectal temperature, and a 20-km time trail performance at 35°C, 37% were assessed before and after the interventions. Post-intervention, both heat acclimation groups experienced a reduction in the resting rectal temperature of 0.3°C, which was significantly different from that (0.1°C) reported in the control group. However, there was no significant difference pre- to post-intervention between the groups in the change in plasma volume, though there was a significant pooled group increase in plasma volume post-intervention (3.6 ± 8.8%). Similarly, no significant pre- to post-intervention difference between the groups in the time trial performance was detected, but pooled group data indicated a 97% likely improvement (1.9 ± 2.5%) in time trial performance.

In the study by Osborne et al., (2021), with a cross-over design, eight unacclimated recreationally trained ($\dot{V}O_{2peak}$ = 49.3 ± 4.9 ml·kg^{-1}·min^{-1}) males completed a five-day training block in a hot (34.9 ± 0.7°C, 53 ± 4% RH) and a five-day training block in a temperate (22.2 ± 2.6°C, 65 ± 8% RH, control condition) environment, with a washout period of minimum two weeks. All training sessions were performed on a cycling ergometer, with the participants cycling daily for 60 minutes at 50% of their peak power output. Before and after each training block, a 20-km time trial test was completed. Although heat acclimation led to 68 seconds faster completion of the second time trial compared to the control training, the difference did not reach statistical significance. It is possible that the researchers failed to detect significant improvement in the time trial performance due to the small sample size. Alternatively, the thermal strain experienced by the participants during the heat acclimation intervention was not sufficient to induce the necessary performance-related heat adaptations. This assumption is based on the available training data showing that the participants were exposed to a degree of hyperthermia equivalent to or higher than 38.5°C for only 20 minutes during the heat acclimation sessions. Recall from section 2.2 (page 9) that maintenance of core temperature at ~38.5°C throughout heat acclimation sessions is considered crucial for effective heat acclimation (Pryor et al., 2019). On the other hand, given that Pethick et al., (2019) observed improved thermoregulation (as evidenced by reduced resting rectal temperature of 0.3°C) post-heat acclimation, it is difficult to explain the lack of improvement in time trail performance with heat acclimation in their study. Although the lack of increase in plasma volume could be pointed out as a reason for not observing improved time trial performance in that study (Pethick et al., 2019),

one must not forget that Garret et al., (2009) reported improved exercise performance in the heat following a five-day heat acclimation intervention despite no increase in plasma volume.

It is important to remember that the ergogenic properties of short-term heat acclimation described in the preceding paragraphs apply to males only. Currently, no data exist on the effects of short-term heat acclimation on endurance performance in women, and the data available on the efficacy of short-term training in the heat to induce heat adaptations in women is inconsistent. Mee et al., (2015) assessed the potential sex differences in the physiological responses to ten days of heat acclimation consisting of 90 minutes of cycling exercise per day in the heat. At baseline, halfway through the intervention (day five) and post-intervention, eight male ($\dot{V}O_{2peak}$ = 3.63 ± 0.69 L·min^{-1}) and eight female ($\dot{V}O_{2peak}$ = 2.69 ± 0.30 L·min^{-1}) physically active volunteers performed a 30-minute continuous running test at 40°C to assess heat adaptations. The researchers found that five heat acclimation sessions reduced exercising rectal temperature and heart rate by 0.39°C and 15 beats·min^{-1}, respectively, in males, but only increased sweat rate in females. Furthermore, it was also revealed that females required ten days to achieve the same magnitude of physiological adaptations as those achieved by males in five days, including reduced body core temperature and heart rate. In their subsequent, cross-over study, Mee et al., (2018) recruited nine non-heat acclimated female athletes ($\dot{V}O_{2max}$ = 50.4 ml·kg^{-1}·min^{-1}) to investigate the effects of wearing a sauna suit for 20 minutes before heat acclimation sessions on physiological adaptations to a five-day heat acclimation intervention. A control condition consisted of 20 minutes of sitting in the thermoneutral environment before each heat acclimation session, which consisted of exercising for 90 minutes at 40°C. While exercising in the heat alone was not able to elicit heat adaptations, adding 20 minutes of passive heating before the heat acclimation sessions resulted in adaptations similar to those reported in males after a five-day heat acclimation (Mee et al., 2015). The findings from these two studies (Mee et al., 2015, 2018) suggest that, compared to males, females require either a greater number of heat exposures or longer daily heat exposure to elicit favourable heat adaptations. In contrast to the findings of the former two studies (Mee et al., 2015; 2018), Garret et al., (2019) reported significantly improved thermoregulatory and cardiovascular responses to a heat stress test following five days of exercise in the heat (90 minutes per day) in ten

moderately trained ($\dot{V}O_{2peak}$ = 43.9 ± 8.6 ml·kg^{-1}·min^{-1}) females who were not accustomed to exercise in the heat. Unfortunately, as neither of these three studies attempted to assess endurance exercise performance in the heat, it is unclear if females can achieve meaningful endurance performance improvements following short-term heat acclimation.

It also needs to be noted that the rate of decay of short-term heat acclimation is faster than that of medium-term heat acclimation. Garret et al., (2009) studied the rate of heat acclimation decay as an additional aim in their first study. For that aim, their participants repeated the heat stress test seven and fourteen days after the post-heat acclimation testing. It was reported that thermoregulatory and cardiovascular adaptations gained from heat acclimation persisted for one week but were completely lost after two weeks of cessation of exercising in the heat. From an applied perspective, these findings indicate that adaptation to heat using active short-term protocol should be completed no later than one week before competing in the heat.

The evidence presented in this section indicates that exercising in the heat for ninety minutes per day over five consecutive days should induce significant heat adaptations. However, the available evidence does not allow a conclusion to be made about the daily 'dose' of exercising in the heat needed to improve cycling performance in the heat following a short-term heat acclimation intervention. Future studies need to address that question since for cyclists residing in a cooler climate and preparing to race in the heat, the use of short-term heat acclimation would be more appropriate during the tapering phase compared with traditional, longer heat acclimation protocols. Nevertheless, the findings from such studies would apply to cyclists who can either train in artificial hot conditions (e.g., environmental chamber) or afford to travel to the race site at least one week in advance and complete the heat acclimation process there. All other cyclists will have to rely on passive heat acclimation strategies such as hot water immersion or sauna bathing (Heathcote et al., 2018).

2.2.4. Hot water immersion as a means for heat acclimation

The idea that submersion in hot water can induce heat acclimation was introduced to scientific literature back in the early 1960s. Brebner et al., (1961) studied the physiological responses of four healthy men to ten days of immersion in a hot bath and reported reduced oral temperature and heart rate during work in the heat following

the immersions. During the next decade, two more studies used hot water immersion to induce heat acclimation (Allan and Wilson, 1971; Bonner et al., 1976). The former study investigated the effects of heat acclimation on sweat sodium concentration in three unacclimated volunteers by having them take a daily hot (40°C) bath for one hour over a period of fifteen days (Allan and Wilson, 1971). During each immersion, the participants remained submerged up to the neck until their aural temperature reached between 37.6°C and 38.6°C, after which the degree of immersion of the body was altered to maintain the aural temperature between 38.0°C and 38.5°C. After completing all immersion sessions, sweat production was increased by 70% and sodium concentration in the sweat was significantly reduced compared to baseline measurements. In the study by Bonner et al., (1976), five healthy unacclimated males completed thirteen one-hour daily hot water immersion sessions during which their aural temperature was maintained at 38.5 ± 0.2°C by sitting alternately in and on the edge of a bath filled with 41°C water. Before and after the thirteen-day intervention period, a heat stress test was performed in an environmental chamber at 33°C, 36% RH consisting of sitting for 155 minutes, followed by light exercise on the bike for 30 minutes. The sweat rate measured during the heat stress testing significantly increased by 72% from before (1.24 ± 0.13 $L \cdot h^{-1}$) to after (2.09 ± 0.15 $L \cdot h^{-1}$) the hot water immersion intervention. Tympanic temperature was consistently lower during the second heat stress test compared to the first one. Post-hot bath intervention, the heart rate was significantly lower during the last 30 minutes of the heat stress test. Hot bathing also led to an expansion of plasma volume of 6.7%.

While several decades will pass after these initial reports (Allan and Wilson, 1971; Bonner et al., 1976; Brebner et al., 1961) before scientists begin promoting hot-water immersion as an alternative to active heat acclimation (Gibson et al., 2020; Kissling et al., 2020; Maloy and Hulsopple, 2021; Pryor et al., 2019; Racinais et al., 2019b), the studies of Bonner et al., (1976) and Brebner et al., (1961) served as a base for Zurawlew and his co-workers (Zurawlew et al., 2016) to develop, what will later become, the most commonly recommended hot water immersion protocol for heat acclimation.

Zurawlew et al., (2016) recruited seventeen physically active, non-heat acclimated males to test the hypothesis that repeated post-exercise hot water immersion induces

heat acclimation and improves exercise performance in temperate and hot conditions. The participants completed a six-day intervention consisting of daily running for 40 minutes at 65% of $\dot{V}O_{2max}$ in temperate conditions (18°C, 40% RH) followed immediately by either hot (40°C; *n* = 10) or thermoneutral (34°C; *n* = 7) water immersion up to the neck. The length of time the participants were able to remain submerged in hot water increased from day to day and ranged from 30 minutes during the first session to 40 minutes during the last session. Two experimental trials under different environmental conditions (18°C, 40% RH and 33°C, 40% RH) and on different days were completed before and after the interventions. The trials were conducted on a treadmill placed inside an environmental chamber and consisted of continuous running for 40 minutes at submaximal intensity followed by an hour of rest and then a 5-km running time trial test. Post-interventions, a significant (*d* = 0.75) reduction in resting rectal temperature (–0.23°C) was noted only in the hot water group. Similarly, only the hot water group demonstrated a significant reduction in rectal temperature at the end of the 40 minutes continuous test following the intervention in a temperate (–0.28°C, *d* = 0.78) and a hot (–0.36°C, *d* = 0.70) condition. The hot water treatment, unlike the control condition, led to reduced skin temperature at the end of the second continuous test at 18°C (*d* = 0.86) and at 33°C (*d* = 0.60) and to reduced rectal temperature at the onset of sweating in both 18°C (*d* = 0.86) and 33°C (*d* = 0.57). The hot water treatment also led to lower ratings of perceived exertion at the end of the second continuous test in both environments (18°C: *d* = 0.74; 33°C: *d* = 0.72) and lower end-exercise heart rate at 18°C (–7 beats·min^{-1}, *d* = 0.52) and at 33°C (–6 beats·min^{-1}, *d* = 0.40), which was not the case with the control group. Finally, compared with the thermoneutral-water condition, the hot-water group demonstrated improved 5-km run time trial performance at 33°C of 4.9% (*d* = 0.42), though the running performance was unchanged at 18°C.

After the first study (Zurawlew et al., 2016), Zurawlew and co-workers carried out a series of studies that investigated several other aspects of their heat acclimation protocol (Zurawlew et al., 2018a; 2018b; 2019).

The first of those studies investigated whether the heat adaptations induced by post-exercise hot water immersion manifested only during the time of the day when the immersions took place (Zurawlew et al., 2018a). For that purpose, ten recreationally

active ($\dot{V}O_{2max}$ = 58.2 ± .8.4 ml·kg^{-1}·min^{-1}) males completed a 40-minute continuous running test at 65% of $\dot{V}O_{2max}$ in an environmental chamber at 33°C, 40% RH (heat stress test) in the morning and the afternoon before and after they were acclimated to heat in the morning using the originally described hot water immersion protocol (Zurawlew et al., 2016). In agreement with the findings of their initial study, the post-exercise hot water immersion protocol resulted in a significant reduction in thermoregulatory and cardiovascular strain during the second compared with the first heat stress test. Importantly, no significant differences were observed in the measured variable between morning and afternoon heat stress tests. It was concluded that heat adaptations by post-exercise hot water immersion are not limited to the clock-time of daily heat exposures.

Their next study compared the effects of post-exercise hot water immersion intervention on heat adaptations in endurance-trained versus recreationally active individuals (Zurawlew et al., 2018b). Eight endurance-trained ($\dot{V}O_{2max}$ = 68 ± 6 ml·kg^{-1}·min^{-1}) males and eight recreationally active ($\dot{V}O_{2max}$ = 54 ± 6 ml·kg^{-1}·min^{-1}) males completed the same heat stress test as in the previous study (Zurawlew et al., 2018a) before and after completing the six-day post-exercise hot water immersion protocol (Zurawlew et al., 2016). Post-interventions, both groups showed a similar reduction in resting rectal temperature (endurance trained: –0.36°C, recreationally active: –0.47°C), reduction in end-heat stress test rectal temperature (endurance trained: –0.17°C, recreationally active: –0.23°C), reduction in rectal temperature at the onset of sweating (endurance trained: –0.22°C, recreationally active: –0.23°C), and reduction in end-heat stress test skin temperature (endurance trained: –0.67°C, recreationally active: –0.75°C). No significant between-group differences were observed in either the ratings of perceived exertion or thermal sensation. However, the reduction in end-heat stress test heart rate was significant in recreationally active individuals (–15 ± 7 beats·min^{-1}) but not in endurance-trained participants (–4 ± 5 beats·min^{-1}). Based on these findings, the researchers concluded that post-exercise hot water immersion is an effective strategy to reduce thermal strain during exercise in the heat in both active and endurance-trained individuals.

The 2019 study investigated the decay of the thermoregulatory and cardiovascular adaptations associated with the post-exercise hot water immersion protocol. Initially,

thirteen recreationally active males were heat acclimated in the same way as in the previous studies. Before and after the heat acclimation intervention, they completed a heat stress test to confirm that heat adaptations were induced. The same heat stress test was repeated one and two weeks after the second test. Post-exercise hot water immersion significantly improved thermoregulatory and cardiovascular functioning during the second heat stress test compared with the baseline test, and the improvements were observable during the third and fourth heat stress test. These findings indicate that heat adaptations gained from short-term heat acclimation induced by hot water immersion last longer than those gained from active short-term heat acclimation (Garret et al., 2009).

More recently, several other studies confirmed the efficacy of hot water immersion to induce heat adaptations in a short period of time when implemented either right after exercise in thermoneutral conditions (Ashworth et al., 2023; McIntyre et al., 2021, 2022; Waldock et al., 2021) or without prior exercise (Greenfield et al., 2021; Kissling et al., 2022).

In a study with a cross-over design, Ashworth et al., (2023) recruited twenty-five recreationally active ($\dot{V}O_{2peak}$ = 51.5 ± 6.4 ml·kg^{-1}·min^{-1}), unacclimated males to compare heat adaptations induced by post-exercise hot water immersion versus post-exercise sauna bathing. Each exercise session (40 minutes) was conducted in a temperate environment (19.2 ± 0.5°C, 63 ± 2% RH) immediately before another 40 minutes of either hot (~40°C) water immersion or sauna (~70°C, 18.5% RH) bathing. Before and after each intervention, which lasted five days, the participants completed a heat (33.4 ± 0.2°C, 77 ± 1% RH) stress test consisting of one-hour of treadmill walking with military gear (20 kg) followed by a GXT. Both interventions resulted in a significant and identical reduction in rectal (0.2°C) and skin (0.2°C) temperature at the end of the second heat stress test compared to the first test. Heart rate at the end of the second heat stress test was reduced by 9 beats·min^{-1} and 11 beats·min^{-1} following the hot water immersion and sauna bathing condition, respectively, with no between-condition differences. There was also a similar reduction in ratings of perceived exertion between the conditions in response to the second heat stress test (hot water immersion: –0.4; sauna bathing: –0.6). Running time was 14% longer during the GXT

after the interventions, though it did not reach statistical significance, nor was it significantly different between the conditions.

McIntyre et al., (2021) compared heat adaptations induced by six days of either post-exercise hot water immersion or exercise in the heat in recreationally active, unacclimated, volunteers. Nine volunteers ($\dot{V}O_{2peak}$ = 53 ± 6 ml·kg^{-1}·min^{-1}) completed the post-exercise hot water immersion intervention introduced by Zurawlew et al., (2016). Another nine volunteers ($\dot{V}O_{2peak}$ = 54 ± 3 ml·kg^{-1}·min^{-1}) were heat acclimated by daily treadmill running for 60 minutes at 65% of $\dot{V}O_{2max}$ in the heat (33°C, 40%). There was also a group ($\dot{V}O_{2peak}$ = 53 ± 4 ml·kg^{-1}·min^{-1}, *n* = 9) that completed six daily 60 minutes of running sessions at 65% of $\dot{V}O_{2max}$ in a thermoneutral environment (19°C, 45%). The previously described heat stress test (Zurawlew et al., 2016) was used to assess heat adaptations, whereas a run to exhaustion at 65% of $\dot{V}O_{2max}$ was used to assess exercise performance in the heat (33°C, 40%). Post-interventions, the hot water immersion group demonstrated a larger reduction in resting rectal temperature (–0.38 ± 0.23°C, *d* = 1.6) compared to exercise in the heat group (–0.14 ± 0.23°C, *d* = 0.6) and exercise in the thermoneutral environment group (–0.12 ± 0.23°C, *d* = 0.5). Similarly, the reduction in rectal temperature at the end of the heat stress test was larger in the hot water immersion group (–0.47 ± 0.23°C, *d* = 2.1) compared to exercise in the heat group (–0.26 ± 0.24°C, *d* = 1.1) and exercise in the thermoneutral environment group (–0.25 ± 0.23°C, *d* = 1.1). The reduction in rectal temperature at the onset of sweating was also larger in the hot water immersion group (–0.42°C) compared to exercise in the heat group (–0.18°C) and exercise in the thermoneutral environment group (–0.11°C). Sweat rate was significantly increased following both heat acclimation interventions (hot water immersion: +0.5 L·h^{-1}; exercise in the heat: +0.2 L·h^{-1}) compared to the group that exercised at 19°C (–0.1 L·h^{-1}), but no significant difference was observed between the former two groups. No intervention had an impact on skin temperature, heart rate, ratings of perceived exertion, thermal sensation, or plasma volume. Finally, there was a non-significant improvement in the run time to exhaustion by 52%, 16%, and 14% following training at 33°C, training at 19°C, and post-exercise hot water immersion, respectively. The finding that the percentage increase in the run time to exhaustion in the active heat acclimation group was almost four-fold higher than that in the post-exercise hot water immersion group is intriguing, given that the latter group experienced a significantly

greater reduction in thermoregulatory strain during the heat stress test than the former group. Interestingly, in their follow-up study, McIntyre et al., (2022) found that extending the six-day hot water immersion protocol to twelve days did not provide additional thermal benefits or improvements in exercise performance.

Waldock et al., (2021) also compared heat and exercise performance adaptations of post-exercise hot water immersion versus exercises in the heat but in recreationally active aged individuals. Each intervention lasted five days. Eight volunteers (seven males and one female: age: 68 ± 3 years) exercised daily on a cycling ergometer for ~120 minutes at 35°C, 50% RH and seven other volunteers (three males and four females; age: 73 ± 3 years) were submerged in 40°C water up to the sternum for 30 minutes immediately following 30 minutes of cycling at 23°C, 36% RH. There was also a group of young volunteers (eight males and three females; age: 22 ± 2 years) who completed the five-day active heat acclimation intervention. A half an hour of cycling heat stress test followed by a six-minute walking test in the heat (35°C, 50% RH) were performed pre- and post-interventions. Post-interventions, all three groups experienced a significant reduction in resting rectal temperature (elderly hot water immersion: –0.33 ± 0.35°C; elderly exercising in the heat: –0.28 ± 0.26°C; young exercising in the heat: –0.04 ± 0.32°C), with no between-group differences detected. Peak skin temperature during the post-intervention heat stress test was also significantly reduced in all three groups, but the magnitude of reduction was greater in the hot water immersion group (–0.28 ± 0.40°C) than in the group with the young participants (–0.17 ± 0.27°C). There was a significant reduction in resting heart rate following heat acclimation only in the young participants (–11 ± 8 beats·min^{-1}). All three groups demonstrated similar decreases in the ratings of perceived exertion, thermal sensation, and thermal comfort during the second heat stress test. Exercise capacity measured by the six-minute walking test in the heat improved by ~10% in both elderly groups and by 4% in the group with the young participants, but no significant difference existed between the groups.

In the study by Greenfield et al., (2021), eight recreationally active ($\dot{V}O_{2max}$ = 46.4 ± 4.0 ml·kg^{-1}·min^{-1}) non-heat acclimated males completed two three-day heat acclimation interventions, with one month washout period between them. One intervention consisted of a daily 40-minute immersion in 40°C water up to the neck,

and the other one of a daily 40-minute cycling at 50% of $\dot{V}O_{2max}$ in the heat (40°C, 40% RH). Heat adaptations were assessed by measuring heart rate, gastrointestinal temperature, ratings of perceived exertion, and thermal sensation before and in response to cycling for 45 minutes at 50% of $\dot{V}O_{2max}$ in an environmental chamber (40°C, 40% RH) pre- and post-interventions. In addition, resting plasma volume was measured before the test. Only the hot water immersion intervention resulted in cardiovascular and thermoregulatory changes. A significant reduction was reported in average heart rate (–7 ± 6 beats·min^{-1}, ES = 1.6), peak heart rate (–10 ± 8 beats·min^{-1}, ES = 1.6), and average core temperature (–0.4 ± 0.3°C, ES = 1.2) during the second heat stress test compared to the first test. Ratings of perceived exertion were significantly reduced between the first and the second heat stress test for the hot water immersion (–1.6 ± 1.4, ES = 1.1) and exercise in the heat (–1.6 ± 1.4, ES = 1.0) condition, with no significant differences between the conditions. On the other hand, thermal sensation during the second heat stress test was significantly reduced only in the hot water immersion condition (–1.3 ± 1.0, ES = 1.0). Resting plasma volume was increased to a similar magnitude after heat acclimation (hot water immersion: 5.9 ± 5.1%; exercise in the heat: 5.4 ± 3.7%).

In a study with a cross-over design conducted on thirteen (eight males and five females) unacclimated moderately trained individuals ($\dot{V}O_{2peak}$ = 54 ± 8 ml·kg^{-1}·min^{-1}), Kissling et al., (2022) compared heat adaptations induced by hot water immersion, sauna bathing, and exercise in the heat. A washout period of at least four weeks separated the treatments. The duration of each intervention and heat acclimation session was five days and one hour, respectively. During the hot water immersion sessions, participants were submerged in 40°C water to the neck until their rectal temperature increased by 1.5°C, after which they were moved to a shallower depth to maintain rectal temperature for the remainder of the sessions. In the sauna, participants were seated upright at 55°C, 54% RH. During the active heat acclimation sessions, participants cycled at 40°C, 52% RH to raise their rectal temperature by 1.5°C. Resting rectal temperature, heart rate, and haematocrit were measured before every heat acclimation session and on the day following the competition of each five-day heat acclimation block. Heat adaptations were assessed as the pooled response over days four to six. Accordingly, the researchers reported a modest reduction in resting rectal temperature (hot water immersion: –0.3°C; sauna bathing: –0.1°C;

exercise in the heat: –0.1°C), which was not significantly different between the conditions. Similarly, there was a modest expansion in plasma volume (hot water immersion: 5%; sauna bathing: 3%; exercise in the heat: 7%), with no significant differences between the heat acclimation modes. No heat acclimation mode had an impact on heart rate.

One study also investigated the effects of post-exercise hot water immersion in partially heat-acclimated endurance athletes training in the heat (Stevens et al., 2021). Participants (four male and nine female racewalkers) were considered partially acclimated to heat because they trained outdoors during summer for one to four weeks before the commencement of the study, which was completed during a fifteen-day training camp in outdoor heat (~34°C). All participants completed the same training and were divided into two groups that, right after eight training sessions, were either immersed in 40°C water to the sternum with submerged arms ($\dot{V}O_{2max}$ = 58.1 ± 7.5 ml·kg^{-1}·min^{-1}, *n* = 7) or rested at 21°C, 50% (control group, $\dot{V}O_{2max}$ = 57.5 ± 3.4 ml·kg^{-1}·min^{-1}, *n* = 6). The duration of the post-training interventions was 30 minutes during the first week and 40 minutes during the second week. Before and after the training camp, participants completed three days of testing, including a half-hour walking heat stress test in an environmental chamber (40°C, 40% RH), walking GXT, and a 10-km outdoor racewalking time trial. During the post-training heat stress test, both groups demonstrated significant reductions in exercising heart rate (hot water immersion: –11 beats·min^{-1}; control group: –11 beats·min^{-1}), significant increases in sweat rate (hot water immersion: 0.55 L·h^{-1}; control group: 0.34 L·h^{-1}), and significant improvements in thermal comfort (hot water immersion: 1.2 AU; control group: 1.5 AU). Both groups also significantly improved the post-training time trial performance (hot water immersion: 5.1%; control group: 6%). However, no significant between-group differences were noted, prompting the researchers to conclude that the addition of post-exercise hot water immersion provides no further enhancement in heat adaptations or exercise performance in partially heat-acclimated endurance athletes. It must be noted, though, that the chosen hot water immersion protocol might not have provided a strong enough stimulus to amplify the heat adaptations, as the rectal temperature at the end of the immersions was only 38.4°C. It is possible that this group of racewalkers would have experienced enhanced heat adaptations had they remained submerged in the bath to the neck, a protocol that typically induces

hyperthermia higher than 39°C (Greenfield et al., 2021; Lovell et al., 2008; Zurawlew et al., 2016, 2018b, 2019).

In summary, the reviewed hot water immersion studies indicate that the thermoregulatory gains induced by this method are greater than those acquired by active heat acclimation protocols. Some of the cited studies (Ashworth et al., 2023; Kissling et al., 2022) also support the use of sauna bathing as an alternative heat acclimation method to exercise in the heat, though their findings are not novel (Kirby et al., 2021; Leppäluoto et al., 1986; Tyka et al., 2008). However, the practical advantage of hot water immersion over sauna bathing is that it requires only a bathtub and hot water, and, as such, it can be conducted by many potential users in their homes. Nonetheless, the findings of Zurawlew et al., (2016) that six consecutive days of post-exercise hot water immersion for 40 minutes improved running performance in the heat are not generalisable beyond the studied population. Therefore, whether Zurawlew's hot water immersion protocol can improve endurance performance in the heat in unacclimated well-trained road cyclists and endurance athletes, in general, is unknown.

2.2.5. Effects of heat acclimation on endurance cycling performance in cool/temperate environments

In the mid-1980s, Sawka et al. (1985) reported that heat acclimation increases peak power output and $\dot{V}O_{2max}$ during a GXT in a thermoneutral environment (21°C, 30% RH). However, it took scientists two decades after that early report to realise that heat acclimation can be used as a tool to improve endurance exercise performance in a non-thermally challenging environment (Corbett et al., 2014).

The first study in this area to show that repeated heat exposure improves endurance performance was conducted by Scoon et al., (2007) on six male competitive distance runners and triathletes. The study took place during the winter period, and the athletes completed three weeks of post-training sauna bathing and three weeks of control training, separated by three weeks. During the sauna bathing training block, participants sat in a sauna (89.9 ± 2.0°C) for 31 ± 5 minutes immediately following running on 12.7 ± 2.1 occasions. A fifteen-minute treadmill run to exhaustion (at participants' current best 5-km run speed) at 18-20°C was completed before and after each training block. In addition, a resting blood sample was collected before each

running test. The researchers reported a 7.1% greater plasma volume and 32% improvement in run time to exhaustion following post-exercise bathing intervention compared with a three-week period of normal training. Based on the observed strong relationship (r = 0.96) between the change in plasma volume and the change in running performance, the researchers suggested that the expansion of plasma volume was responsible for the performance enhancement. Physiological mechanisms by which increased plasma volume could have improved running performance in that study include reduced exercising heart rate and increased $\dot{V}O_{2max}$ secondary to increased maximal cardiac stroke volume and cardiac output (Convertino, 1991; Guy et al., 2015). Furthermore, Scoon et al., (2007) speculated that adaptations other than plasma volume expansion that result in increased oxygen delivery to the muscles could have contributed to the improved running performance. While it is not clear what adaptations the researchers were referring to, there is evidence that chronic passive heat exposure increases muscle capillarization (Hesketh et al., 2019), an adaptation that leads to improved delivery of oxygen to mitochondria (Prior et al., 2004).

A few years after Scoon's study, Lorenzo et al., (2010), in the study cited earlier in the text (page 13), confirmed the ergogenic effects of heat acclimation for endurance cycling performance in nonthermally challenging conditions. In order for Lorenzo et al., (2010) to assess the effects of heat acclimation on cycling performance in a cool environment, the same testing (the GXT and one-hour time trial test) that was completed pre/post interventions in the heat was also completed in 13°C, 30% RH. Compared with training in a cool condition, heat acclimation resulted in a significant increase in power output at lactate threshold by 5% (3.88 ± 0.82 watts/kg versus 4.09 ± 0.76) and at $\dot{V}O_{2max}$ by 5% (66.8 ± 2.1 versus 70.2 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$) and it also significantly improved the time trial performance by 5% (879.8 ± 48.5 versus 934.7 ± 50.9 kJ). As possible mechanisms underpinning the ergogenic effects of heat acclimation on time trial performance in cool conditions, the researchers pointed out reduced glycogenolysis, reduced accumulation of blood lactate, and improved cardiac performance. The researchers also discussed that the last two possible physiological events could have been partially mediated by the expansion of plasma volume.

One more already mentioned study (Keiser et al., 2015) in the text also assessed the effects of heat acclimation on cycling performance in a thermoneutral environment. In

that study, the previously described testing in the heat (a GXT and a 30-minute time trial test) was also performed at 18°C, 30% RH. Unlike Lorenzo et al., (2010), Keiser et al., (2015) failed to observe improvement in either $\dot{V}O_{2max}$ or time trial performance following ten days of training in the heat despite the comparable increase in plasma volume (6%) to that of the former study. These findings suggest that plasma volume expansion associated with heat acclimation does not necessarily translate into improved temperate exercise performance. Although reported in a separate paper (Karlsen et al., 2015), neither the cyclists described previously by Racinais et al., (2015b) managed to improve their 43 km time trial performance in cool conditions (~5-13°C) following two weeks of heat acclimation.

Another four cycling studies also reported no performance benefits following short- (Neal et al., 2016), medium- (Corbett et al., 2022), and long- (Mikkelsen et al., 2019; Rønnestad et al., 2021) term heat acclimation interventions in non-heat acclimated cyclists. In the study by Neal et al., (2016), ten trained ($\dot{V}O_{2max}$ = 63.3 ± 4.0 ml·kg^{-1}·min^{-1}) male cyclists and triathletes completed a cycling heat stress test at 40°C, 50% RH and three additional tests (twenty-minute cycling at fixed intensity, cycling GXT, and 20-km time trial) in a temperate environment (22°C, 60% RH) before and after five days of controlled hyperthermia heat acclimation protocol described by Garret et al., (2009). Heat acclimation significantly reduced cardiovascular and thermal strain in both temperate and hot conditions. Power output at the lactate threshold and peak power output measured during the post-heat acclimation GXT were increased by 6.3% (ES = 0.28) and 1.8% (ES = 0.18), but the time trial performance was unchanged (p = 0.37). Corbett et al., (2022) allocated twenty-four trained ($\dot{V}O_{2max}$ = 56.7 ± 7.5 ml·kg^{-1}·min^{-1}) males into two different groups that trained for eleven days (60-90 minutes per day) in either a hot (40°C, 50% RH, n = 16) or a cool (11°C, 60% RH, control condition, n = 8) environment. Both groups completed a cycling heat stress test (40°C, 50% RH) and a GXT and 30-minute time trial test in a temperate environment (22°C, 50% RH) before and after the training interventions. The results from the heat stress test showed a significantly improved thermoregulation following the training only in the heat acclimation group. However, heat adaptations had no impact on the variables measured during the GXT (i.e., lactate threshold, gross efficiency, $\dot{V}O_{2max}$, peak power output) or time trial performance. Mikkelsen et al., (2019) subjected twenty-one trained ($\dot{V}O_{2max}$ = 58.1 ± 5.3 ml·kg^{-1}·min^{-1}) male cyclists to a 15-km time trial test in 14°C, 54%

RH before and after they completed five and a half weeks of either heat acclimation (*n* = 12) or training in cool conditions (<15°C, *n* = 9). The training volume and intensity throughout the study were consistent across the groups, but the heat acclimation group substituted part of their cool training with 28 ± 2 one-hour cycling training sessions in the heat (35-40°C, 30% RH) where the rectal temperature was increased above 39°C. Both groups demonstrated similar improvement in the time trial performance following the training blocks. Likewise, studying elite ($\dot{V}O_{2max}$ = 76.2 ± 7.6 ml·kg^{-1}·min^{-1}) male cyclists, Rønnestad et al., (2021) reported that five weeks of heat acclimation (twenty-four 50-minute sessions in 37.8 ± 0.5°C, 65.4 ± 1.8% RH) were not superior in improving fifteen minutes cycling time trial performance in a temperate environment (16-19°C) compared to training in a cool environment (15.5 ± 0.1°C, 25.1 ± 0.0% RH).

In contrast to the findings of Corbett et al., (2022), Karlsen et al., (2015), Keiser et al., (2015), Mikkelsen et al., (2019), Neal et al., (2016), and Rønnestad et al., (2021), there are studies (Maunder et al., 2021; Rendell et al., 2017; Rønnestad et al., 2022) that support the findings of Lorenzo et al., (2010).

Maunder et al., (2021) investigated whether three weeks of training in the heat (33°C, 60% RH) will lead to greater mitochondrial adaptations and cycling performance improvement compared to training in a temperate environment (18°C, 60% RH). Participants (seventeen trained [$\dot{V}O_{2peak}$ = 4.3 ± 0.7 L·min^{-1}] non-heat acclimated male cyclists and triathletes) were allocated into two groups that completed five supervised training sessions per week (two 90-minute moderate-intensity rides and three interval training sessions) in either hot or temperate conditions. Before and after the three-week training, participants completed a cycling GXT and a thirty-minute cycling time trial test preceded by a two-hour ride at 80% of power output corresponding to the first ventilatory threshold in an environment chamber set at 18°C, 60% RH. In addition, a resting biopsy was taken from the *vastus lateralis* at baseline and post-intervention to assess the activity of citrate synthase, which is the most commonly used marker for mitochondrial content (Bishop et al., 2019). Time trial performance was significantly improved in both groups but to a greater magnitude in the heat acclimation group (30 ± 13 watts versus 16 ± 5 watts). Power output at 2 and 4 mmol·L^{-1} lactate threshold and at the first and second ventilatory threshold was also significantly increased

following the training, with no significant difference between the groups. The activity of citrate synthase was significantly increased only following the training in the heat (fold-change, 1.25 ± 0.25).

Rendell et al., (2017) recruited eight trained ($\dot{V}O_{2max}$ = 58.6 ± 8.9 ml·kg^{-1}·min^{-1}) non-heat acclimated male cyclists/triathletes/runners to examine whether heat acclimation can improve endurance performance in temperate conditions and whether supplementing heat acclimation with a daily hypoxic stimulus can have an influence on performance. For the purpose of the study, each participant underwent two eleven-day controlled hyperthermia (90 minutes per day at 40°C, 50% RH) heat acclimation interventions (once with overnight hypoxia and once without), separated by three to seven months. Heat adaptations were assessed by a one-hour cycling heat stress test (40°C, 50% RH), whereas cycling performance at 22°C, 55% RH was assessed by a cycling GXT and a half an hour cycling time trial test, all performed before and after each intervention. Both heat acclimation interventions induced heat adaptations. Heat acclimation significantly improved power output at the lactate threshold (+15 watts) and peak power output (+12 watts) during the GXT and also significantly improved the work done during the time trial (+12 kilojoules). Interestingly, supplementing heat acclimation with an overnight hypoxic stimulus did not increase exercise performance significantly more than heat acclimation alone. It should be noted that the limitation of this study is the lack of a control group that underwent training in colder conditions. Given that, it remains unclear whether the observed performance improvement stems from heat acclimation or owes simply to a training effect.

Rønnestad et al., (2022) compared the effects of two different heat acclimation methods with training in a cool environment on hemoglobin mass and temperate (16-19°C) endurance cycling performance. Thirty-eight male non-heat acclimated cyclists were allocated into three groups that underwent a five-week intervention consisting of either five x 50-minute cycling sessions per week in an environmental chamber (~35°C, ~60% RH) in addition to training in a cool environment ($\dot{V}O_{2max}$ = 77.5 ± 4.3 ml·kg^{-1}·min^{-1}, *n* = 12), cycling in a cool environment but wearing a heat-suit for 50 minutes per training session on five weekly occasions ($\dot{V}O_{2max}$ = 77.8 ± 5.7 ml·kg^{-1}·min^{-1}, *n* = 13) or cycling training only in a cool environment (control group, $\dot{V}O_{2max}$ = 75.4 ± 3.2 ml·kg^{-1}·min^{-1}, *n* = 13). During the five-week intervention, there was no significant

difference in the training volume and intensity between the groups. Both heat acclimation groups experienced a significant increase in hemoglobin mass after the training compared to the control group, with no significant difference being noted between the heat acclimation groups. Performance index (composed of power output at 4 mmol·L^{-1} lactate threshold and peak power output determined during a cycling GXT and a 15-minute cycling time trial power output) was significantly improved following training with the heat-suit (3.1%), but not in the other two groups. However, when the performance data from both heat acclimation groups were pooled, there was a greater improvement in the performance index compared to the control group (4.9 ± 3.2% versus 1.7 ± 1.1%).

The inconsistency in the research findings presented in this section precludes making a definitive conclusion regarding the efficacy of heat acclimation in improving endurance cycling performance in cool/thermoneutral conditions. It is surprising that no study attempted to examine the effects of heat acclimation on endurance exercise performance in moderately warm climatic conditions. Addressing that question would reveal whether athletes can use heat acclimation to improve their exercise performance when competing during the first warm days of the spring or under unseasonably warm environmental conditions.

2.3. Inflammatory responses to exercise in the heat

Inflammation occurs in response to infection or injury, and it is characterised by capillary dilatation, the production of pathological blood-borne soluble components, and increased body temperature (Moldoveanu et al., 2001). Mediated by a small group of proteins called cytokines, the process of inflammation isolates and destroys noxious compounds, limits tissue damage, and activates tissue repair. More specifically, cytokines are produced in immune and non-immune cells in response to an inflammatory stimulus and are subsequently released at the site of inflammation, where they facilitate an influx of white blood cells (leucocytes) (Smith, 2003). The latter process participates in the clearing of antigens and tissue healing. There are more than a hundred cytokines and based on their physiological effects, they are classified as either 'pro' or 'anti-inflammatory' (Smith, 2003). The most studied pro-inflammatory cytokines are tumour necrosis factor (TNF)-α and interleukin (IL)-1β. The major anti-inflammatory cytokines include IL-4, IL-10, and IL-1 receptor antagonist (IL-1ra), and

their action restricts the production of pro-inflammatory cytokines (Moldoveanu et al., 2001). IL-6 can act as both a pro- and anti-inflammatory cytokine, depending on the situation. A balance between pro- and anti-inflammatory cytokines is crucial for maintaining tissue homeostasis. Insufficient or excessive cytokine production plays a role in the pathophysiology of a range of disorders (Oberholzer et al., 2000), including potentially fatal heat stroke (Heled et al., 2013). For instance, a study on 28 heat stroke patients found significantly increased plasma levels of IL-1β, IL-6, and interferon-gamma (INF-γ) at the time of hospital admission, and the level of IL-6 correlated with the severity of the illness (r = 0.51) (Bouchama et al., 1993).

In the context of exercise, various stimuli (i.e., hyperthermia, hypoxia, and mechanical, hormonal, and oxidative stress) can cause a release of cytokines and acute inflammation (Fehrenbach and Schneider, 2006). Furthermore, the combination of mode, intensity, and duration of exercise influences cytokine responses (Peake et al., 2015). Endurance sporting events such as marathon running (Nieman et al., 2001; Suzuki et al., 2003) and road cycling racing (Luk et al., 2016; Nieman et al., 2012) have been shown to induce cytokinaemia. Environmental conditions under which a person exercises may also modulate cytokine production (Shephard et al., 1998). Given the purpose of the thesis, only studies that have focused on the association between exercise-heat stress and plasma cytokines will be reviewed (Hosick et al., 2010; Peake et al., 2008; Rhind et al., 2004; Selkirk et al., 2008; Starkie et al., 2005).

Rhind et al., (2004) investigated the cytokine (TNF-α, IL-6, IL-12, and IL-1ra) and stress hormone (epinephrine, norepinephrine, growth hormone, and cortisol) responses to exertional hyperthermia in ten healthy male volunteers ($\dot{V}O_{2peak}$ = 48.0 ± 12.4 ml·kg^{-1}·min^{-1}). On two separate occasions, participants completed two 40-minute cycling exercise trials at 65% $\dot{V}O_{2peak}$ while immersed to mid-chest in either cold (18°C) or hot (39°C) water. Body temperature was continuously measured during the trials using a rectal probe. Venous blood samples were collected before, during, and 30- and 120-minute after exercise. During exercise in hot water, the rectal temperature rose from 37.2 ± 0.2°C to 39.1 ± 0.2°C. When participants exercised in cold water, the increase in their rectal temperature was limited to 0.36 ± 0.13°C. Exercising in hot water increased plasma norepinephrine, epinephrine, growth hormone, and cortisol by ~390%, ~580%, 300%, and 75%, respectively, above resting levels. In contrast,

exercising in cold water blunted increases in all hormones. When participants exercised in hot water, increases in plasma concentrations of TNF-α and IL-12 were significant after 20 minutes of exercise, with peak increments of >90% and >150%, respectively, by the end of the exercise. During the same condition, plasma IL-1ra and IL-6 demonstrated respective increases of >50% and >150% at the end of the 40-minute exercise but did not attain their peak values (>150% and >400%, respectively) until 30 minutes post-exercise. Exercising in cold water eliminated cytokine increase. The increase in rectal temperature during the hot condition was significantly related to an increase in TNF-α (r = 0.49), IL-12 (r = 0.21), and IL-1ra (r = 0.41). Furthermore, plasma IL-6 concentration was significantly related to plasma norepinephrine (r = 0.24), growth hormone (r = 0.37), and cortisol (r = 0.41). Plasma IL-1ra was also significantly related to cortisol (r = 0.51) and growth hormone (r = 0.32) levels. Based on the results, the researchers suggested that an increase in body temperature greater than >0.5°C during exercise in the heat evokes the release of stress hormones and sequential cytokine production.

Starkie et al., (2005) examined the effect of prolonged exercise in the heat on plasma TNF-α, IL-6, norepinephrine, epinephrine, and cortisol levels. Using a cross-over design, the researchers had seven endurance-trained ($\dot{V}O_{2peak}$ = 4.7 ± 0.4 L·min^{-1}) men completing a 90-minute cycling trial at 70% of $\dot{V}O_{2peak}$ in 15°C, 30% RH (control condition) and 35°C, 30% RH. Rectal temperature and blood samples were collected before, during and after the trials. Participants experienced significantly (p < 0.05) higher rectal temperature at the end of the trial in the heat than the trial at 15°C (~39.2°C and ~38.5°C, respectively). Increases in plasma norepinephrine and epinephrine were significantly higher during the exercise in the heat compared to the control condition. However, heat stress did not affect plasma cortisol levels compared with the control condition. Exercise at 35°C significantly increased plasma concentrations of TNF-α and IL-6 but not at 15°C. It was concluded that the production of cytokines is potentiated by heat stress.

In the study by Peake et al., (2008), ten well-trained ($\dot{V}O_{2max}$ = 4.8 ± 1.3 L·min^{-1}) male cyclists completed an exercise trail consisting of ninety minutes of cycling at 60% of $\dot{V}O_{2max}$ followed immediately by a 16.1 km time trial in two environmental conditions: 18°C and 32°C. Before, during, and after the trials, venous blood samples were

collected to assess serum concentrations of TNF-α, IL-6, IL-8, IL-10, IL-1ra, epinephrine, norepinephrine, and cortisol. Rectal temperature was monitored throughout the trials. The rectal temperature measured at the end of the trial in the heat was significantly higher than at 18°C (~38.9°C and ~38.5°C, respectively). Serum concentrations of norepinephrine and epinephrine significantly increased in response to cycling in both conditions, but only norepinephrine concentration was significantly higher following the trial in the heat compared to 18°C. Serum cortisol concentration significantly decreased throughout the trials, but there was no difference between the trials. Both trials induced similar increases in serum concentrations of IL-6, IL-8, and IL-10. Serum IL-8 and IL-10 concentrations at the end of the trial in the heat were significantly higher compared to 18°C. Only the cycling trial in the heat led to the release of TNF-α and IL-1ra.

Selkirk et al., (2008) compared cytokine (TNF-α, IL-6, IL-10, and IL-1ra) response to exercise-induced hyperthermia in twelve endurance-trained ($\dot{V}O_{2peak}$ = 70 ± 2 ml·kg^{-1}·min^{-1}) versus eleven untrained ($\dot{V}O_{2peak}$ = 50 ± 1 ml·kg^{-1}·min^{-1}) individuals. Wearing protective clothing, participants walked on a treadmill at 4.5 km with 2% elevation inside an environmental chamber (40°C, 30% RH) until volitional exhaustion. Venous blood was sampled at rest and at every 0.5°C increment in rectal temperature during the trial. At baseline, no significant between-group difference was observed for TNF-α, IL-6, and IL-10, but IL-1ra concentration was higher in athletes, and it remained elevated throughout the heat stress test compared to untrained participants. During the test, a significant increase in serum TNF-α concentration was observed at a lower rectal temperature in untrained participants than in athletes (38°C versus 38.5°C). There was also a difference in the rectal temperatures between the untrained (38°C) and trained (39.5°C) group at which the serum IL-1ra concentration significantly increased during the test. Serum IL-6 significantly rose at a rectal temperature of 38°C in both groups. Only athletes demonstrated a significant increase in serum IL-10, which occurred at a rectal temperate of 38.5°C. The researchers suggested that the inflammatory activation during exercising in the heat is triggered at lower body temperature in untrained compared with trained individuals.

In a study with a design similar to that of Rhind et al., (2004), Hosick et al., (2010) examined the relationship between the exercise-induced increase in rectal

temperature and cortisol and TNF-α in eight recreationally active ($\dot{V}O_{2peak}$ = 52.8 ± 3.7 ml·kg^{-1}·min^{-1}) men. Participants completed two 40-minute trials of cycling at 65% $\dot{V}O_{2peak}$ immersed to mid-chest: one in 25°C water and the other one in 38°C water. The rectal temperature during cycling in warm water was significantly higher than cycling in 25°C water (2.4°C versus 0.6°C). Neither TNF-α nor cortisol changed significantly following cycling in 25°C water, but both variables were significantly increased in the warm water trial. There was a strong (r = 0.83) but non-significant relationship between the change in cortisol and TNF-α. Results from the multiple regression analysis revealed that the change in rectal temperature was related to the change in cortisol but not to the change in TNF-α. The researchers concluded that (1) the increase in rectal temperature of at least 1°C above rest during exercise induces stress and inflammatory responses; (2) the change in rectal temperature during exercise has more of an influence on cortisol than TNF-α.

Besides the cited acute studies (Hosick et al., 2010; Peake et al., 2008; Rhind et al., 2004; Selkirk et al., 2008; Starkie et al., 2005), several other studies have looked into cytokine behaviour in response to repeated heat exposure (Barberio et al., 2014; Costello et al., 2018; Hailes et al., 2011; Kuennen et al., 2011; Willmott et al., 2018).

Fifteen unacclimated recreationally-trained ($\dot{V}O_{2max}$ = 54 ± 6 ml·kg^{-1}·min^{-1}) males took part in the study by Hailes et al., (2011) who examined inflammatory response during five consecutive days of exercise in the heat. Each session consisted of cycling in an environmental chamber (38°C, 40% RH) at 70% of $\dot{V}O_{2max}$ until volitional exhaustion or a rectal temperature of 39.5°C, whichever came first. Venous blood samples collected before and after the first and fifth sessions were analysed for eighty inflammatory markers. The duration of exercise of the first session (38.0 ± 9.4 minutes) was similar to that of the fifth session (40.7 ± 11.7 minutes). Resting rectal temperature before the fifth session (36.8 ± 0.6°C) was significantly lower than before the first session (37.1 ± 0.3°C). Of the fifty-two inflammatory markers that increased from before to after exercise on the first day, twenty-seven showed reduced response after exercise on the fifth day compared to after exercise on the first day. Resting values of eighteen, both pro- and anti-inflammatory markers were increased on the fifth day compared to the first day. The researchers concluded that individuals exposed to consecutive days of exercise in the heat experienced increased concentrations of

inflammatory markers associated with heat illness but also showed a reduced inflammatory response to a subsequent bout of exercise in the heat.

Kuennen et al., (2011) had eight non-heat acclimated men ($\dot{V}O_{2max}$ = 55.6 ± 2.3 ml·kg^{-1}·min^{-1}) completing a seven-day heat acclimation protocol. Heat acclimation was induced by exercising in an environmental chamber set at 46.5°C, 20% RH for 2 x 50 minutes, with 10 minutes of recovery between the bouts. The first 50-minute exercise bout induced an increase in rectal temperature to ≥39°C, and the rectal temperature was maintained above 39°C during the second 50-minute exercise bout. A heat stress test (46.5°C, 20% RH) consisting of exercising for 45 minutes at 50% of $\dot{V}O_{2max}$ was implemented before and after heat acclimation to assess heat adaptations. Venous blood was sampled before and after exercise on day one and day seven of the intervention, and the samples were analysed for TNF-α, IL-6, and IL-10. Heat acclimation led to reduced heart rate, skin and rectal temperatures, and increased sweat rate (p = 0.04) and plasma volume (16 ± 4%). There was no significant increase in plasma TNF-α concentration in response to the first heat acclimation session, but both plasma IL-6 and IL-10 concentrations were significantly increased during the session. Plasma TNF-α concentration remained unaffected by one week of exercising in the heat. Resting plasma IL-6 concentration was unaffected after seven days of exercise in the heat, though the response of this cytokine to the seventh heat acclimation session was similar to that of the first session. In contrast to the finding on day one, the plasma concentration of IL-10 did not increase during the seventh heat acclimation session.

In the study by Barberio et al., (2014), cytokine (TNF-α, IL-6, IL-10, and IL-1ra) response was examined in eight unacclimated endurance-trained ($\dot{V}O_{2max}$ = 55.3 ± 3.6 ml·kg^{-1}·min^{-1}) males during a five-day heat acclimation intervention. Participants exercised on a treadmill in an environmental chamber (40°C, 40% RH) at an intensity corresponding to 4 mmol·L^{-1} lactate threshold until volitional exhaustion or until rectal temperature increased by 2°C. Venous blood samples were collected before and after the first, third, and fifth heat acclimation sessions. A significantly lower resting rectal temperature was noted before the fifth (36.8 ± 0.2°C) and fourth (36.8 ± 0.3°C) heat acclimation sessions compared to the first session (37.1 ± 0.2°C). The rectal temperature at the end of the fifth (38.7 ± 0.2°C) and fourth (38.7 ± 0.2°C) heat

acclimation sessions was also significantly lower compared to that of the first heat acclimation session (39.2 ± 0.2°C). Heat acclimation had no significant impact on heart rate. A non-significant increase in plasma TNF-α concentration from rest to after exercise was observed during the first and third heat acclimation sessions. On day five, a significant decrease in plasma TNF-α concentration was observed from rest to one hour after exercise. Plasma IL-6 concentration increased non-significantly during the first, third, and fifth heat acclimation sessions. During all three heat acclimation sessions, when cytokines were measured, plasma IL-10 concentration was significantly higher one hour after exercise than at rest. Plasma IL-1ra concentration also peaked, though non-significantly, one hour after exercise during each of the three heat acclimation days.

Costello et al., (2018) examined the acute and chronic effects of hydration status during eleven days of heat acclimation on the levels of cortisol and a few inflammatory markers (C-reactive protein [CRP] and IL-6). In that study, eight unacclimated trained ($\dot{V}O_{2max}$ = 56.9 ± 7.2 ml·kg^{-1}·min^{-1}) males completed two heat acclimation interventions, once drinking to remain euhydrated during heat acclimation sessions and once with restricted drinking to induce body water loss of 2.35 ± 0.89% by the end of heat acclimation sessions. On both occasions, heat acclimation was induced in an environmental chamber (40°C, 50% RH) by applying the controlled hyperthermia protocol (Garret et al., 2009). Venous blood was collected immediately before and after heat acclimation sessions on days one, two, and eleven. During the first heat acclimation session, plasma IL-6 concentration increased from 1.0 ± 0.9 pg·mL^{-1} to 1.8 ± 1.0 pg·mL^{-1} in the hypohydrated group and from 1.0 ± 1.4 pg·mL^{-1} to 1.6 ± 2.1 pg·mL^{-1} in the euhydrated group, but no significant difference in IL-6 was observed between the groups. Neither exercise nor hydration status significantly impacted plasma CRP concentration during the first heat acclimation session. In a hypohydrated condition, plasma cortisol was significantly increased from 473.7 ± 388.6 nmol·L^{-1} to 1113.0 ± 983.1 nmol·L^{-1} during the first heat acclimation session, which was not the case with the euhydrated condition. Eleven days of heat acclimation did not affect either resting or exercising plasma IL-6, CRP, or cortisol, irrespective of the participant's hydration status.

The study by Willmott et al., (2018) compared the effects of ten heat acclimation (45°C, 20% RH) sessions to ten training sessions in a temperate environment (22°C, 40% RH) on plasma TNF-α, IL-6, and cortisol. Forty non-heat acclimated moderately-trained males were allocated to four separate groups that completed ten 60-minute training sessions administered either: over ten consecutive days in the heat (*n* = 10), over five non-consecutive days (twice-daily sessions) in the heat (*n* = 10), over ten consecutive days in a temperate environment (*n* = 10), or over five non-consecutive days (twice-daily sessions) in a temperate environment (*n* = 10). Venous blood was collected before and after sessions one, five, and ten. Plasma TNF-α, IL-6, and cortisol were significantly increased from pre- to post-exercise on days one, five, and ten in all groups. However, inflammatory and stress responses were significantly greater in heat acclimation groups compared to temperate training groups. There was no evidence of chronic effects on plasma TNF-α, IL-6, or cortisol over the course of the study. Accordingly, the researchers concluded that heat acclimation interventions had no adverse effects on inflammatory or stress responses.

To sum up, acute inflammation is vital in host defence and promotes tissue repair, but chronic inflammation can exert harmful health effects. Significant cytokinaemia is induced when non-heat acclimated, endurance-trained individuals perform intensive physical activity in the heat. Some of the cited studies indicate that heat acclimation may attenuate the cytokine response to subsequent exercise heat-stress exposure. However, Hailes et al., (2011) show that heat acclimation can increase resting inflammation. It is possible that their participants did not have enough recovery time between the heat acclimation sessions to re-establish homeostasis, leading to a state of chronic inflammation. The finding that heat acclimation may result in chronic inflammation is concerning because this condition may hinder athletic performance, impair the immune system, and contribute to the development of overtraining syndrome (Cheng et al., 2020; Smith, 2003). Unfortunately, the potential clinical consequences associated with the increased inflammation following heat acclimation in the study by Hailes et al., (2011) remain unknown because the researchers did not attempt to assess participants' health or exercise performance. Nevertheless, the idea that heat acclimation may induce fatigue secondary to the development of chronic inflammation helps to explain the symptoms of overreaching experienced by some of the participants in the heat acclimation study by McIntyre et al., (2021). The evidence

that heat acclimation has the potential to induce chronic inflammation and knowing what consequences this could have on athletes' health and performance justifies the need to examine cytokine response to the six-day post-exercise hot water immersion protocol.

2.4. Effects of heat stress on the antioxidant status

It is well-documented that one by-product of cellular respiration is the formation of reactive oxygen species (Verhagen et al., 2006). Although these free radicals play a vital role in cell-signalling and immune-mediated defence (Pham-Huy et al., 2008; Verhagen et al., 2006), their overproduction is potentially harmful because it can cause damage to all living cells and impair their functioning (Halliwell and Chirico, 1993). The process of uncontrolled free radical production, known as oxidative stress, has also been implicated in Alzheimer's disease, cancer, cardiovascular disease, cataracts, rheumatoid arthritis, type two diabetes, and aging (Pham-Huy et al., 2008; Verhagen et al., 2006). By definition, oxidative stress occurs as a consequence of an imbalance between the production of free radicals and the ability of the antioxidant defence system to scavenge them (Sies, 1997). The mechanisms responsible for the detoxification of free radicals include numerous enzymatic and non-enzymatic antioxidants. The three major classes of antioxidant enzymes are catalase, glutathione peroxidase, and superoxide dismutase, whereas some of the non-enzymatic antioxidants include vitamins C and E, beta carotene, and glutathione (Sies, 1997).

At resting conditions or during low-intensity physical exercise, the body's antioxidant defence mechanisms can easily deal with the production of free radicals and maintain oxidative balance (Finaud et al., 2006). However, the evidence of increased oxidative stress markers during intensive or/and prolonged sporting events indicates that participation in such events may overwhelm the antioxidant system (Bloomer et al., 2005; Briviba et al., 2005; Duca et al., 2006; Kaikkonen et al., 1998; Mastaloudis et al., 2001; Pinho et al., 2010). Exercise-induced oxidative stress is thought to be involved in the onset of premature fatigue (Allen et al., 2008; Powers and Jackson, 2008), initiation of muscle damage (Aoi et al., 2004; Gómez-Cabrera et al., 2003), and the development of overtraining syndrome (Cheng et al., 2020; Tiidus, 1998). Besides exercise, environmental factors such as heat stress can also induce increased free

radical production. The following several studies (McAnulty et al., 2005; Ohtsuka et al., 1994; Pilch et al., 2014; Sureda et al., 2015) illustrate the latter notion.

Ohtsuka et al., (1994) examined the effects of passive heat exposure *via* hot water immersion on antioxidative defence status and oxidative stress. The researchers allocated twenty-one healthy male students into three groups that were immersed for ten minutes in either 25°C, 39°C or 42°C water. Erythrocytes (RBC) from venous blood, taken immediately before and after the immersions, were analysed for glutathione metabolism and lipid peroxidation (a marker of oxidative stress). No significant changes were observed in any measured variables during immersion in 39°C water. A significant decrease in plasma glutathione concentration from 2.44 ± 0.14 to 1.80 ± 0.10 μmol·ml RBC^{-1} was noted following the immersion at 42°C. In contrast, glutathione concentration significantly increased from 2.46 ± 0.17 to 2.91 to 0.17 μmol·ml RBC^{-1} following the immersion at 25°C. Activities of glutathione peroxidase significantly decreased from 35.90 ± 1.83 to 34.33 ± 1.66 IU·g Hb^{-1} following the immersion at 42°C. During the immersion in 25°C water, the activities of glutathione peroxidase were slightly increased from 38.22 ± 1.90 to 39.65 ± 2.44 IU·g Hb^{-1}. The concentration of lipid peroxidation significantly increased from 1.87 ± 0.03 to 2.06 ± 0.04 μmol·ml RBC^{-1} following immersion at 42°C, but no significant change in lipid peroxidation was observed during the immersion in 25°C water. The researchers concluded that heat stress alters glutathione metabolism and causes intracellular oxidative damage.

McAnulty et al., (2005) compared the activity of oxidative stress markers in response to exercise at 35°C versus 25°C. In a cross-over manner, six moderately trained ($\dot{V}O_{2max}$ = 40.6 ± 1.9 ml·kg^{-1}·min^{-1}) males exercised on a treadmill at 50% of $\dot{V}O_{2max}$ either in the heat (35°C, 70%) until their rectal temperature increased to 39.5°C or for an equivalent time at 25°C, 40%. The rectal temperature during the latter condition rose to 38.1°C, and the average exercise time during both conditions was 49.8 ± 4.6 minutes. Oxidative stress was determined by measuring plasma F2 isoprostanes and lipid hydroperoxides before and after the exercise bouts. Lipid hydroperoxides were significantly increased after exercise in both conditions, but the increase tended to be higher in hot compared to neutral conditions. Similarly, F2 isoprostanes were significantly increased after exercise in both conditions. However, the magnitude of

increase in F2 isoprostanes was significantly higher in the hot compared to the 25°C condition. Because no significant difference in oxygen consumption (the main source of free radical production) was observed between the conditions, the researchers concluded that hyperthermia increases oxidative stress independently of oxygen consumption.

Pilch et al., (2014) compared the effects of exercise in the heat to passive heat exposure on prooxidant-antioxidant balance in ten trained ($\dot{V}O_{2max}$ = 60.5 ± 13.51 ml·kg^{-1}·min^{-1}) and ten untrained ($\dot{V}O_{2max}$ = 44.5 ± 8.98 ml·kg^{-1}·min^{-1}) men. All participants first completed an exercise session on a cycling ergometer with a load of 53% $\dot{V}O_{2max}$ in the heat (33 ± 1°C, 70% RH) until their rectal temperature increased by 1.2°C. Following a month after the completion of the exercise session, participants were exposed to sauna bathing (96 ± 2°C, 16 ± 5% RH) aimed to induce an increase in their rectal temperature by 1.2°C. Immediately before and after the interventions, venous blood was sampled to assess the antioxidant status and lipid peroxidation products. Exercise in the heat led to a reduction in antioxidant status by 24.3% from 220.42 ± 49.50 µmol·l^{-1} to 166.85 ± 53.80 µmol·l^{-1} and an increase in lipid peroxidation by 61.7% from 113.17 ± 42.57 µmol·l^{-1} to 183.00 ± 69.81 µmol·l^{-1} in untrained individuals. Trained individuals experienced a drop in antioxidant status by 21.3% from 242.40 ± 37.10 µmol·l^{-1} to 190.55 ± 37.80 µmol·l^{-1} and an increase in lipid peroxidation by 88.9% from 67.20 ± 27.43 µmol·l^{-1} to 127.00 ± 71.63 µmol·l^{-1} after exercise in the heat. Sauna bathing reduced the antioxidant status in untrained individuals by 65.4% from 200.57 ± 44.57 µmol·l^{-1} to 69.14 ± 23.57 µmol·l^{-1} and increased lipid peroxidation by 38.4% from 139.62 ± 52.53 µmol·l^{-1} to 193.25 ± 66.28 µmol·l^{-1}. In trained individuals, sauna bathing reduced the antioxidant status by 20.7% from 250.25 ± 44.58 µmol·l^{-1} to 198.25 ± 23.57 µmol·l^{-1} and increased lipid peroxidation by 56.2% from 65.28 ± 20.16 µmol·l^{-1} to 102.00 ± 29.96 µmol·l^{-1}. A significant between-group difference in the values both before and after the intervention was observed. It was concluded that trained individuals have higher resting antioxidant status, and the disturbance of their prooxidant-antioxidant balance in response to heat stress remained lower compared to untrained individuals.

Nine endurance-trained ($\dot{V}O_{2max}$ = 61.4 ± 2.8 ml·kg^{-1}·min^{-1}) male athletes partook in the study by Sureda et al., (2015), who measured markers of antioxidant status and

oxidative stress in response to exercise in the heat versus cool environments. Participants completed two 45-minute exercise sessions on a treadmill at 75-80% of $\dot{V}O_{2max}$ in an environmental chamber under two conditions: one in 10-12°C, 40-55% RH and the other one in 30-32°C, 75-78% RH. Body core temperature was measured continuously throughout the exercise *via* an ingestible telemetric sensor. Venous blood and urine samples were collected immediately before and after exercise and after two hours of recovery. Blood was analysed for markers of antioxidative defence (catalase, superoxide dismutase, and paraoxonase-1) and oxidative stress (malondialdehyde and protein carbonyl derivatives), whereas concentrations of 8-Hydroxy-2′-deoxyguanosine (oxidative stress marker) were determined in urine. Exercise in the heat resulted in a significantly higher increase in gastrointestinal temperature than in the cool environment (~39.8°C versus ~38.3°C). There were no significant changes in the protein carbonyl index after exercise in either condition. Plasma malondialdehyde concentration was significantly influenced by the air temperature, with a 44% higher value observed after recovery in the heat compared to the cool environment (2.56 ± 0.26 μmol·l^{-1} versus 2.00 ± 0.27 μmol·l^{-1}). A significant change in urinary 8-Hydroxy-2′-deoxyguanosine concentration was observed only in the hot condition, where this marker increased from 93 ± 11 μg·l^{-1} at rest to 156 ± 21 μg·l^{-1} after two hours of recovery. Exercise in the heat significantly impacted antioxidant enzymes, which was not the case with exercise in a cool environment. Catalase activity was increased by 59% during exercise in the heat. Paraoxonase-1 activity demonstrated a 29% higher value after recovery compared to rest. However, no significant change was observed in superoxide dismutase activity. It was concluded that exercising in the heat increased oxidative stress but also activated the antioxidant defence compared to exercising in a cool environment.

One study also examined the changes in oxidative stress during repeated exposure to exercise in heat (Kaldur et al., 2014). In that study, twenty-one unacclimated physically active ($\dot{V}O_{2peak}$ = 53.8 ± 7.1 ml·kg^{-1}·min^{-1}) young men were heat acclimated by exercising on a treadmill for ten consecutive days (110 minutes per day) into an environmental chamber at 42°C and 18% RH. Before and after heat acclimation, participants completed an endurance capacity test in the heat (42°C, 18% RH) consisting of exercising on a treadmill at 60% of $\dot{V}O_{2peak}$ until volitional exhaustion or until the rectal temperature reached 40°C. Venous blood samples were collected

immediately before and after pre- and post-heat acclimation testing. The researchers determined total peroxide concentration and oxidised low-density lipoproteins (both are markers of oxidative stress) in blood samples and the total antioxidant capacity. The percent ratio of the total peroxide concentration of plasma to the total antioxidant capacity of plasma was accepted as the oxidative stress index and an indicator of the extent of oxidative stress. Ten days of exercise in the heat improved endurance capacity by 82.7%. Furthermore, heat acclimation increased resting total peroxide concentration by 24.2% and oxidative stress index by 36.7% and reduced resting oxidised low-density lipoproteins by 9.2% but had no significant impact on the resting total antioxidant capacity. The baseline endurance capacity test increased total peroxide concentration by 27% and oxidative stress index by 29%, whereas oxidised low-density lipoproteins and the total antioxidant capacity remained unchanged. Post-heat acclimation, a decrease in oxidative stress index by 17.7% was noted during the endurance capacity test. Other variables were unchanged during the second test. Based on the findings, the researchers concluded that the ten-day heat acclimation intervention increased resting oxidative stress, but it also induced some beneficial adaptations that reduced oxidative stress during acute exhaustive endurance exercise in the heat performed post-heat acclimation.

Collectively, the cited studies (McAnulty et al., 2005; Ohtsuka et al., 1994; Pilch et al., 2014; Sureda et al., 2015) indicated that acute hyperthermia, induced either by exercising in the heat or by passive heat exposure, is a factor that can disrupt the prooxidant-antioxidant balance. However, our understanding of the potential of repeated heat exposure to chronically alter prooxidant-antioxidant balance is based on only one study (Kaldur et al., 2014). That study showed that ten days of active heat acclimation increased resting oxidative stress. An open question is whether shorter heat acclimation interventions, such as the six-day post-exercise hot water immersion protocol, would cause chronic disruption in the prooxidant-antioxidant balance. Addressing that question is important, given the negative health and performance consequences associated with chronic oxidative stress.

2.5. Monitoring body temperature during heat stress

The human body is equipped with a well-developed thermoregulation system that maintains its resting internal temperature at around 37°C for optimal function of organ

systems (Kjertakov and Epstein, 2013). However, when thermally challenged, the body establishes thermal homeostasis at a higher internal temperature. Indeed, a magnitude of hyperthermia between 38°C and 40°C is a normal physiological response during prolonged exposure to either exercise in the heat (Barnett and Maughan, 1993; Jentjens et al., 2002; Naperalsky et al., 2010; Ryan et al., 1989) or heavy passive heat load (Dumke et al., 2015; Ihsan et al., 2020; Périard et al., 2014). Yet, both scenarios also significantly increase the risk for the development of extreme hyperthermia and associated potentially fatal heat stroke (Chen et al., 2014; Erarslan et al., 2012; Rav-Acha et al., 2004; Shibolet et al., 1967; Ubaldo et al., 2020; Zhuang et al., 2017). Therefore, an accurate measurement of body core temperature in a situation when the risk of heat stroke is high is essential for preventing heat casualties (Moran and Mendal, 2002). In laboratory settings, sports scientists most commonly use rectal probes to ensure participants' safety during trials that impose a risk of heat stroke. Ingestible telemetric capsule systems are other means for monitoring core temperature scientists use. Although both products allow accurate measurement of core temperature (Lim et al., 2008), their use is not feasible for athletes outside of the laboratory due to the invasive nature of rectal probes and the prohibitive cost of temperature pills. Given those limitations, researchers have examined the suitability of several other tools as an alternative to rectal probes and temperature pills. Studies in this research area have been conducted on exercising healthy individuals (Casa et al., 2007; Fenemor et al., 2020; Ganio et al., 2009; Morán-Navarro et al., 2019; Otani et al., 2020) and hyperthermic patients (Morrissey et al., 2021).

Casa et al., (2007) assessed the validity of seven different temperature devices during outdoor exercise in the heat in twenty-five (fifteen men and ten women) physically active individuals. The temperature measuring devices included: expensive (SureTemp model 679; Welch Allyn Medical Products, Skaneateles Falls, NY) and inexpensive (model VT-801BWT; Walgreen Co, Deerfield, IL) oral digital thermometers; expensive (Data-Therm model 00703; RG Medical Diagnostics, Southfield, MI) and inexpensive (model VT-801BWT; Walgreen Co) devices for measuring axillary temperature; a tympanic thermometer (Braun Thermoscan ExacTemp model IRT 4520; Braun, South Boston, MA); a forehead sticker (SportsTemp; Greenwood Village, CO); and a temporal artery scanner (model 2000C; Exergen Corp, Watertown, MA). All these devices were compared with rectal

temperature, and to be deemed valid, a mean bias of a given device should not have been higher than ±0.27°C from rectal temperature. Temperatures were measured after 60, 120, and 180 minutes of exercise. The mean bias of all seven studied devices was higher than the accepted limit of ±0.27°C: temperature measured *via* expensive oral device (–1.20°C), inexpensive oral device (–1.67°C), expensive axillary device (–2.58°C), inexpensive axillary (–2.07°C), tympanic device (–1.00°C), temporal method (–1.46°C), and forehead temperature (–0.60°C). Similar results were reported when the same devices were tested during indoor exercise in the heat (Ganio et al., 2009).

Morán-Navarro et al., (2019) compared oral, skin, and tympanic temperatures with gastrointestinal temperature during 60 minutes of cycling exercise in the heat (40.1 ± 0.5°C, 39.5 ± 3.4% RH) in twelve well-trained ($\dot{V}O_{2max}$ = 62.2 ± 3.4 ml·kg^{-1}·min^{-1}) male cyclists and triathletes. Every ten minutes during the trial, body temperatures were measured with an oral thermometer (model PRT 2000, Braun, Switzerland), three types of skin-surface thermometers (model FTN B, Medisana, Germany; model VisioFocus 06400, Tecnimed, Italy; model JPD-FR100, Etekcity, Germany), and two types of tympanic thermometers (model IRT 6520, Braun, Switzerland; model JPD-FR100, Etekcity, Germany). The gastrointestinal temperature was measured with an ingestible telemetric capsule system (model CorTemp, HQinc, USA) and served as a reference against which the other thermometers were tested. The researchers also examined the impact of wind and sweat during exercise on the thermometers' reading by creating different wind/sweat conditions each time a temperature was measured. As a result, the following four conditions were simulated: 'sweat and wind', 'sweat without wind', 'no sweat with wind', and 'no sweat, no wind'. However, only the performance of skin-surface thermometers was accessed under the former two conditions. A difference in body temperature of less than 0.3°C between the reading of the gastrointestinal pill and a given device was considered acceptable. According to that criterion, tympanic thermometer model IRT 6520 was deemed valid during exercise in hot, wind-still and windy conditions because it underestimated gastrointestinal temperature by only 0.1°C and 0.2°C, respectively. Skin thermometer model FTN B showed acceptable mean bias during exercise in wind-still conditions with (0.2°C) and without a sweat (0.1°C), whereas skin thermometer model VisioFocus 06400 was shown valid during exercise in wind-still conditions where sweating was absent (mean bias 0.2°C). The mean bias of these two skin thermometers during

exercise under the other conditions and the mean bias of model JPD-FR100 during exercise under all conditions ranged from 0.3°C to 3.2°C. The mean bias of the oral thermometer and tympanic thermometer model JPD-FR100 during exercise in wind-still and windy conditions were 0.5°C and 0.6°C, respectively and 0.9°C and 0.6°C, respectively.

Fenemor et al., (2020) recruited fifteen (nine male and six female) recreational athletes to examine the validity of the Braun ThermoScan® 7 IRT 6520 tympanic thermometer for measuring core temperature during exercise in the heat. Participants exercised on a cycling ergometer for half an hour at self-paced power output in an environmental chamber set at 35°C and 60% RH. During the trial, body temperature was simultaneously measured using the above-mentioned tympanic thermometer and a gastrointestinal temperature pill, which participants had to swallow five hours beforehand. Body temperature measured *via* the tympanic thermometer was not significantly different than that obtained by the temperature pill. There was also a strong ($r = 0.74$) correlation between devices. Furthermore, the tympanic thermometer underestimated gastrointestinal temperature by only 0.1°C, which was within the allowed limit of ±0.3°C set by the researchers.

Eight healthy ($\dot{V}O_{2max}$ = 47.3 ± 7.0 $ml \cdot kg^{-1} \cdot min^{-1}$) males participated in the study by Otani et al., (2020) who compared body temperature measured by Genius™ 2 (Covidien, Mansfield, MA, USA) tympanic thermometer to rectal temperature during exercise in the heat with variations in solar radiations. Participants completed three separate cycling trials at 70% of peak power output until exhaustion in a heated (30°C, 50% RH) environmental chamber under solar radiation of 0, 250, and 500 watts/m^2. No significant difference was observed between the devices during the trials. Strong ($r_s = 0.73$ for the 0 watts/m^2 trial; $r_s = 0.77$ for the 500 watts/m^2 trial) and very strong ($r_s = 0.86$ for the 250 watts/m^2 trial) correlations were observed between devices. The agreement between tympanic and rectal temperatures during exercise was –0.11°C in the 0 watts/m^2 trial, –0.13°C in the 250 watts/m^2 trial, and –0.03°C in the 500 watts/m^2 trial. The researchers concluded that Genius™ 2 tympanic thermometer is acceptable for monitoring core temperature during exercise in the heat when the solar radiation is below 500 watts/m^2.

In the study by Morrissey et al., (2021), body temperature measured by Braun Pro 6400 (Kronberg, Germany) tympanic thermometer was compared to rectal temperature in twenty-six (fifteen men and eleven women) heat stroke patients. All patients were runners who participated in an 11.3-km foot race in a warm environment (27.0 ± 2.8°C, 75.5 ± 12% RH). Tympanic and rectal temperatures were initially measured when the runners were brought to the triage area. Subsequently, rectal temperature was continuously monitored during the cold-water treatment, whereas tympanic temperature was measured only once during the treatment. Initial average tympanic and rectal temperatures were 38.8 ± 1.1°C and 41.1 ± 0.8°C, respectively. Rectal temperature was significantly greater than tympanic temperature. The mean bias between tympanic and rectal temperatures was 2.4 ± 0.96°C. Tympanic and rectal temperatures during the cold-water treatment were 38.0 ± 1.2°C and 40.4 ± 1.0°C, respectively. Again, rectal temperature was significantly greater than tympanic temperature. The mean bias between devices during the treatment was 2.4 ± 1.0°C. It was concluded that using a tympanic thermometer to measure body temperature in a case of heat stroke incident would lead to misdiagnosis of the disorder, which in turn can result in catastrophic outcomes.

The findings that the Genius™ 2 (Otani et al., 2020) and Braun ThermoScan® 7 IRT 6520 (Fenemor et al., 2020; Morán-Navarro et al., 2019) tympanic thermometers accurately tracked core temperature during exercise in the heat suggest that they might be suitable tools for detecting dangerous hyperthermia in a situation when the body is exposed to heavy heat load, such as during the head-out 40°C water immersion protocol. There is also a potential for a few other models of the Braun (Pro 3000, IRT 1020, and IRT 4000) tympanic thermometers to be used for that purpose, as they were validated for monitoring core temperature in clinical settings (Bock et al., 2005; Kocoglu et al., 2002; van Staaij et al., 2003). However, since the highest degree of induced hyperthermia in the above studies was ~38.5°C, further validation of those tympanic thermometers during more severe heat stress is required.

Chapter three: Effects of post-exercise hot water immersion on heat acclimation and endurance cycling performance in warm and hot conditions

Abstract

Background: Heat acclimation is a proven strategy for enhancing endurance exercise performance in the heat in non-heat acclimated athletes. There is evidence that repeated post-exercise hot water immersion induces heat acclimation and improves endurance performance in the heat in physically active individuals. However, whether this heat acclimation strategy could improve endurance performance in the heat in trained endurance athletes is unknown.

Objective: To examine the effects of a six-day post-exercise head-out immersion in 40°C water on physiological and exercise performance outcomes in warm and hot environmental conditions in a group of well-trained non-heat acclimated male endurance athletes.

Methods: Sixteen male cyclists completed a six-day intervention involving a daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot water immersion (HWI; n = 8) or thermoneutral water immersion (CON; n = 8) for 40 minutes. At baseline and post-intervention, participants completed two 30-minute continuous cycling tests followed by 20-km time trial tests (one at 27°C, 40% RH and the other one at 35°C, 40% RH) on two different days separated by 48 hours of rest.

Results: The HWI group showed a significantly decreased peak heart rate (-7.37 ± 5.21 beats·min^{-1}, p = 0.03), peak thermal sensation (-0.56 ± 0.41 arbitrary units, p < 0.01), and rating of perceived exertion (-1.00 ± 0.75 arbitrary units, p = 0.02) during the 30-minute continuous test at 27°C. The HWI group also showed a significantly decreased peak thermal sensation (-0.50 ± 0.53 arbitrary units, p = 0.03) and peak rating of perceived exertion (-1.62 ± 1.06 arbitrary units, p = 0.01) during the 30-minute continuous test at 35°C. None of these variables were altered in the CON group. Furthermore, the HWI group showed only a tendency for improved 20 km time trial performance at 27°C (p = 0.06) and 35°C (p = 0.06).

Conclusion: The findings from the current study indicate that a six-day post-exercise hot water immersion reduces cardiovascular and perceptual strain during exercise at 27°C and reduces only perceptual strain during moderate-intensity exercise at 35°C. Although the post-exercise hot water immersion intervention did not significantly improve the 20 km time trial performance either at 27°C or 35°C, the reduction in the completion time of the latter test by 1.77% in the HWI group can be considered practically significant.

Keywords: well-trained endurance athletes, heat acclimation, hot water immersion

3.1. Introduction

Studies have shown that five 90-minute daily exercise sessions in the heat are sufficient to improve cycling, running, and rowing time trial performance in hot conditions in previously non-heat acclimated athletes (Garret et al., 2012; James et al., 2017; Wingfield et al., 2016). Nonetheless, the findings from those studies apply to athletes who either have access to facilities that can simulate a hot environment (e.g., an environmental laboratory) or afford to travel to the competition venue at least one week in advance and complete the heat acclimation process there. All other athletes will have to rely on passive heat acclimation strategies such as hot water immersion.

Zurawlew et al., (2016) demonstrated that hot water immersion could be an effective heat acclimation strategy. These researchers recruited seventeen physically active, non-heat acclimated, males who completed a six-day intervention consisting of daily running for 40 minutes in temperate conditions followed immediately by either hot (40°C; n = 10) or lukewarm (34°C; n = 7) water immersion up to the neck for 40 minutes. Before and after the intervention, the participants completed a 40-minute continuous running test at submaximal intensity followed by a 5-km run time trial in hot (33°C, 40% RH) conditions. Post-intervention, a significant reduction in body temperature and heart rate during the 40-minute continuous test was observed only in the hot water group. Similarly, only the hot-water group demonstrated improvement in the 5-km running performance (4.9%). Subsequently, the same research group also showed that their heat acclimation strategy induces heat adaptations in endurance-trained individuals (Zurawlew et al., 2018b). Unfortunately, as the latter study did not include an endurance performance test, it is unclear if endurance-trained athletes can achieve meaningful endurance performance improvements following Zurawlew's heat acclimation protocol. Given that, the first aim of this study was to investigate whether the six-day post-exercise hot water immersion intervention induces heat acclimation and improves endurance performance in the heat in a group of well-trained road cyclists.

In general, heat acclimation is recommended as an ergogenic strategy for exercise performance in climates where air temperature exceeds 30°C. However, the evidence that the thermoregulation stress increases and the capacity to perform endurance

exercise declines as the air temperature increases above 20°C (Galloway and Maughan, 1997) suggests that heat acclimation may be a useful strategy even when the competitive environmental conditions are cooler (Corbett et al., 2014). To test this idea, Neal et al., (2016) had ten trained male cyclists complete a 20-km cycling time trial test and a twenty-minute constant load test at 22°C before and after a five-day active heat acclimation intervention. Although the thermoregulatory responses during exercise at 22°C were significantly improved after the intervention, the time trial performance was not changed, which led the researchers to conclude that longer heat acclimation protocols may be needed to improve temperate performance. This may hold true as Rendell et al., (2017) reported an enhanced time trial performance under an identical air temperature as in Neal's study after eleven days of training in the heat. Nevertheless, a question that remains open is whether short-term heat acclimation can improve endurance performance in moderately warm conditions. In a recent study, Faulkner et al., (2019) showed improved cycling time trial performance at 27°C and 35°C but not at 24°C after a pre-cooling intervention consisting of wearing an ice vest and sleeves for 20 minutes before the start of the nine-minute warm up for the time trial. The beneficial effects of pre-cooling on time trial performance were attributed to the reduced skin temperature and thermal sensation before and during the initial phase of the time trials. Given that repeated hot water immersion can also lead to favourable alterations in both skin temperature and thermal sensation (Greenfield et al., 2021; Waldock et al., 2021; Zurawlew et al., 2016), in addition to reduced heart rate and body temperature, it is reasonable to expect that the six-day post-exercise hot water immersion protocol would improve endurance performance at 27°C. Addressing the latter was an additional aim of this study.

3.2. Methodology and Procedures

3.2.1. Participants

Sixteen well-trained cyclists aged between 18 and 45 who have not been exposed to (1) exercise in the heat or (2) sauna or spa bathing at least one month before the commencement of the study were invited to participate. Participants' baseline demographic characteristics and fitness levels are presented in Table 3.1. Based on their PPO values, participants were classified as well-trained (De Pauw et al., 2013). *A priory* sample size calculation using G*power 3.1.9.2 software was based on the paper by Zurawlew et al. (2018b), who found a significantly reduced end-exercise rectal temperature by 0.38°C in endurance-trained male athletes following six post-exercise hot water immersion sessions. From these findings, it was determined that a minimum of 14 participants (7 participants per group) would be required to achieve a statistical power of 0.80 and a significance level of 0.05.

Table 3.1. Participants' characteristics at baseline. Data expressed as mean ± SD.

	HWI (n = 8)	CON (n = 8)	p values
Age (years)	29.00 ± 7.85	32.00 ± 6.76	0.51
Height (cm)	178.37 ± 3.88	178.75 ± 4.71	0.81
Body mass (kg)	75.72 ± 8.22	75.30 ± 5.77	0.92
$\dot{V}O2_{max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	63.75 ± 7.46	61.02 ± 6.67	0.35
PPO (W)	416.25 ± 43.15	393.75 ± 45.80	0.16

Abbreviations: HWI – hot water immersion group, CON – control group, $\dot{V}O2_{max}$ – maximal oxygen consumption, PPO – peak power output, W – watts.

3.2.2. Experimental design

The study was conducted between late autumn and early spring to minimise seasonal acclimatisation effects. Participants completed a six-day intervention involving a daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot water immersion (HWI; n = 8) or thermoneutral water immersion (CON; n = 8) for 40 minutes. At baseline and post-intervention, participants completed two 30-minute continuous cycling tests followed by 20-km time trial tests (one in a warm environment and one in a hot environment) on two different days separated by 48 hours of rest. A familiarization trial of the time trial test was completed five days before the baseline

testing to increase the reliability of the subsequent testing (Zavorsky et al., 2007). A graded exercise test (GXT) was completed two days before the familiarisation trial. An overview of the study design is displayed in Figure 3.1 below.

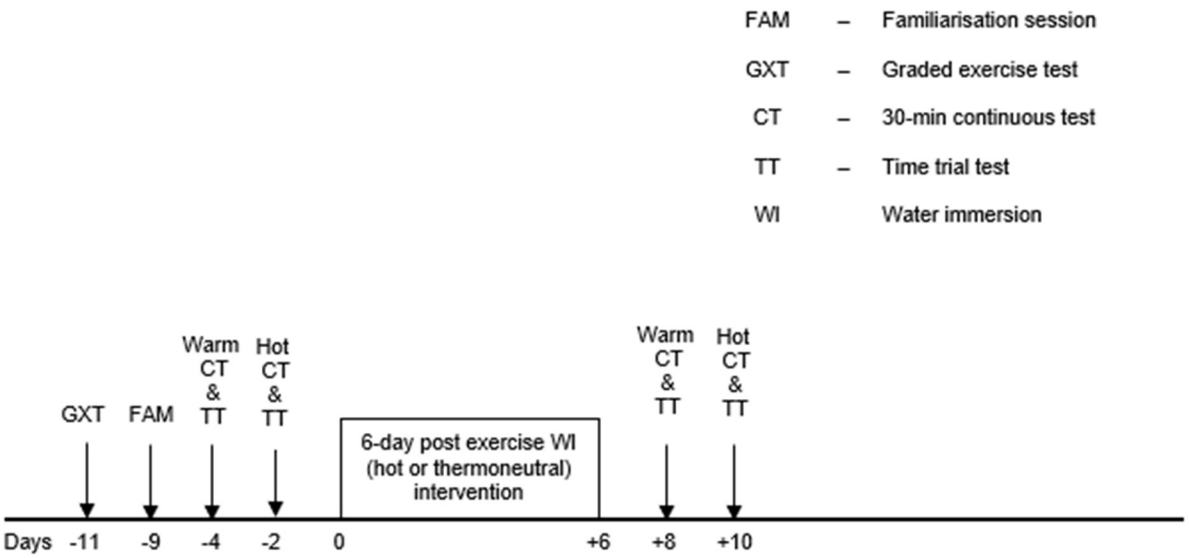

Figure 3.1. Study overview

3.2.3. Graded exercise test

A GXT was conducted on an electronically-braked cycle ergometer (Lode Excalibur Sport, v2.0, Groningen, The Netherlands) at ~19°C to determine each participant's $\dot{V}O_{2max}$ and peak power output. The test started with a 4-minute warm-up at 100 W, and the workload was subsequently increased by 25 W each minute until exhaustion. Oxygen consumption and carbon dioxide production were continuously measured with a gas analyser (Quark, Cosmed, Rome, Italy), and $\dot{V}O_{2max}$ was defined as the highest recorded reading over a 30-s period. Peak power output was defined as the power output of the last completed stage of the test. When the last exercise stage was not completed, peak power output was calculated according to the equation described by Kuipers et al., (1985):

Peak power output = W_{com} + (t/60 x 30)

Where W_{com} is the power output (watts) of the last completed stage; t is the time (in seconds) that the final, uncompleted exercise stage was sustained.

3.2.4. 30-minute continuous cycling test

This test served to quantify the potential physiological benefits that the intervention (i.e., post-exercise HWI) conferred during exercise in a warm and hot environment. One day before each continuous test, participants were provided with a packaged temperature pill (CorTemp, HQInc, Palmetto, FL, USA) and were instructed to swallow the pill at least four hours before reporting to the laboratory the following day. The temperature pill allowed us to monitor participants' body core temperature during the testing using a handheld digital monitor. Each continuous and time trial test was completed on the same day, as previously described (McIntyre et al., 2021). For each participant, the testing was completed at the same time of the day to avoid possible diurnal effects (Reilly et al., 1984). Participants were instructed to refrain from intensive or prolonged exercise for 72 hours (Rose Chrismas et al., 2017) and caffeine for 12 hours before each test. Participants were also asked to record their dietary intake for the 24 hours preceding the familiarisation trial and to repeat the same nutritional intake before each subsequent test. To ensure euhydration of participants on the day of the testing, they were recommended to drink 500 ml of water 2 hours before arriving at the laboratory. Pre-testing hydration status was measured via urine specific gravity (USG) using a handheld refractometer (URC-NE, Atago, Japan), and the hypohydration threshold was set at USG ≥ 1.020 (Sawka et al. 2007). Upon confirmation of euhydration, body mass was measured to the nearest 10g, after which participants were fitted with a heart rate monitor (Polar Electro OY, Kempele, Finland) to their upper torso. In addition, skin temperature sensors (Thermodata Pty, Ltd, QLD, Australia) were attached to the chest, arm, thigh, and calf, which allowed the calculation of mean skin temperature using the equation from Ramanathan (1964). Participants then moved into a heated room where the 30-minute continuous test was conducted. The room's air temperature/relative humidity was maintained at 27°C and 40% RH, or 35°C and 40% RH during the test in a warm or hot environment, respectively, and participants were fan-cooled during each test. After 10 minutes of seated rest, baseline heart rate, core temperature, skin temperature, thermal sensation (Toner et al., 1986) and perceived exertion (Borg, 1982) were recorded. Once these measurements were taken, participants cycled (Velotron, RacerMate Inc., Seattle, WA, USA) for 30 minutes at 40% peak power output (determined during the GXT) during the test in both environmental conditions. No fluid was allowed during this test because fluid ingestion may influence the temperature reading (Wilkinson et al.,

2008). Physiological and perceptual measures were recorded every 5 minutes during the test and at the end of the test. Gastrointestinal and heart rate data collected during the test were also used to calculate the physiological strain index by applying the equation described by Takaishis et al., (2002). Upon completing the test, participants removed the heart rate monitor and skin temperature sensors and then towel-dried before their body weight was re-measured. Sweat rate was determined by the difference in body weight measured before and after the test, but no corrections were made for respiratory fluid loss and weight loss due to fuel oxidation, as these were likely negligible (Baker et al., 2009; Maughan et al., 2007). Subsequently, participants were given half an hour to relax at room temperature (~20°C). During the recovery period, participants consumed an amount of cold (~7°C) sports drink (Powerade®, The Coca-Cola Company, USA) equal to 100% of their body mass loss during the test.

3.2.5. 20 km time trial test

The time trial test was used to assess the effects of the intervention on endurance exercise performance in a warm (27°C, 40% RH) and a hot (35°C, 40% RH) environment. This test was preceded by a warm-up consisting of cycling for 5 minutes at an intensity corresponding to 40% peak power output, which was determined during the GXT. Participants were instructed to ride the test as fast as possible. In line with the research methodology of previous heat acclimation studies in cyclists (Keiser et al., 2015; Racinais et al., 2015b), during the test, participants had access to power output, speed, heart rate, and distance data. They were also allowed to adjust the power output throughout the test and to drink *ad libitum*. The time to complete the test, average power, and average heart rate were recorded. Participants were fan-cooled during the test. Participants' sweating-induced fluid loss during the time trial was determined by measuring their body weight before and after the test, and post-testing, they were provided with an amount of sports drink (Powerade®, The Coca-Cola Company, USA) equal to 150% of their body mass loss during the test.

3.2.6. Wellness questionnaire

Upon arrival at the laboratory for testing and before assessing USG the participants also completed a validated wellness questionnaire (Nässi et al., 2017) including perceived sleep quality (1 = very poor, 5 = very good); muscle soreness (1 = minimal, 10 = severe); fatigue (1 = not at all, 10 = very fatigued); general health (1 = poor, 10 =

excellent); mood (1 = poor, 10 = excellent); physical readiness to exercise (1 = not at all, 10 = completely); and mental readiness to exercise (1 = not at all, 10 = completely).

3.2.7. Training/water immersion sessions

Before each training session, participants' body weight was measured to the nearest 10g. All training sessions were performed on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA, USA), and each session consisted of cycling for 40 minutes at 50% of peak power output in a cool environment (14°C, 40% RH). In addition, participants were fan-cooled during the training sessions. After each training session, participants were given 2 minutes to change out of their cycling gear. Thereafter, they were immersed up to their neck in either hot (40°C; HWI group) or thermoneutral (34°C; CON group) water for 40 minutes. The 34°C water temperature for the control condition was chosen based on observation (Zurawlew et al., 2016) that this water temperature has no effect on body core temperature. During the first and the last HWI session, body core temperature was monitored by temperature pill (CorTemp, HQInc, Palmetto, FL, USA) in the same manner as during the 30-minute continuous test. This also allowed us to accurately assess participants' internal heat load at the beginning and the end of the HWI intervention. The gastrointestinal temperature was also measured during the first session in the CON group to confirm that immersion in 34°C water did not impose thermal stress. A tympanic thermometer was used to measure body temperature every 5 minutes during the 2nd, 3rd, 4th, and 5th, and HWI sessions. Although a tympanic thermometer does not measure core temperature with the same precision as temperature pills, the device is accurate enough to monitor body temperature during heat exposure and prevent an increase in body temperature to dangerous levels (Otani et al., 2020). Water was available *ad libitum* during exercise but not during immersions. Following the immersions, participants' body mass was measured again. Since participants from the HWI group lost a considerable amount of fluid via sweating during the HWI sessions, they were provided with a volume of electrolyte drink (Hydralyte Sports®, Care Pharmaceuticals Pty Ltd, NSW, Australia) equal to 150% of their body mass loss during the sessions, as recommended by the current hydration guidelines (McDermott et al., 2017). Participants were also asked to avoid any prolonged heat exposures (e.g., sauna or spa bathing) outside the laboratory during the study.

3.2.8. Statistical analysis

Statistical analyses were conducted using GraphPad Prism (version 9.5.1, GraphPad Software Inc., La Jolla, CA, USA). Initially, all the data were tested for normality of distribution using the Shapiro-Wilk test and data that did not pass normality testing were log-transformed before analyses. Paired *t*-test was used to compare mean demographic characteristics and fitness levels between the groups. A two-way repeated measures ANOVA was used for all other analyses. Where significant main effects or interactions occurred, a Bonferroni post-hoc analysis was applied. A statistical significance level was set at $p < 0.05$. In addition, the between-group effect size was calculated and reported for all primary outcomes to assist interpretation of the results. Effect sizes were calculated according to Dankel and Loenneke, (2021) and interpreted using the following thresholds (Batterham and Hopkins, 2006): trivial (<0.2), small (0.2-0.59), moderate (0.6-1.19), large (1.2-1.99), and very large (>2.0). Unless otherwise stated, descriptive data are presented as mean ± standard deviation (SD) and 95% confidence intervals (CI).

3.3. Results

3.3.1. Intervention sessions

All sixteen participants completed the six-day intervention, and at each visit, all HWI participants completed the full 40-minute immersion in 40°C water. Figure 3.2 shows the kinetics of gastrointestinal temperature in both groups during the first session. However, the results of the CON group are based on $n = 7$ due to the inability to obtain the reading of the telemetric pill in one of the participants in this group. At rest, there was no statistically significant difference in gastrointestinal temperature between the HWI (37.38 ± 0.29°C) and CON (37.06 ± 0.29°C) groups. Likewise, cycling for 40 minutes at 14°C induced a similar ($p > 0.05$) increase in gastrointestinal temperature in both groups (HWI: 0.90 ± 0.23°C; CON: 1.07 ± 0.25°C). Post-exercise immersion in 34°C water for 40 minutes allowed the return of gastrointestinal temperature to pre-exercise resting level. In contrast, after the initial drop, the gastrointestinal temperature in the HWI group increased progressively during the 40-minute immersion period, peaking at 39.47 ± 0.14°C. Comparison of gastrointestinal temperature responses to the first and sixth post-exercise hot water immersion sessions showed no significant between-session differences ($F_{(1, 14)} = 2.61$, $p = 0.12$) (Figure 3.3), indicating that the adaptive stimulus provided by hot water immersion was not significantly changed over time. Yet, the overall mean gastrointestinal temperature during the sixth session was 0.23 ± 0.07°C lower than that of the first session, very likely because of an adaptive response to repeated high hyperthermia. Although the hot water immersion intervention had no significant effect on the gastrointestinal temperature, a significant effect was observed for the sweat rate ($p = 0.01$) and exercising heart rate ($p = 0.01$). Indeed, the participants from the HWI group sweated significantly more during the sixth (1.78 ± 0.34 $L \cdot h^{-1}$) than the first (1.65 ± 0.41 $L \cdot h^{-1}$) sessions and their average heart rate during exercise was significantly reduced from 136.5 ± 15.49 $beats \cdot min^{-1}$ on day one to 132.3 ± 14.54 $beats \cdot min^{-1}$ on day six. On the other hand, neither sweat rate ($p > 0.99$) nor exercising heart rate ($p = 0.16$) were significantly changed over time in the CON group, though a significant between-group difference was observed only in the former ($F_{(1, 14)} = 71.06$, $p < 0.01$) but not the latter variable ($F_{(1, 14)} = 0.00$, $p = 0.97$).

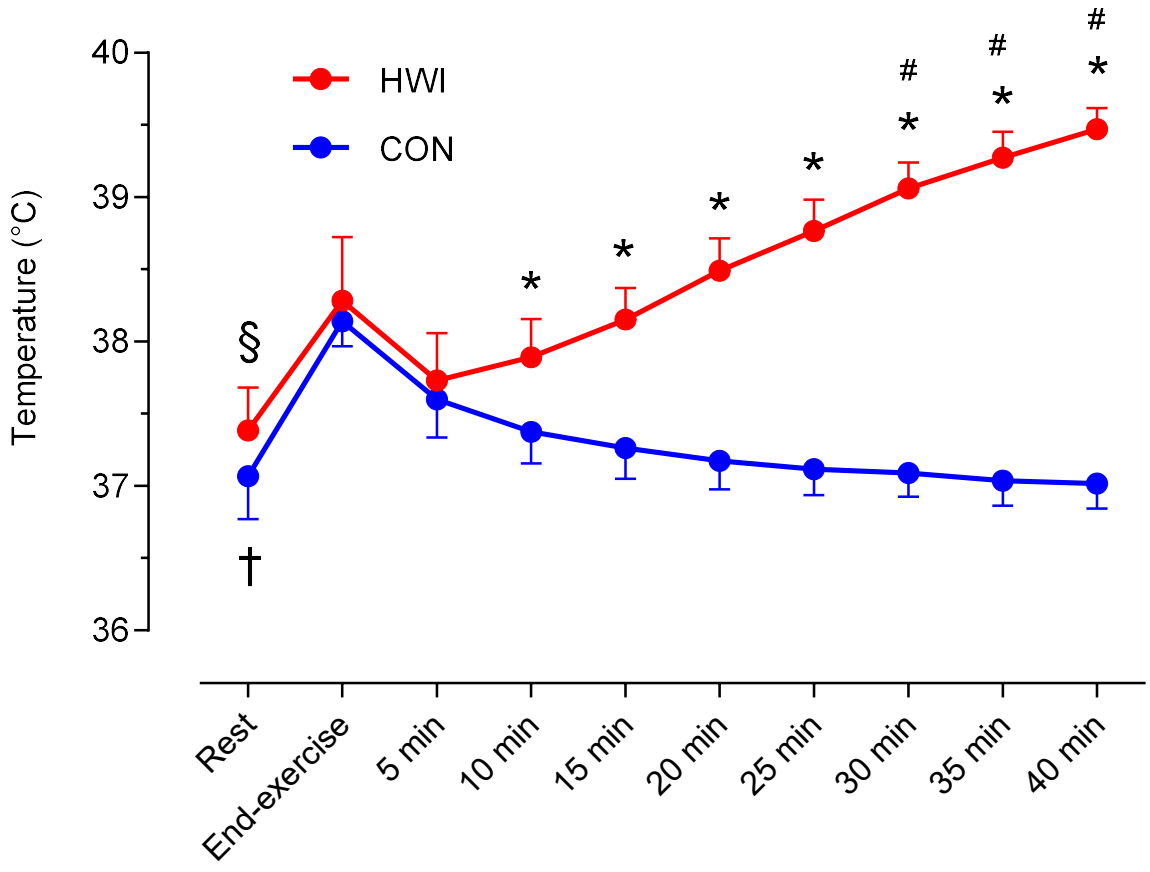

Figure 3.2. Gastrointestinal temperature measured during the first session in both groups. Measurements were taken at rest, after exercise, and at 5-minute intervals throughout the post-exercise water immersion period. Data expressed as mean ± SD.

*A significant between-group difference, $p < 0.05$.

#Significantly different from the end-exercise time point, $p < 0.05$.

§Significantly different from the end-exercise and 15 to 40-minute time points, $p < 0.05$.

†Significantly different from the end-exercise and 5-minute time points, $p < 0.05$.

Note: Significant within-group differences between certain time points during the immersion period were also observed, but those findings are considered irrelevant, so they are not indicated in the figure.

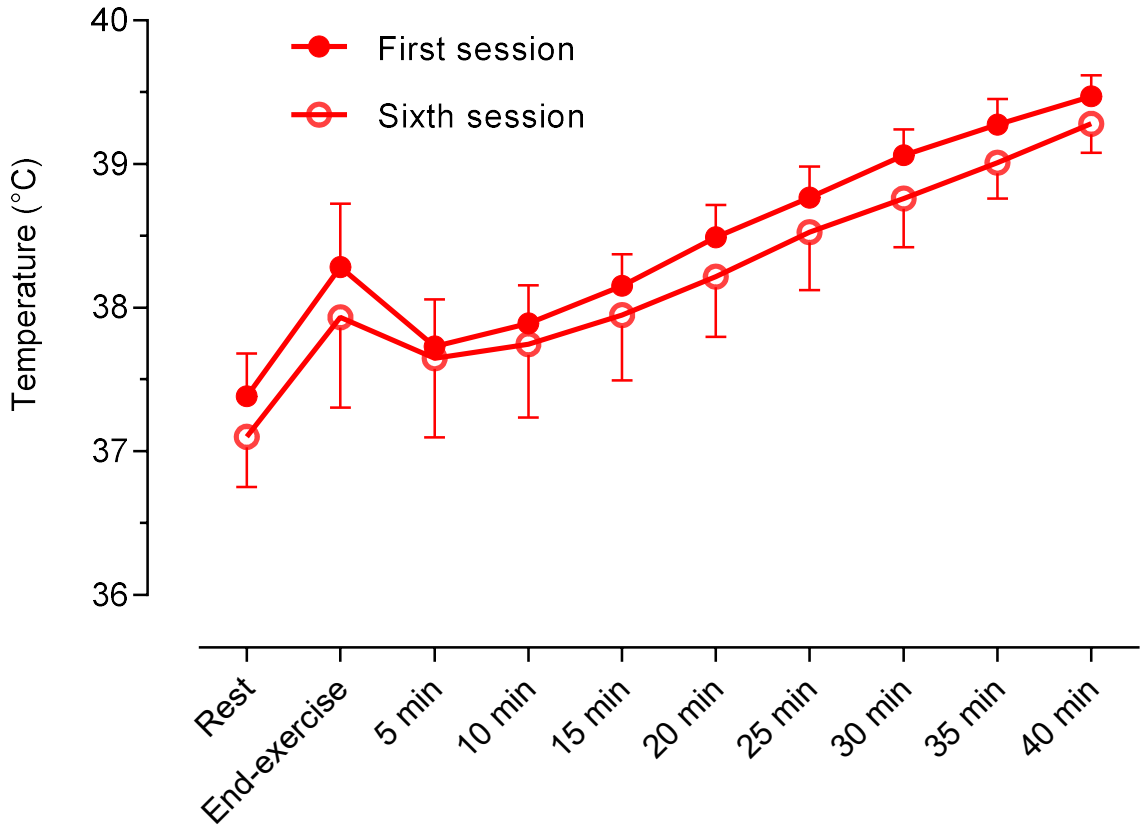

Figure 3.3. Gastrointestinal temperature measured in the HWI group during the first and sixth sessions. Measurements were taken at rest, after exercise, and at 5-minute intervals throughout the post-exercise hot water immersion period. Data expressed as mean ± SD.

3.3.2. 30-minute continuous tests at 27°C and 35°C

The results from the wellness questionnaire and urine samples collected before each testing session are presented in Tables 3.2 and 3.3, respectively (Appendix). There was no statistically significant between- or within-group difference in self-reported wellness data (all $p > 0.05$). A time x group interaction was found for urine specific gravity, as indicated in Table 2. Post-hoc analysis revealed that this was a result of a statistically significant difference in urine specific gravity between the third and fourth time points within the HWI group, between the third time point in the HWI group and the same time point in the CON group, and between the second and fourth time points within the CON group. Nonetheless, those results can be disregarded since all the values of urine specific gravity were below the hypohydration threshold of 1.020.

Table 3.4 summarises the physiological and perceptual responses during the 30-minute continuous test at 27°C before and after the intervention in the HWI and CON groups. Examination of the gastrointestinal temperature data showed neither significant main effect of time ($F_{(1, 14)}$ = 0.16, p = 0.68), group ($F_{(1, 14)}$ = 0.72, p = 0.40), or time × group interaction ($F_{(1, 14)}$ = 0.12, p = 0.73) for resting nor significant main effect of time ($F_{(1, 14)}$ = 0.54, p = 0.47), group ($F_{(1, 14)}$ = 2.67, p = 0.12), or time × group interaction ($F_{(1, 14)}$ = 0.03, p = 0.86) for peak values of this variable. Although no significant main effect of time ($F_{(1, 14)}$ = 2.97, p = 0.10), group ($F_{(1, 14)}$ = 0.50, p = 0.49), or time × group interaction ($F_{(1, 14)}$ = 1.94, p = 0.18) was found for resting heart rate, descriptive statistics indicated a meaningful reduction in mean resting heart rate (5.2 beats·min^{-1}) in the HWI group post-intervention. In addition, the between-group effect size (0.67) revealed a moderate benefit of the HWI group vs. the CON group. In contrast to resting heart rate, peak heart rate showed a significant main effect for time ($F_{(1, 14)}$ = 7.36, p = 0.01), and the post-hoc analysis revealed that the HWI group experienced a statistically significant reduction in mean peak heart rate from pre- to post-intervention by 7.37 ± 5.21 beats·min^{-1} (p = 0.03). However, there was no significant between-group difference for peak heart rate ($F_{(1, 14)}$ = 0.04, p = 0.82). Furthermore, no significant main effect of time ($F_{(1, 14)}$ = 0.15, p = 0.70), group ($F_{(1, 14)}$ = 1.42, p = 0.25), or time × group interaction ($F_{(1, 14)}$ = 0.02, p = 0.87) was found for resting skin temperature, nor a significant main effect of time ($F_{(1, 14)}$ = 0.12, p = 0.72), group ($F_{(1, 14)}$ = 0.25, p = 0.61), or time × group interaction ($F_{(1, 14)}$ = 2.43, p = 0.14) was found for peak skin temperature. Similarly, the physiological strain index was unaffected by either time ($F_{(1, 14)}$ = 2.22, p = 0.15) or group ($F_{(1, 14)}$ = 4.21, p = 0.05), and the same was the case for sweat rate, as evidenced by no significant main effect of time ($F_{(1, 14)}$ = 3.86, p = 0.06), group ($F_{(1, 14)}$ = 0.38, p = 0.54), or time × group interaction ($F_{(1, 14)}$ = 0.02, p = 0.88). Analyses of the resting thermal sensation data showed that the difference in this variable between the groups approached statistical significance ($F_{(1, 14)}$ = 3.94, p = 0.05), and the post-hoc testing revealed that the resting thermal sensation at post-intervention testing was significantly (p = 0.04) lower in the HWI group (3.87 ± 0.23 AU) versus the CON group (4.25 ± 0.37 AU). Peak thermal sensation, on the other hand, showed a main effect for time ($F_{(1, 14)}$ = 16.16, p < 0.01) and time × group interaction ($F_{(1, 14)}$ = 6.72, p = 0.02). The post-hoc comparisons revealed a statistically significant (p < 0.01) decrease in peak thermal sensation over time only in the HWI group. Peak rating of perceived exertion is another perceptual

variable that showed a significant main effect for time ($F_{(1, 14)}$ = 5.50, p = 0.03). The post-hoc analysis indicated a statistically significant (p = 0.02) decrease in peak rating of perceived exertion by 1.00 ± 0.75 AU from pre- to post-intervention in the HWI group. However, no significant effect of group ($F_{(1, 14)}$ = 0.45, p = 0.50) or group X time interaction ($F_{(1, 14)}$ = 3.33, p = 0.08) was found.

Table 3.4. Physiological and perceptual variables pre- and post-intervention during the 30-min continuous test at 27°C. Data expressed as mean ± SD (95%CI*).

	Pre-intervention		Post-intervention		Pre-post intervention change*			ANOVA's *p* values		
	HWI	CON	HWI	CON	HWI	CON	Between-group ES	Time	Group	T x G
Rest G_T (°C)	37.34 ± 0.23	37.19 ± 0.25	37.26 ± 0.42	37.19 ± 0.36	-0.08 ± 0.47 (-0.48, 0.31)	-0.00 ± 0.37 (-0.31, 0.30)	0.18	0.68	0.40	0.73
Peak G_T (°C)	38.24 ± 0.20	37.98 ± 0.46	38.30 ± 0.34	38.02 ± 0.37	0.06 ± 0.31 (-0.20, 0.32)	0.03 ± 022 (-0.1, 0.22)	0.11	0.47	0.12	0.86
Rest HR (beats·min^{-1})	65.50 ± 4.59	65.00 ± 7.05	59.63 ± 9.65	64.38 ± 6.09	-5.20 ± 8.20 (-12.0, 1.65)	-0.62 ± 7.05 (-6.51, 5.26)	0.67	0.10	0.49	0.18
Peak HR (beats·min^{-1})	144 .3 ± 14.01	140.6 ± 12.25	136.9 ± 13.56$^{\#}$	137.8 ± 11.74	-7.37 ± 5.21 (-11.7, -2.99)	-2.87 ± 9.31 (-10.6, 4.90)	0.58	0.01	0.82	0.25
Rest S_T (°C)	31.83 ± 0.73	31.35 ± 1.15	31.74 ± 0.77	31.31 ± 0.57	-0.09 ± 0.52 (-0.53, 0.35)	-0.03 ± 0.75 (-0.67, 0.59)	0.09	0.70	0.25	0.87
Peak S_T (°C)	33.15 ± 0.64	33.01 ± 0.56	32.83 ± 0.63	33.21 ± 0.48	-0.32 ± 0.76 (-0.96, 0.32)	0.20 ± 0.54 (-0.25, 0.65)	0.17	0.72	0.61	0.14
PSI (AU)	6.11 ± 0.19	5.71 ± 0.61	6.24 ± 0.36	5.89 ± 0.41	0.13 ± 0.35 (-0.16, 0.43)	0.14 ± 0.39 (-0.18, 0.47)	-0.02	0.15	0.05	0.95
Sweat rate (L·h^{-1})	0.46 ± 0.07	0.48 ± 0.08	0.49 ± 0.66	0.51 ± 0.06	0.03 ± 0.07 (-0.02, 0.08)	0.03 ± 0.06 (-0.01, 0.08)	-0.07	0.06	0.54	0.88
Rest TS (AU)	4.12 ± 0.23	4.18 ± 0.37	3.87 ± 0.23	4.25 ± 0.37	-0.25 ± 0.37 (-0.56, 0.06)	0.06 ± 0.62 (-0.45, 0.58)	0.59	0.40	0.05	0.16
Peak TS (AU)	5.75 ± 0.46	5.50 ± 0.53	5.18 ± 0.65$^{\#}$	5.37 ± 0.44	-0.56 ± 0.41 (-0.91, -0.21)	-0.12 ± 0.32 (-0.31, 0.06)	1.12	0.00	0.90	0.02
Peak RPE (AU)	11.75 ± 2.12	12.00 ± 2.50	10.75 ± 1.90$^{\#}$	11.88 ± 1.72	-1.00 ± 0.75 (-1.63, -0.36)	-0.12 ± 1.12 (-1.06, 0.81)	0.85	0.03	0.50	0.08

Abbreviations: G_T – gastrointestinal temperature, HR – heart rate, S_T – skin temperature, PSI – physiological strain index, TS – thermal sensation, RPE – rating of perceived exertion, AU – arbitrary units, ES – effect size, T x G – time and group interaction. $^{\#}$A significant within-group difference, $p < 0.05$.

Table 3.5 summarises the physiological and perceptual responses during the 30-minute continuous test at 35°C before and after the intervention in the HWI and CON groups. The gastrointestinal temperatures during testing at 35°C behaved similarly to testing at 27°C. Indeed, no significant main effect of time ($F_{(1, 14)}$ = 3.10, p = 0.09), group ($F_{(1, 14)}$ = 1.19, p = 0.30), or time X group interaction ($F_{(1, 14)}$ = 0.31, p = 0.58) was found for resting gastrointestinal temperature, nor a significant main effect of time ($F_{(1, 14)}$ = 0.03, p = 0.85), group ($F_{(1, 14)}$ = 1.02, p = 0.32), or time X group interaction ($F_{(1, 14)}$ = 0.01, p = 0.91) was found for peak gastrointestinal temperature. Somewhat surprisingly, the HWI group did not display the same beneficial changes in resting and peak heart rates during testing at 35°C as at 27°C. From pre to post-intervention, the mean resting and peak heart rates in the HWI group decreased by only 3.50 beats·min^{-1} and 2.12 beats·min^{-1}, respectively. Statistically, neither significant main effect of time ($F_{(1, 14)}$ = 2.62, p = 0.12), group ($F_{(1, 14)}$ = 0.20, p = 0.65), or time X group interaction ($F_{(1, 14)}$ = 0.15, p = 0.69) was found for resting, nor a significant main effect of time ($F_{(1, 14)}$ = 0.05, p = 0.81), group ($F_{(1, 14)}$ = 0.00, p = 0.98), or time X group interaction ($F_{(1, 14)}$ = 1.13, p = 0.30) was found for peak heart rate. Furthermore, resting skin temperature showed no significant main effect for time ($F_{(1, 14)}$ = 0.18, p = 0.67), group ($F_{(1, 14)}$ = 2.14, p = 0.16), or time X group interaction ($F_{(1, 14)}$ = 0.42, p = 0.52). In contrast, a significant time X group interaction ($F_{(1, 14)}$ = 5.76, p = 0.03) was observed for peak skin temperature, resulting from a significantly (p = 0.03) lower peak skin temperature in the HWI group (35.34 ± 0.40°C) compared to the CON group (35.69 ± 0.14°C) during the post-intervention testing. The same variable showed no significant main effect for time ($F_{(1, 14)}$ = 1.06, p = 0.31) or group ($F_{(1, 14)}$ = 1.58, p = 0.22), but the effect size analysis indicated a moderate benefit of the HWI group vs. the CON group on peak skin temperature (effect size = 1.02). The response of the physiological strain index to testing at 35°C mirrored that during testing at 27°C, showing no significant main effect of time ($F_{(1, 14)}$ = 0.00, p = 0.94), group ($F_{(1, 14)}$ = 0.72, p = 0.40), or time X group interaction ($F_{(1, 14)}$ = 0.00, p = 0.98). The same also applies to sweat rate, given that no significant main effect of time ($F_{(1, 14)}$ = 0.75, p = 0.40), group ($F_{(1, 14)}$ = 3.98, p = 0.06), or time X group interaction ($F_{(1, 14)}$ = 0.00, p = 0.94) was observed for this variable. A significant main effect of time ($F_{(1, 14)}$ = 5.03, p = 0.04) but no group ($F_{(1, 14)}$ = 3.15, p = 0.09) or time X group interaction ($F_{(1, 14)}$ = 1.46, p = 0.24) was found for resting thermal sensation. However, the post-hoc analysis of the time effect identified only a tendency (p = 0.05) for resting thermal sensation to decrease in the HWI group. There was also

Table 3.5. Physiological and perceptual variables pre- and post-intervention during the 30-min continuous test at 35°C. Data expressed as mean ± SD (95%CI*).

	Pre-intervention		Post-intervention		Pre-post intervention change*			ANOVA's *p* values		
	HWI	CON	HWI	CON	HWI	CON	Between-group ES	Time	Group	T x G
Rest G_T (°C)	37.28 ± 0.25	37.12 ± 0.24	37.15 ± 0.30	37.05 ± 0.26	-0.13 ± 0.24 (-0.34, 0.07)	-0.06 ± 0.20 (-0.24, 0.10)	0.28	0.09	0.30	0.58
Peak G_T (°C)	38.23 ± 0.30	38.13 ± 0.25	38.22 ± 0.33	38.10 ± 027	-0.00 ± 0.48 (-0.40, 0.39)	-0.02 ± 0.26 (-0.24, 0.19)	-0.05	0.85	0.32	0.91
Rest HR (beats·min^{-1})	67.75 ± 3.80	68.25 ± 6.90	64.25 ± 8.86	66.13 ± 4.32	-3.50 ± 7.57 (-9.83, 2.83)	-2.12 ± 6.24 (-7.34, 3.09)	0.20	0.12	0.65	0.69
Peak HR (beats·min^{-1})	148.8 ± 13.35	145.9 ± 13.96	146.6 ± 16.59	149.3 ± 9.86	-2.12 ± 9.79 (-10.3, 6.05)	3.37 ± 10.8 (-5.67, 12.4)	0.53	0.81	0.98	0.30
Rest S_T (°C)	34.12 ± 0.49	33.91 ± 0.43	34.29 ± 0.62	33.88 ± 0.54	0.17 ± 0.60 (-0.33, 0.67)	-0.03 ± 0.65 (-0.58, 0.51)	0.22	0.67	0.16	0.52
Peak S_T (°C)	35.65 ± 0.17	35.57 ± 0.28	35.34 ± 0.40	35.69 ± 0.14	-0.30 ± 0.35 (-0.60, -0.00)	0.12 ± 0.35 (-0.17, 0.42)	1.02	0.31	0.22	0.03
PSI (AU)	6.03 ± 0.60	5.88 ± 0.51	6.02 ± 0.48	5.86 ± 0.38	-0.00 ± 0.83 (-0.70, 0.69)	0.71 ± 2.11 (-1.05, 2.47)	0.00	0.94	0.40	0.98
Sweat rate (L·h^{-1})	0.68 ± 0.10	0.57 ± 0.11	0.70 ± 0.12	0.59 ± 0.13	0.02 ± 0.13 (-0.08, 0.13)	0.02 ± 0.08 (-0.04, 0.10)	-0.03	0.40	0.06	0.94
Rest TS (AU)	5.66 ± 0.53	5.87 ± 0.51	5.06 ± 0.49	5.68 ± 0.75	-0.62 ± 0.44 (-0.99, -0.25)	-0.56 ± 0.72 (-1.72, 0.04)	0.75	0.04	0.09	0.24
Peak TS (AU)	6.87 ± 0.44	6.75 ± 0.46	6.37 ± 0.44$^{\#}$	6.62 ± 0.44	-0.50 ± 0.53 (-0.94, -0.05)	-0.12 ± 0.51 (-0.55, 0.37)	0.70	0.03	0.73	0.17
Peak RPE (AU)	12.50 ± 2.33	13.00 ± 2.50	10.88 ± 2.41$^{\#}$	13.00 ± 2.33	-1.62 ± 1.06 (-2.51, -0.73)	0.00 ± 1.85 (-1.54, 1.54)	0.96	0.04	0.26	0.04

Abbreviations: G_T – gastrointestinal temperature, HR – heart rate, S_T – skin temperature, PSI – physiological strain index, TS – thermal sensation, RPE – rating of perceived exertion, AU – arbitrary units, ES – effect size, T X G – time and group interaction. $^{\#}$A significant within-group difference, $p < 0.05$.

a moderated effect size (0.75) in favour of the HWI group. Similar to resting thermal sensation, a significant main effect of time ($F_{(1, 14)}$ = 5.03, p = 0.04) but not group ($F_{(1, 14)}$ = 3.15, p = 0.09) or time X group interaction ($F_{(1, 14)}$ = 1.46, p = 0.24) was observed for peak thermal sensation. The post-hoc analysis revealed a statistically significant (p = 0.03) decrease in peak thermal sensation by 0.50 ± 0.53 AU from pre- to post-intervention in the HWI group. Finally, the peak rating of perceived exertion demonstrated a statistically significant difference over time ($F_{(1, 14)}$ = 4.63, p = 0.04) and time X group interaction ($F_{(1, 14)}$ = 4.63, p = 0.04). Follow-up analysis indicated that post-intervention, the peak rating of perceived exertion was significantly reduced in the HWI group (-1.62 ± 1.06 AU, p = 0.01) but not in the CON group (0.00 ± 1.85 AU).

3.3.3. 20 km time trial tests at 27°C and 35°C

A significant main effect of time ($F_{(1, 14)}$ = 5.66, p = 0.03) was observed for the average power output maintained during the 20 km time trial at 27°C. Compared to the initial time trial test, power output during the post-intervention test was increased by 10 ± 11.40 W (95 CI: 0.46, 19.53) in the HWI group (Pre: 283.3 ± 45.46 W vs. Post: 293.3 ± 45.47 W) and by 5.15 ± 13.89 W (95 CI: -6.48, 16.74) in the CON group (Pre: 266.9 ± 61.86 W vs. Post: 272.0 ± 49.82 W) (Figure 3.4. A). However, these increases were not statistically significant (p = 0.08, p = 0.54, respectively), nor was the between-group difference statistically significant ($F_{(1, 14)}$ = 0.55, p = 0.47). The between-group effect size (0.38) indicated a small benefit of the HWI vs. the CON group. A significant main effect of time ($F_{(1, 14)}$ = 5.66, p = 0.03) was also observed for the average completion time of the 20 km time trial at 27°C. Follow-up analysis revealed that that effect resulted from a non-significant improvement in the time trial performance by 1.07 ± 2.19% (CI: -2.90, 0.76) in the HWI group (Pre: 31.62 ± 2.09 minutes vs. Post: 31.11 ± 1.87 minutes, p = 0.06) and by 1.01 ± 1.70% (CI: -2.44, 0.40) in the CON group (Pre: 32.53 ± 2.74 minutes vs. Post: 32.13 ± 2.21 minutes, p = 0.18) (Figure 3.4. B). Again, the between-group difference was not statistically significant ($F_{(1, 14)}$ = 0.75, p = 0.39), but there was a small effect size (0.20) in favour of the HWI group.

Analyses of the power output data during the 20 km time trial test at 35°C showed no significant main effect of time ($F_{(1, 14)}$ = 3.12, p = 0.09), group ($F_{(1, 14)}$ = 0.47, p = 0.50), or time X group interaction ($F_{(1, 14)}$ = 1.53, p = 0.23). Yet, numerically, the average power output maintained during the time trail test increased over time by 10.63 ± 13.42

W (95 CI: -0.59, 21.84) in the HWI group (Pre: 265.1 ± 51.10 W vs. Post: 275.8 ± 48.76 W) and by only 1.87 ± 14.84 W (95 CI: -10.53, 14.28) in the CON group (Pre: 251.4 ± 56.35 W vs. Post: 253.3 ± 55.76 W) (Figure 3.5. A). Additionally, the effect size analysis revealed a moderate benefit of the HWI group vs. the CON group on power output (effect size = 0.60). Analyses of the time trial performance data revealed a significant main effect of time ($F_{(1, 14)}$ = 5.78, p = 0.03), but not group ($F_{(1, 14)}$ = 0.64, p = 0.43), or time X group interaction ($F_{(1, 14)}$ = 0.82, p = 0.38). Although the post-hoc analysis of the time effect did not reveal any statistically significant differences, it was observed that both groups completed the post-intervention time trial test faster than at baseline (Figure 3.5. B). However, the time to complete the test was reduced to a greater extent in the HWI group (-1.77 ± 1.93% [CI: -3.38, 0.15]) compared to the CON group (-0.73 ± 2.28% [CI: -2.64, 1.16]). In the HWI group, the finish time of the time trial was reduced from 32.46 ± 2.43 minutes at baseline to 31.87 ± 2.24 minutes post-intervention (p = 0.06), whereas the corresponding times in the CON group were 33.30 ± 2.77 minutes and 33.03 ± 2.56 minutes (p = 0.61). Finally, there was a small effect size (0.46) in favour of the HWI group.

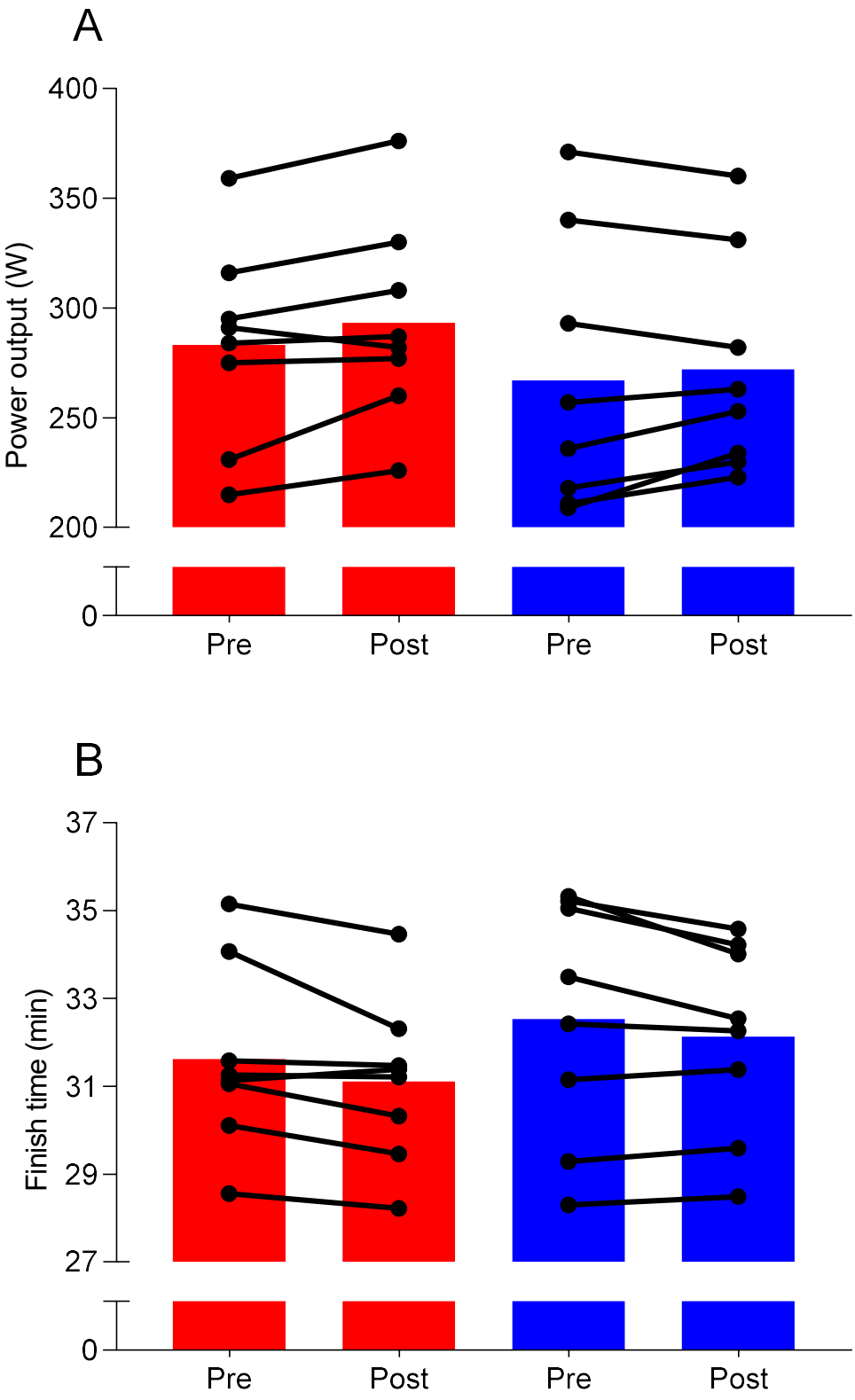

Figure 3.4. Individual data points (black lines) and mean values (bars: - HWI, - CON) for power output during the 20 km time trial test (A) and time to complete the 20 km time trial test (B) at 27°C, before (Pre) and after (Post) the intervention period.

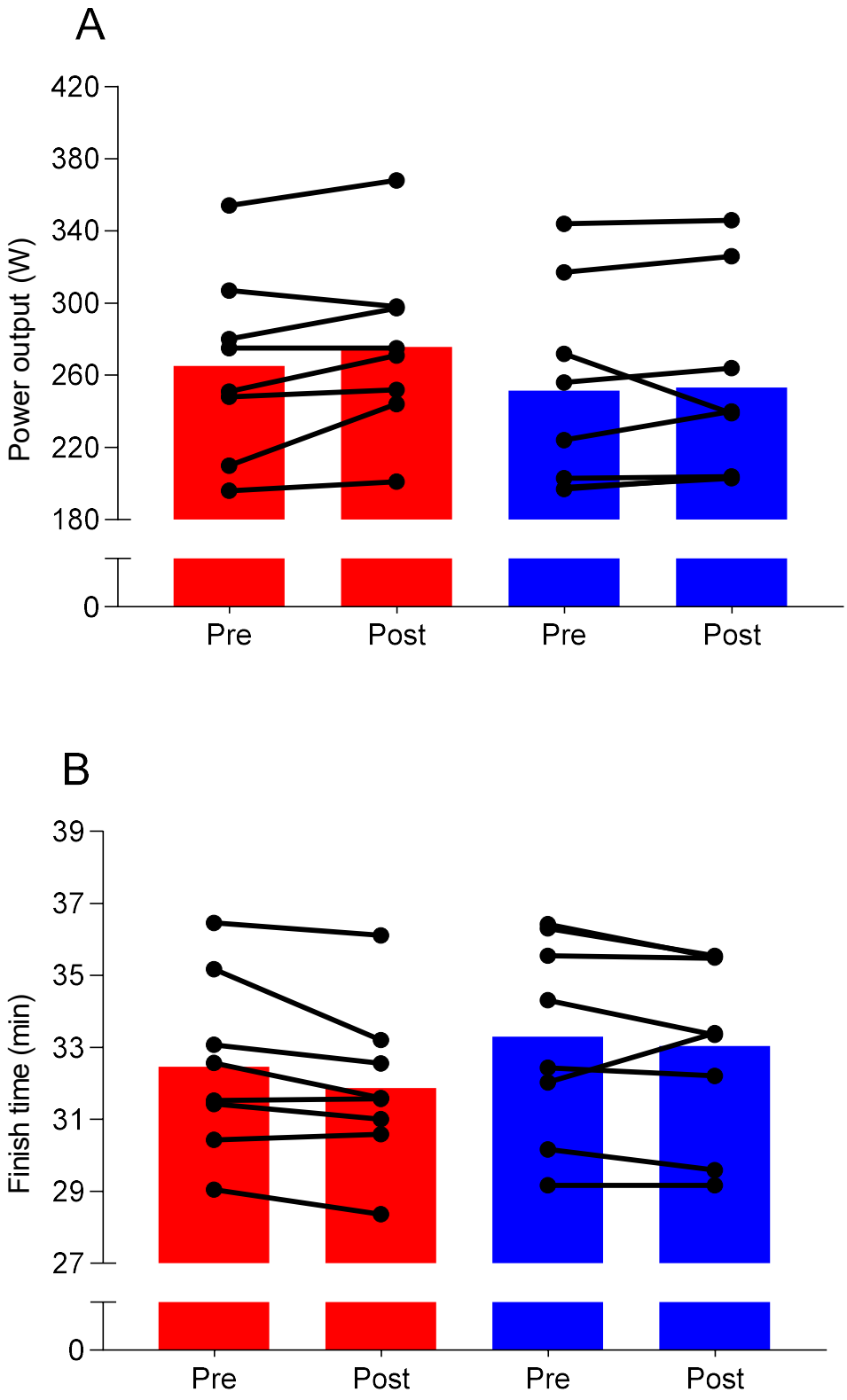

Figure 3.5. Individual data points (black lines) and mean values (bars: - HWI, - CON) for power output during the 20 km time trial test (A) and time to complete the 20 km time trial test (B) at 35°C, before (Pre) and after (Post) the intervention period.

3.4. Discussion

This study examined the effects of repeated post-exercise hot water immersion for six days on physiological and exercise performance outcomes in warm (27°C) and hot (35°C) environmental conditions in a group of trained but non-heat acclimated cyclists. The main findings are that the HWI group showed 1) significantly (p > 0.05) increased sweat rate and decreased exercising heart rate between the first and the sixth training sessions; 2) significantly (p > 0.05) decreased peak heart rate, peak thermal sensation, and rating of perceived exertion during the 30-minute continuous test at 27°C; 3) significantly (p > 0.05) decrease peak thermal sensation and peak rating of perceived exertion during the 30-minute continuous test at 35°C; 4) only a tendency for improved 20 km time trial performance at 27°C (p = 0.06) and 35°C (p = 0.06).

The novel finding of the current study is that the six-day post-exercise hot water immersion intervention induced favourable changes in several variables measured during the 30-minute continuous test at 27°C. Compared to baseline testing, there was a non-significant but meaningful decrease in resting heart rate (-5.20 ± 8.20 beats·min^{-1}, p = 0.08), a statistically significant decrease in peak heart rate (-7.37 ± 5.21 beats·min^{-1}, p = 0.03), peak thermal sensation (-0.56 ± 0.41 AU, p < 0.01), and peak rating of perceived exertion (-1.00 ± 0.75 AU, p = 0.02) during post-intervention testing. These findings were not unexpected given that Zurawlew et al., (2016) reported significantly reduced heart rate (-7 beats·min^{-1}, p = 0.02) and rating of perceived exertion (~-1 AU, p = 0.01) at the end of a continuous running test in less thermally demanding conditions (18°C) than in the current study following post-exercise hot water intervention. However, Zurawlew et al., (2016) also observed a significant decrease in rectal (-0.28°C, p < 0.01) and skin (-1°C, p < 0.01) temperatures at the end of the same test in the HWI group. Although a direct comparison between the current and Zurawlew's study is difficult due to the considerable difference in environmental conditions under which the testing was performed, the inconsistency in the findings could be attributed to the training status of participants. The current study was conducted on well-trained endurance individuals, whereas Zurawlew et al., (2016) recruited physically active individuals in their study, and the former population is considered to have less room for heat adaptations. Interestingly, thermal sensation after the continuous test at 18°C in Zurawlew's study was unaffected (p > 0.05) by hot water immersion intervention, probably because the testing environment was not

thermally challenging. The physiological mechanism behind reduced resting and peak heart rate during testing at 27°C following intervention in the HWI could have been increased stroke volume brought about by expanded plasma volume. Indeed, increased plasma volume is a typical heat acclimation outcome (Kissling et al., 2020) and is commonly implicated in enhanced cardiovascular stability following heat acclimation (Périard et al., 2016). Nonetheless, as plasma volume was not assessed in the current study (due to technical issues), the notion that expansion of plasma volume could have induced a reduction in heart rate via increased stroke volume is purely speculative. Regardless of the mechanism underlying reduced cardiovascular strain, this physiological change and a reduced peak thermal sensation likely led to reduced peak rating of perceived exertion during the post-intervention testing in the HWI group. It should also be stressed that the absence of a significant between-group difference in peak heart rate and peak rating of perceived exertion during post-intervention testing at 27°C lessens the impact of the findings associated with the effects of hot water immersion on those variables.

Despite a significantly ($p < 0.05$) reduced cardiovascular strain, peak thermal sensation, and peak rating of perceived exertion from pre- to post-intervention testing at 27°C in the HWI group, there was only a trend ($p = 0.06$) of improved 20 km time trial performance at 27°C following hot water immersion intervention. The study upon which the idea to investigate the latter was based found a 2.6% improvement ($p < 0.05$) in time trial performance at 27°C after applying a pre-cooling vest for 20 minutes before the start of the nine-minute warm-up for the time trial (Faulkner et al., 2019). Compared to the control condition, the pre-cooling intervention resulted in a significantly ($p < 0.05$) lower skin temperature during the warm-up and first 20% of the time trial. The pre-cooling also led to a significant ($p < 0.05$) reduction in thermal sensation, though the effect did not last beyond the warm-up phase. Since pre-cooling had no significant effects on either gastrointestinal temperature or heart rate during the time trial, the researchers suggested that the ergogenic effects of pre-cooling were driven by the alterations in skin temperature and thermal sensation (Faulkner et al., 2019). Given that repeated hot water immersion reduces skin temperature and thermal sensation, at least in a hot environment (Greenfield et al., 2021; Waldock et al., 2021; Zurawlew et al., 2016), it was expected that the six-day post-exercise hot water immersion protocol would lead to favourable alterations in both variables and, as a

result, significantly improve time trial performance at 27°C. A question that remains open in this study is whether the time trial performance would have been significantly improved in the HWI group if their peak skin temperature during exercise at 27°C was significantly reduced over time. One may also assume that the lack of significant improvement in the time trial performance in the HWI group was due to a type II error. However, even if that was the case, the percent (-1.07 ± 2.19%) change in the time to complete the test from pre to post-intervention suggests that hot water immersion intervention had no meaningful effects on endurance permeance knowing that the test used in the present study has a coefficient of variation of 0.6% when applied to well-trained cyclists (Zavorsky et al., 2007). Zurawlew et al., (2016) reported that a six-day post-exercise hot water immersion intervention did not significantly improve time trial performance in thermoneutral conditions (18°C), and the findings from this study showed that the intervention is not ergogenic for endurance performance in warm conditions. Nonetheless, the idea that heat acclimation might improve endurance performance in warm climates should not be dismissed.

Numerous studies to date have demonstrated that repeated hot water immersion can induce a range of heat adaptations (Allan and Wilson, 1971; Ashworth et al., 2023; Barry et al., 2020; Bonner et al., 1976; Brazaitis and Skurvydas, 2010; Brebner et al., 1961; Gass and Gass, 2001; Greenfield et al., 2021; Kissling et al., 2022; McIntyre et al., 2021; Shin et al., 2013; Waldock et al., 2021; Zurawlew et al., 2016, 2018a, 2018b, 2019) among which the most common ones relevant to athletes are reduced heart rate, core and skin temperatures, thermal sensation, and rating of perceived exertion during exercise in hot conditions (Ashworth et al., 2023; Bonner et al., 1976; Brebner et al., 1961; Greenfield et al., 2021; McIntyre et al., 2021; Zurawlew et al., 2016, 2018a, 2018b, 2019). However, except one (Zurawlew et al., 2018b), all other cited studies were conducted on either sedentary or physically/recreationally active participants. The only previous study in this area conducted on non-heat acclimated endurance athletes showed a significant change in several markers of heat acclimation during a 40-minute continuous running test at 65 $\dot{V}O_{2max}$ in a hot condition (33°C) performed before and after a six-day post-exercise hot water immersion intervention (Zurawlew et al., 2018b). Post-intervention, a significant decrease was found in resting rectal temperature (-0.17°C), rectal temperature at the onset of sweating (-0.22°C), end-exercise rectal temperature (-0.36°C), end-exercise skin temperature (-0.67°C), end-

exercise thermal sensation (-1 AU), end-exercise ratings of perceived exertion (-1 AU), and end-exercise physiological strain index (-1 AU). In contrast to those findings, the HWI group in the present study showed a significant reduction only in peak thermal sensation (-0.50 AU) and peak ratings of perceived exertion (-1.62 AU) during the 30-minute continuous test at 35°C following the intervention. It is surprising that peak heart rate was unchanged during the testing at 35°C given that there was a significant decrease in heart rate during exercise sessions at 14°C and testing at 27°C, inconsistency in the findings which is difficult to explain. It is also somewhat surprising that the end-exercise heart rate during the testing following heat acclimation in the former study (Zurawlew et al., 2018b) was unchanged, knowing that cardiovascular changes are the earliest adaptive response to chronic heat exposure (Armstrong and Maresh, 1991; Périard et al., 2015). Perhaps the expansion in plasma volume (4%) observed by Zurawlew et al., (2018b) in their endurance-trained participants was insufficient to induce a significant decrease in heart rate. Interestingly, the magnitude of reduction of end-exercise rectal temperature (-0.36°C) in the endurance-trained athletes following post-exercise water immersion intervention (Zurawlew et al., 2018b) was similar to that (-0.3°C, p = 0.01) reported by Garrett et al., (2012) in highly trained rowers following five 90-minute daily exercise sessions in the heat. Those findings suggest that the passive heat acclimation strategy originally introduced by Zurawlew et al., (2016) is equally effective as active heat acclimation in inducing thermoregulatory gains in endurance athletes. Unfortunately, the present study could not support the latter notion since no significant changes over time were observed in either resting or peak gastrointestinal temperatures in the HWI group. Collectively, the results from the 30-minute continuous test at 35°C revealed perceptual but not physiological benefits of hot water immersion intervention in well-trained cyclists during exercise in the heat.

The finding that the HWI group did not significantly improve endurance exercise performance in the heat (35°C) post-intervention is in line with the study by McIntyre et al., (2021) but in contrast with Zurawlew et al., (2016), who found significantly improved 5 km running time trial performance (4.9%, p = 0.01) in their HWI group consisting of non-heat acclimated physically active males. However, the reduction (1.77%) in the completion time of the 20 km time trial test from the first to second visits in the HWI group can be regarded as meaningful (Peiffer and Abbiss, 2011) even when

factoring in the coefficient of variation of the test (0.6%) (Zavorsky et al., 2007). Given that the time trial performance change over time in the CON group was only -0.73%, one could be confident that the performance gains in the HWI group were induced by the hot water immersion intervention and are likely attributable to reduced perceptual strain. It should also be mentioned that when the same statistical test (one-tailed *t*-test) used by Zurawlew et al., (2016) was applied to the current time trial data sets, the change in the completion time of the test between the visits became significant in the HWI group (p = 0.02), whereas in the CON group remained non-significant (p = 0.17). Clearly, the magnitude of improvement of time trial performance reported by Zurawlew et al., (2016) is considerably greater than that in the current study, but it would be unreasonable to expect well-trained endurance individuals to show the same performance benefits from the six-day post-exercise hot water immersion intervention as physically active individuals. A considerably greater improvement in cycling time trial performance in the heat than in the present study was also reported in studies that employed active heat acclimation interventions. For instance, Lorenzo et al., (2010) trained 12 highly-trained ($\dot{V}O_{2max}$ = 66.9 ± 2.1 ml·kg^{-1}·min^{-1}) non-heat acclimated male cyclists for 10 consecutive days (90 minutes per session) at 40°C and found an 8% increase (p = 0.014) in total work completed after 1-hour time trial test at 38°C. In another study, 9 well-trained ($\dot{V}O_{2max}$ = 4.8 ± 0.2 L·min^{-1}) non-heat acclimated male cyclists performed a 43 km time trial test before and after two weeks of heat acclimation consisting of training in a naturally hot environment (34 ± 3°C and 18 ± 5% RH) for 13 ± 1 hour per week (Racinais et al., 2015b). Heat acclimation improved time trial performance by 18% at ~37°C (p < 0.01). Since the training status of the participants in the latter two studies and the current study was similar, the differences in time trial performance improvement could be attributed to the duration of heat exposure. Indeed, the total dose of heat stress (240 minutes) received by the current participants was 3.7 and 6.5 times lower than participants in the studies by Lorenzo et al., (2010) and Racinais et al., (2015b), respectively. Nonetheless, the change in time trial performance by 1.77% in the HWI group is large enough to be considered worthwhile for competitive road cyclists (Paton and Hopkins, 2006).

In conclusion, this is the first study to examine the effects of a six-day post-exercise head-out immersion in 40°C water on exercise performance outcomes in warm (27°C) and hot (35°C) environmental conditions in non-heat acclimated well-trained

endurance athletes. The HWI group showed significantly reduced cardiovascular and perceptual strain during moderate-intensity exercise at 27°C and significantly reduced perceptual strain during moderate-intensity exercise at 35°C. Furthermore, the HWI group showed only a tendency for improved 20 km time trial performance at 27°C (p = 0.06) and 35°C (p = 0.06). However, a 1.77% reduction in the finishing time of the time trial test in the heat is practically significant. Finally, these findings may not apply to female athletes since they do not respond in the same way to short-term heat acclimation as their male counterparts (Mee et al., 2015), which is a limitation of the study.

Chapter four: Acute and chronic effects of post-exercise hot water immersion on plasma cytokines, oxidative stress, and antioxidant capacity

Abstract

Background: Some previous studies showed that active heat acclimation induces chronic inflammation and oxidative stress. However, the impact of passive heat acclimation strategies such as post-exercise hot water immersion on plasma cytokines and prooxidant-antioxidant balance is unknown. Addressing those questions is relevant, given that chronically elevated inflammation and oxidative stress markers are associated with impaired exercise performance.

Objective: To determine the acute and chronic effects of exposure to post-exercise head-out immersion in 40°C water on plasma cytokine response, oxidative stress, and antioxidant capacity.

Methods: Fourteen well-trained male cyclists completed a six-day intervention involving daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot water immersion (HWI; n = 7) or thermoneutral water immersion (CON; n = 7) for 40 minutes. Ten mL of venous blood was taken before and after the first session to assess acute inflammatory responses, oxidative stress, and total antioxidant capacity. Resting venous blood was also sampled 48 hours after the last session to assess the chronic impact of post-exercise hot water immersion on inflammation, oxidative stress, and total antioxidant capacity. The inflammatory response was assessed by measuring the following cytokines: tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), IL-6, and IL-10. Oxidative stress was determined by measuring thiobarbituric acid-reactive substances (TBARS), whereas total antioxidant capacity (TAC) was measured as a sum of all antioxidants in the plasma.

Results: Acute post-exercise hot water immersion had no significant effects on any of the measured biomarkers (all $p > 0.05$). Acute post-exercise immersion in 34°C water significantly increased IL-6 ($p < 0.01$). Post-exercise hot water immersion over six consecutive days significantly increased resting plasma IL-1β concentration ($p < 0.01$).

Conclusion: This study suggests that chronic heat stress imposed by post-exercise hot water immersion induces an inflammatory response but not oxidative stress in well-trained male endurance athletes.

Keywords: well-trained endurance athletes, hot water immersion, cytokines, lipid peroxidation

4.1. Introduction

It is well-documented that exercise in the heat is associated with a significantly higher cardiovascular and thermoregulatory strain compared with exercise in thermoneutral conditions (Logan-Sprenger et al., 2012; Logan-Sprenger et al., 2013). However, it has also been shown that exercise in the heat may induce a significantly greater inflammatory response (Peake et al., 2008; Rhind et al., 2004; Starkie et al., 2005) and oxidative stress (McAnulty et al., 2005; Pilch et al., 2014; Sureda et al., 2015) than exercise in less thermally stressful conditions.

Although acute inflammation is vital in host defence and promotes tissue repair, chronic inflammation can exert harmful health effects. Significant cytokinaemia is induced when non-heat acclimated individuals perform intensive physical activity under heat stress. For instance, Starkie et al., (2005) measured change in plasma concentration of tumour necrosis factor (TNF)-α and interleukin (IL)-6 in response to a 90-minute cycling trial at 15°C versus 35°C in seven non-heat acclimated endurance athletes and found that only cycling in the heat induced production of these cytokines. One study also documented that heat acclimation may attenuate the cytokine response to subsequent exercise heat-stress exposure (Barberio et al., 2014). However, Hailes et al., (2011) show that heat acclimation can increase inflammation. Of the eighty inflammatory markers examined by the researchers (Hailes et al., 2011), resting values of eighteen were significantly increased following five consecutive days of exercise at 38°C until the rectal temperature reached 39.5°C (~40 minutes). The finding that heat acclimation may result in chronic inflammation is concerning because this condition may hinder athletic performance, impair the immune system, and contribute to the development of overtraining syndrome (Cheng et al., 2020; Smith, 2003).

Assuming that the increase in core temperature to 39.5°C during the heat acclimation sessions in the study by Hailes et al., (2011) was mainly responsible for increased inflammation, one may suggest that any heat acclimation intervention associated with a degree of hyperthermia similar to that in the former study may increase the risk of chronic inflammation. One heat acclimation method that usually induces an increase in core temperature to ~39.5°C is taking a hot bath right after exercise in a temperate environment (Greenfield et al., 2021; Lovell et al., 2008; Zurawlew et al., 2016, 2018b,

2019). This heat acclimation approach, as originally described by Zurawlew et al., (2016), consists of daily head-out immersion in 40°C water for 40 minutes after a 40-minute exercise session at 18°C over a period of six days. Due to its efficacy in inducing heat acclimation and high practical value, post-exercise hot water immersion is a highly recommended heat acclimation method (Gibson et al., 2020; Heathcote et al., 2018; Kissling et al., 2020; Maloy and Hulsopple, 2021; Pryor et al., 2019; Racinais et al., 2019b). Given the popularity of this method, it would be of interest to many potential users to examine its inflammatory response.

As already mentioned, exercising in the heat may also cause oxidative stress (McAnulty et al., 2005; Pilch et al., 2014; Sureda et al., 2015). In one of those studies, McAnulty et al., (2005) compared the activity of oxidative stress markers (plasma F2 isoprostanes and lipid hydroperoxides) in response to exercise at 35°C versus 25°C. In a cross-over manner, six moderately trained males exercised on a treadmill at 50% of $\dot{V}O_{2max}$ either in the heat (35°C, 70%) until their rectal temperature increased to 39.5°C or for an equivalent time at 25°C, 40%. The rectal temperature during the latter condition rose to 38.1°C, and the average exercise time during both conditions was 49.8 ± 4.6 minutes. It was found that the increase in plasma concentration of both markers was greater following the exercise in the heat than at 25°C. Sureda et al., (2015) showed that increased oxidative stress during exercise in the heat was accompanied by increased activity of antioxidative enzymes, implying that in the situation of increased oxidative stress, the body activates the antioxidant defence in order to maintain the prooxidant-antioxidant balance. However, there is evidence that repeated exposure to exercise in the heat may disrupt the prooxidant-antioxidant balance. Indeed, Kaldur et al., (2014) reported that ten days of heat acclimation consisting of 110 minutes of exercise per day at 42°C, 18% RH increased resting oxidative stress, but total antioxidant capacity remained unchanged. An open question is whether shorter heat acclimation interventions, such as the six-day post-exercise hot water immersion protocol, would cause chronic disruption in the prooxidant-antioxidant status. Addressing that question is important, given the negative health (Pham-Huy et al., 2008) and exercise performance (Cheng et al., 2020; Tiidus, 1998) consequences associated with chronic oxidative stress.

This study aimed to fill the gaps mentioned in the literature by examining the acute and chronic effects of Zurawlew's post-exercise hot water immersion protocol on plasma cytokine response, oxidative stress, and antioxidant capacity. With regard to the inflammatory responses, it was decided to investigate the production of TNF-α and interleukin (IL)-1β, as they are considered to be classic pro-inflammatory cytokines (Docherty et al., 2022), and IL-10 and IL-1 receptor antagonist (IL-1ra), as they are major anti-inflammatory cytokines. IL-6, which can act as both a pro- and anti-inflammatory cytokine, was also included. Oxidative stress was determined by measuring thiobarbituric acid-reactive substances (TBARS), whereas total antioxidant capacity (TAC) was measured as a sum of all antioxidants in the plasma.

4.2. Methodology and Procedures

4.2.1. Experimental design

This study was completed as a part of the first study, and the intervention involved in this study has been explained in the previous chapter. Briefly, participants completed a six-day intervention involving daily cycling exercise for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH) followed immediately by either hot (40°C) water immersion (HWI; n = 7) or thermoneutral (34°C) water immersion (CON; n = 7) for 40 minutes. A venous blood sample was taken immediately before and 20 minutes after the first session to assess acute inflammatory responses, oxidative stress, and TAC. Resting venous blood was also sampled 48 hours after the last session to assess the chronic impact of post-exercise hot water immersion on inflammation, oxidative stress, and TAC. The participants were asked to abstain from any antioxidant supplements throughout the study because these supplements were shown to inhibit cytokine release (Fischer et al., 2004; Vassilakopoulos et al., 2003) and reduce oxidative stress (Alessio et al., 1997; Vincent et al., 2006) during exercise. Dietary supplement use was assessed before the study using a previously developed questionnaire (Kjertakov et al., 2013), which helped to identify the possible use of antioxidant supplements among the participants.

4.2.2. Blood sampling and analysis

Ten mL of venous blood was sampled into an EDTA blood collection tube after 10 minutes of resting in a supine position. All samples were centrifuged for 10 minutes at 4000 revs·min^{-1} and 4°C within 1 minute of being drawn. The separated plasma was frozen at – 80°C until analysed. Commercially available ELISA kits were used to measure IL-6, IL-10, and TNF-α (R&D Systems, Minneapolis, MN, USA), IL-1β (Invitrogen, Massachusetts, USA), IL-1ra (Abcam, Cambridge, UK), TBARS and TAC (Cayman Chemical Company, Michigan, USA). All cytokines, TBRAS, TAC, and their standards were measured in duplicate, and all assays were performed according to the manufacturer's instructions. Absorbance was read using an automated microplate photometer (SpectraMax® i3x, Molecular Devices, CA, USA).

4.2.3. Statistical analysis

Statistical analyses were conducted using GraphPad Prism (version 9.5.1, GraphPad Software Inc., La Jolla, CA, USA). All the data were initially tested for normality of

distribution using the Shapiro-Wilk test, and data that did not pass normality testing were log-transformed before analyses. A two-way repeated measures ANOVA was applied to both acute and chronic data sets. Where significant main effects or interactions occurred, a Bonferroni post-hoc analysis was applied. A statistical significance level was set at $p < 0.05$. The between-group effect size was calculated according to Dankel and Loenneke (2021) and interpreted using the following thresholds (Batterham and Hopkins, 2006): trivial (<0.2), small (0.2-0.59), moderate (0.6-1.19), large (1.2-1.99), and very large (>2.0). Unless otherwise stated, descriptive data are presented as mean ± standard deviation (SD) and 95% confidence intervals (CI).

4.3. Results

Table 4.1 shows the markers of inflammation, TBARS, and TAC measured before and after the first session in the HWI and CON groups. IL-1β showed a significant main effect for group ($F_{(1, 12)}$ = 6.19, p = 0.02), but post-hoc analysis did not reveal any significant difference. There was no significant main effect of time ($F_{(1, 12)}$ = 0.05, p = 0.81), group ($F_{(1, 12)}$ = 0.31, p = 0.58), or time X group interaction ($F_{(1, 12)}$ = 0.68, p = 0.42) for IL-1ra. IL-6 showed a significant main effect of time ($F_{(1, 12)}$ = 16.58, p < 0.01), and the post-hoc analysis revealed that IL-6 significantly (p < 0.01) increased by 253% from pre- to post-session in the CON group. However, there was no significant between-group difference for IL-6 ($F_{(1, 12)}$ = 1.76, p = 0.20). A significant main effect of group ($F_{(1, 24)}$ = 17.95, p < 0.01) but no time ($F_{(1, 24)}$ = 0.65, p = 0.42) or time X group interaction ($F_{(1, 24)}$ < 0.01, p = 0.94) was found for IL-10. The follow-up analysis indicated that plasma IL-10 concentration at both time points was significantly higher in the CON group compared to the HWI group (p = 0.01). Similarly, a significant main effect of group ($F_{(1, 12)}$ = 10.87, p < 0.01) but no time ($F_{(1, 12)}$ < 0.01, p = 0.96) or time X group interaction ($F_{(1, 12)}$ = 2.43, p = 0.14) was found for TNF-α. The follow-up analysis indicated that plasma TNF-α concentration at pre-session was significantly higher in the CON group vs. the HWI group (p < 0.01). The same was the case for TBARS, as evidenced by a significantly higher plasma concentration of this marker at pre-session in the CON group vs. the HWI group (p = 0.02), but no significant main effect of time ($F_{(1, 24)}$ = 2.94, p = 0.09) or time X group interaction ($F_{(1, 24)}$ = 2.38, p = 0.13). Finally, no significant main effect of time ($F_{(1, 24)}$ = 0.01, p = 0.90), group ($F_{(1, 24)}$ < 0.01, p = 0.94), or time X group interaction ($F_{(1, 24)}$ = 1.05, p = 0.31) was found for TAC.

Table 4.2 shows the markers of inflammation, TBARS, and TAC measured before and after the six-day intervention in the HWI and CON groups. IL-1β demonstrated a significant main effect for time ($F_{(1, 12)}$ = 15.01, p < 0.01), and the post-hoc analysis revealed that IL-1β significantly increased from 0.03 pg/mL at baseline to 10.08 pg/mL post-intervention in the HWI group (p < 0.01). However, there was no significant between-group difference for IL-1β ($F_{(1, 12)}$ = 1.27, p = 0.28). No significant main effect of time ($F_{(1, 12)}$ = 0.10, p = 0.74), group ($F_{(1, 12)}$ = 0.12, p = 0.72), or time X group interaction ($F_{(1, 12)}$ = 0.31, p = 0.58) was found for IL-1ra, nor a significant main effect of time ($F_{(1, 12)}$ = 0.81, p = 0.38), group ($F_{(1, 12)}$ = 2.25, p = 0.15),

Table 4.1. Acute changes in plasma cytokines, TBARS, and TAC in response to post-exercise hot (HWI) and thermoneutral (CON) water immersion.

	Pre-session		Post-session		Pre-post session change*			ANOVA's *p* values		
	HWI	CON	HWI	CON	HWI	CON	Between-group ES	Time	Group	T x G
IL-1β (pg/mL)	0.03 ± 0.00	4.58 ± 5.34	0.03 ± 0.00	4.87 ± 6.09	0.00 ± 0.00 (-0.00, 0.00)	0.29 ± 5.69 (-4.91, 5.49)	0.07	0.89	0.02	0.89
IL-1ra (pg/mL)	296.8 ± 301.0	290.8 ± 183.8	361.1 ± 191.7	255.4 ± 83.82	64.29 ± 186 (-107, 236)	-35.4 ± 248 (-296, 225)	0.46	0.81	0.58	0.42
IL-6 (pg/mL)	0.83 ± 1.77	1.02 ± 0.84	2.10 ± 1.31	3.61 ± 1.81$^{\#}$	1.27 ± 1.08 (0.27, 2.26)	2.59 ± 2.26 (0.49, 4.69)	-0.72	0.00	0.20	0.18
IL-10 (pg/mL)	1.82 ± 2.06	7.20 ± 4.72§	2.81 ± 2.19	8.32 ± 3.81§	0.95 ± 2.24 (-1.12, 3.02)	1.12 ± 6.41 (-4.80, 7.06)	0.03	0.42	0.00	0.94
TNF-α (pg/mL)	1.07 ± 2.10	8.06 ± 3.44§	2.52 ± 3.20	6.51 ± 4.78	1.45 ± 2.96 (-1.28, 4.19)	-1.54 ± 3.91 (-5.65, 2.56)	0.82	0.96	0.00	0.14
TBARS (μM)	0.03 ± 0.04	0.14 ± 0.16§	0.02 ± 0.02	0.04 ± 0.01	-0.00 ± 0.03 (-0.03, 0.02)	-0.10 ± 0.16 (-0.25, 0.05)	0.83	0.09	0.03	0.13
TAC (mM)	0.52 ± 0.21	0.43 ± 0.23	0.45 ± 0.18	0.52 ± 0.16	-0.07 ± 0.30 (-0.35, 0.21)	0.09 ± 0.31 (-0.23, 0.41)	0.53	0.90	0.94	0.31

Note: Data expressed as mean ± SD (95%CI*). $^{\#}$A significant within-group difference, $p < 0.05$. §A significant between-group difference, $p < 0.05$

or time X group interaction ($F_{(1, 12)}$ = 1.94, p = 0.18) was found for IL-6. A significant main effect of group ($F_{(1, 12)}$ = 13.58, p < 0.01) was detected for IL-10, resulting from a significantly higher plasma IL-10 concentration at baseline (p < 0.01) and post-intervention (p = 0.01) in the CON group vs. the HWI group. Likewise, there was a significant main effect of group ($F_{(1, 12)}$ = 24.59, p < 0.01) for TNF-α, with post-hoc testing revealing a significantly higher plasma TNF-α concentration at baseline (p < 0.01) and post-intervention (p < 0.01) in the CON group than the HWI group. Finally, there was no significant main effect of time ($F_{(1, 12)}$ = 4.20, p = 0.06), group ($F_{(1, 12)}$ = 4.29, p = 0.06), or time X group interaction ($F_{(1, 12)}$ = 2.72, p = 0.12) for TBARS, nor a significant main effect of time ($F_{(1, 12)}$ < 0.01, p = 0.94), group ($F_{(1, 12)}$ = 0.18, p = 0.67), or time X group interaction ($F_{(1, 12)}$ = 0.40, p = 0.53) for TAC.

Table 4.2. Chronic changes in plasma cytokines, TBARS, and TAC in response to post-exercise hot (HWI) and thermoneutral (CON) water immersion.

	Pre-intervention		Post-intervention		Pre-post intervention change*			ANOVA's *p* values		
	HWI	CON	HWI	CON	HWI	CON	Between-group ES	Time	Group	T x G
IL-1β (pg/mL)	0.03 ± 0.00	4.58 ± 5.34	10.08 ± 8.43[#]	10.14 ± 3.93	10.04 ± 8.43 (2.24, 17.85)	5.55 ± 6.50 (-0.45,11.5)	0.61	0.00	0.28	0.28
IL-1ra (pg/mL)	296.8 ± 301.0	290.8 ± 183.8	223.6 ± 251.9	309.6 ± 239.3	-73.21 ± 179 (-239, 93.17)	18.75 ± 391 (-391, 429)	-0.32	0.74	0.72	0.58
IL-6 (pg/mL)	0.83 ± 1.77	1.02 ± 0.84	0.53 ± 0.81	2.38 ± 2.61	-0.29 ± 1.61 (-1.78, 1.19)	1.36 ± 2.70 (-1.13, 3.86)	-0.84	0.38	0.15	0.18
IL-10 (pg/mL)	1.82 ± 2.06	7.20 ± 4.72[§]	1.81 ± 2.23	6.67 ± 2.76[§]	-0.05 ± 1,35 (-1.30, 1.20)	-0.52 ± 4.78 (-4.95, 3.89)	0.13	0.76	0.00	0.80
TNF-α (pg/mL)	1.07 ± 2.10	8.06 ± 3.44[§]	1.45 ± 1.77	6.98 ± 3.05[§]	0.38 ± 2.35 (-1.79, 2.56)	-1.07 ± 2.92 (-4.15, 1.96)	0.55	0.64	0.00	0.34
TBARS (μM)	0.03 ± 0.04	0.14 ± 0.16	0.01 ± 0.02	0.03 ± 0.00	-0.01 ± 0.03 (-0.04, 0.02)	-0.11 ± 0.15 (-0.26, 0.03)	-0.71	0.06	0.06	0.12
TAC (mM)	0.52 ± 0.21	0.43 ± 0.23	0.46 ± 0.09	0.48 ± 0.26	-0.05 ± 0.26 (-0.30, 0.18)	0.04 ± 0.31 (-0.28, 0.37)	-0.33	0.94	0.67	0.53

Note: Data expressed as mean ± SD (95%CI*). [#]A significant within-group difference, $p < 0.05$. [§]A significant between-group difference, $p < 0.05$

4.4. Discussion

This study examined the acute and chronic effects of exposing well-trained, non-heat acclimated male cyclists to post-exercise head-out immersion in either 40°C or 34°C water for 40 minutes on plasma cytokine (IL-1β, IL-1ra, IL-6, IL-10, and TNF-α) responses, oxidative stress measured by TBARS, and TAC. The main findings are that 1) acute post-exercise hot water immersion had no significant effects on any of the measured biomarkers (all $p < 0.05$); 2) acute post-exercise immersion in 34°C water (control group) induced a significant increase in IL-6 ($p < 0.01$); 3) post-exercise hot water immersion over six consecutive days significantly increased resting plasma IL-1β concentration ($p < 0.01$).

Prolonged or strenuous physical activity is a potent activator of the immune system, resulting in cytokine production from various cell types and upregulating the inflammatory cascade. Typically, the body initially releases pro-inflammatory cytokines such as IL-1β and TNF-α, followed by a production of IL-6 and anti-inflammatory cytokines, including IL-1ra and IL-10, whose actions suppress the release of pro-inflammatory cytokines (Pedersen, 2000). A balance between pro- and anti-inflammatory cytokines release is essential for preventing excessive inflammation and maintaining tissue homeostasis. While the intensity of effort is the strongest predictor of the acute inflammatory response to prolonged exercise (Nieman et al., 2012), the evidence also suggests that the hyperthermia, induced during exercise plays a role in the inflammatory process. For instance, Rhind et al., (2004) reported that an increase in rectal temperature to 39.1°C induced by cycling for 40 minutes in 39°C water was associated with a significant increase in plasma concentrations of TNF-α, IL-6, IL-12, and IL-1ra, whereas the same duration of exercise performed in 18°C water blunted the increase in rectal temperature and eliminated cytokine release. In another study, Starkie et al., (2005) compared cytokine (TNF-α and IL-6) response to 90 minutes of moderate-intensity cycling at 15°C vs. 35°C in seven endurance-trained men. They found that only the cycling trial in the heat led to the release of TNF-α and IL-6 ($p < 0.05$). Furthermore, the rectal temperature at the end of the trial in the heat was significantly ($p < 0.05$) higher than the trial at 15°C (~39.2°C and ~38.5°C, respectively). Available evidence also indicates that hyperthermia on its own can induce cytokinaemia. Indeed, submersion of 8 healthy volunteers up to the neck in water set at a temperature 2°C above their resting oesophageal temperatures for one

hour induced a significant increase in plasma concentrations of IL-1ra, IL-6, and IL-8 (Leicht et al., 2015).

In contrast to the cited studies (Leicht et al., 2015; Rhind et al., 2004; Starkie et al., 2005) and several other similar studies (Cosio-Lima et al., 2011; Hosick et al., 2010; Peake et al., 2008; Sureda et al., 2015), the current study observed no significant change in any of the measured cytokines following the first post-exercise hot water immersion session. These findings are somewhat surprising given that core temperatures, measured via an ingestible telemetric pill, in all HWI participants at the end of the immersion period met the threshold of core temperature (~38.5°C) above which alterations in the inflammatory markers are expected to occur in endurance-trained male individuals (Selkirk et al., 2008). It is possible that, despite causing an increase in mean gastrointestinal temperature to 39.4°C (Figure 3.2, Chapter Three), the duration of heat exposure (40 minutes) in the current study might not have been sufficient to trigger immunological responses in the well-trained cyclists. It is also possible that cytokinaemia did occur in the hours following hot water immersion, but the study failed to detect it since the blood was sampled too soon post-immersion (20 minutes) (Suzuki et al., 2002). The decision for blood sampling to take place 20 minutes post-immersion was based on the findings that all the cytokines examined by Rhind et al., (2004) in response to cycle exercise during hot water immersion were significantly increased in the blood samples collected 20 minutes post-trial. A more intriguing finding than the previous one is the significant increase in IL-6 post-session in the CON group ($p < 0.01$) but not in the HWI group ($p = 0.16$). That is especially true since acute passive heat exposure using hot water immersion (Faulkner et al., 2017; Hoekstra et al., 2018; Leicht et al., 2015; Mansfield et al., 2021), sauna bathing (Kaldur et al., 2016), and hot water-perfused suit (Hoekstra et al., 2021) has repeatedly been shown to stimulate increased release of endogenous IL-6 in the blood. For instance, Mansfield et al., (2021) recently found in nine healthy males a significant increase ($p < 0.01$) in plasma concentration of IL-6 immediately following 60 minutes of immersion up to the waist in 42°C water, whereas immersion in thermoneutral (36°C) water did not affect this cytokine. Similar results were previously reported by Hoekstra et al., (2018) in a study that had ten sedentary men submerged up to the neck in 39°C water for one hour or resting for one hour at ambient temperature (control condition). Plasma IL-6 was significantly increased immediately following the immersion but unchanged

during the control condition. However, it should be noted that the acute change in plasma IL-6 concentration in the HWI group in the present study was numerically higher (+1.27 pg·ml^{-1}) compared to that (+1.14 pg·ml^{-1}) reported by Hoekstra et al., (2018) and particularly than that (+0.5 pg·ml^{-1}) of Mansfield et al., (2021). While the lack of a significant change in IL-6 following acute post-exercise hot water immersion could have been due to a type II error, the finding that the magnitude of change in IL-6 was higher in the CON group (+2.59 pg·ml^{-1}) is difficult to explain. An *in vitro* study showed that human peripheral blood mononuclear cells produced less ($p < 0.01$) IL-6 at 39°C compared with 37°C (Kappel et al., 1991), but the available evidence do not support a negative feed-back mechanism between hyperthermia and the production of IL-6 *in vivo*. Again, the finding that IL-6 was significantly increased during the first session in the CON group is intriguing, but it is not concerning, as the literature describes an acute subclinical increase in IL-6 as a favourable physiological change (Docherty et al., 2022; Steensberg, 2003). The observation of significantly higher mean resting plasma concentrations of IL-10 and TNF-α in the CON group vs. the HWI group is also not concerning. The upper limits of IL-10 and TNF-α for healthy individuals are 16 pg·ml^{-1} (Richardson et al., 2023) and 75 pg·ml^{-1} (Damas et al., 1989), respectively, which are well above the values measured in the CON participants (Table 4.1).

A transient increase in inflammation following exercise is vital for regenerative processes and adaptive remodelling of skeletal muscles (Cheng et al., 2020). On the other hand, a chronic inflammatory response may result in muscle weakness, maladaptation, chronic fatigue, and adverse health outcomes (VanderVeen et al., 2019). One scenario that may lead to chronically elevated cytokine levels is the process of heat acclimation, and this has been well-documented in the study by Hailes et al., (2011) mentioned in the Introduction. Given that head-out 40°C water immersion induces hyperthermia comparable to that observed during the heat acclimation sessions in the former study, it is not surprising that the HWI group in the current study showed a significant ($p < 0.01$) increase in one of the measured inflammatory markers (i.e., IL-1β) following the six-day intervention. The increased appearance of IL-1β in the plasma, which was above the healthy value for this cytokine (Richardson et al., 2023), and the lack of a significant increase in either IL-1ra or IL-10 indicate that the intervention induced a pro-inflammatory environment in the HWI participants. This

outcome could be a cause for concern, knowing what consequences chronic inflammation could have on athletes' exercise performance, particularly since IL-1β promotes muscle protein breakdown (Moldoveanu et al., 2001). Yet, whether the elevation of IL-1β in the HWI group was sufficiently high to affect the cells or exercise performance remains unknown. Increased resting inflammatory response has also been associated with increased susceptibility to heat injury (Hailes et al., 2011), but none of the participants in the HWI group displayed any signs or symptoms of heat illness. In the context of heat stroke, the post-intervention mean resting plasma level of IL-1β in the HWI group was three times lower than that observed in heat stroke patients (Bouchama et al., 1993). Interestingly, the magnitude of increase in resting IL-1β during the intervention in the HWI group was nine times higher compared to that reported by Gill et al., (2015) in response to a five-day ultra-marathon running race in hot conditions. However, along with the increased resting IL-1β ($p < 0.01$), Gill et al., (2015) also observed increased ($p < 0.01$) release of IL-1ra, IL-6, and IL-10, which likely limited the extent of pro-inflammatory response in their participants. The reason why the increased production of IL-1β was not compensated by an increased release of anti-inflammatory cytokines in the HWI participants is unclear. It is also unclear what was the source of the increase in plasma IL-1β.

Besides cytokinaemia, oxidative stress is another byproduct associated with acute strenuous physical activity (Briviba et al., 2005; Duca et al., 2006; Mastaloudis et al., 2001; Pinho et al., 2010) or passive heat stress (Laitano et al., 2010; Ohtsuka et al., 1994; Pilch et al., 2014). By definition, oxidative stress occurs when the production of reactive oxygen species overwhelms the body's antioxidant defence (Sies, 1997), causing damage to various cellular components such as lipids, proteins, and nucleic acids (Verhagen et al., 2006). Since cellular lipids are particularly susceptible to uncontrolled free radicals' production (Halliwell and Chirico, 1993), a typical tool for quantification of oxidative stress in exercise science studies is measuring lipid peroxidation biomarkers (Fisher-Wellman et al., 2009; Vollaard et al., 2005). Furthermore, the literature indicates that TBARS is the most commonly used lipid-peroxidation marker (Fisher-Wellman et al., 2009). With regard to the biomarkers of antioxidant defence system, the use of TAC is widespread (Belviranli et al., 2016; Diaz et al., 2011; Duthie et al., 1990; Jackson et al., 2010; Magalhães et al., 2007; Nielsen et al., 2004; Paik et al., 2009; Park and Nickerson, 2022; Pilch et al., 2014; Silva et al.,

2013; Vezzoli et al., 2014; Vider et al., 2001) since it provides an overall value corresponding to the sum of all antioxidants in the plasma (Finaud et al., 2006). Antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, are other markers utilised by researchers to quantify the activity of the antioxidant defence system (Andrews and Kantor, 2010; Laitano et al., 2010; Ohtsuka et al., 1994; Pinho et al., 2010; Robertson et al., 1991; Sureda et al., 2015; Sutkowy et al., 2014; Zivkovic et al., 2013).

The present study showed that acute post-exercise hot water immersion had no significant impact on TBARS. Likewise, hot water immersion had no significant effect on TAC, which raises a question regarding the potential mechanism behind attenuated oxidative stress following hot water immersion. Hypothetically, if the lack of evident oxidative damage in the HWI group was due to the effective neutralisation of free radicals by plasma-based antioxidants, TAC should have been significantly reduced post-session. One possible explanation for the absence of lipid peroxidation following post-exercise hot water immersion in the current study is that the enzymatic antioxidant defence system eliminated the excessively produced free radicals during the immersion and protected cell membranes from oxidative damage (Sies, 1997). Alternatively, the quantity of free radicals produced during the hot water immersion was insufficient to challenge plasma antioxidant defences. One may also suggest that the TBARS assay was not sensitive enough to detect lipid peroxidation (Fisher-Wellman et al., 2009; Vollaard et al., 2005), but that would not explain why TAC was unchanged. Indeed, even when TBARS are unaffected by conditions documented to induce peroxidation of lipids, TAC significantly changes (Diaz et al., 2011; Duthie et al., 1990). As for the potential issues regarding the blood sample timing, Pilch et al., (2014) reported significantly ($p < 0.05$) increased lipid peroxidation products and significantly ($p < 0.05$) decreased TAC immediately following a 31-minute Finnish sauna bathing session in 10 male long-distance runners. Given all the above, it is reasonable to suggest that the lack of a significant change in TABRS and TAC 20 minutes following acute post-exercise hot water immersion reflects an intact prooxidant-antioxidant balance. This could also help partially to explain the lack of a significant change in cytokines in response to the acute post-exercise hot water immersion since oxidative stress is an independent trigger of cytokinaemia (Esposito et al., 2002; Kosmidou et al., 2002).

The present study also showed that repeated post-exercise hot hater immersion over six days had no significant effect on resting TBARS and TAC, suggesting that this heat acclimation strategy does not induce chronic disruption in the prooxidant-antioxidant balance in well-trained male endurance athletes. The finding that the oxidative stress marker remained unchanged over time in the HWI group contrasts with the observation of Kaldur et al., (2014). Their study examined the changes in oxidative stress in response to ten consecutive days of exercise (110 minutes per day) at 42°C in 21 unacclimated physically active men. Venous blood samples collected before and after the heat acclimation period were analysed for total peroxide concentration, oxidised low-density lipoproteins, and TAC. The percent ratio of the total peroxide concentration of plasma to TAC of plasma was accepted as the oxidative stress index and an indicator of the extent of oxidative stress. It was reported that heat acclimation increased resting total peroxide concentration by 24.2% ($p < 0.05$) and oxidative stress index by 36.7% ($p < 0.05$) but did not alter resting TAC ($p > 0.05$). The difference between the current study and the study by Kaldur et al., (2014) could be explained by the differences in the markers used to quantify oxidative stress, the nature of the heat acclimation sessions, the duration of the heat acclimation intervention, and the fitness level of participants. Unfortunately, the lack of a group that underwent the same exercise intervention but in a thermoneutral environment makes it impossible to determine the contribution of heat stress to the development of increased resting oxidative stress in the study by Kaldur et al., (2014). Opposite results to that of Kaldur et al., (2014) were reported by Fujita et al., (2011) and Masuda et al., (2004) following repeated passive heat exposure. In the study by Masuda et al., (2004), 28 patients with at least one coronary risk factor were allocated to two weeks of either resting in a far infrared-ray dry sauna (60°C) for 15 minutes, seven days per week ($n = 14$) or a control group ($n = 14$). Resting oxidative stress was measured by analysing morning urine samples collected before and after the intervention for 8-epi-prostaglandin F2α as a marker for lipid peroxidation. The urinary 8-epi-prostaglandin F2 level was significantly ($p < 0.01$) decreased after two weeks in the sauna bathing group but not in the control group. Fujita et al., (2011) found significantly ($p < 0.01$) decreased resting plasma concentration of hydroperoxide (oxidative stress marker) in 20 patients with chronic heart failure following a heating treatment consisting of 5 weekly 15-minute sauna (60°C) bathing sessions performed over 4 weeks. The plasma concentration of hydroperoxide was unchanged in the control group ($n = 20$). The findings of these two

studies (Fujita et al., 2011; Masuda et al., 2004) suggest that chronic passive heat exposure can induce adaptations in the mechanisms responsible for preventing oxidative stress. Nevertheless, it is unlikely that those findings would apply to well-trained endurance athletes whose antioxidant defence system is already well developed (Robertson et al., 1991), as likely was the case with the HWI participants.

This study was not without limitations. An obvious limitation is the lack of blood sampling at multiple time points following the first session. Therefore, it is possible that some of the measured biomarkers were significantly changed after the blood sample was collected. Another limitation is the small sample size, which could have prevented reaching statistical significance in some of the comparisons. A priory sample size calculation was not performed as there was no previous data upon which the calculation could have been based on. Furthermore, analysing a range of other biomarkers (e.g., epinephrine, growth hormone, cortisol, and heat shock proteins) could have provided mechanistic insights into the chronically increased plasma IL-1β. Likewise, the inclusion of biomarkers related to protein oxidation or DNA base modification and measuring the activity of antioxidant enzymes would have provided a more comprehensive insight into the prooxidant-antioxidant response to post-exercise hot water immersion. Finally, the results from this study may not apply to less trained individuals and the female sex.

In conclusion, chronic inflammation and oxidative stress are associated with impaired exercise performance, and some previous studies indicated that active heat acclimation induces chronically elevated cytokine levels and oxidative stress markers. The current study examined whether the heat acclimation strategy introduced by Zurawlew et al., (2016) would impact plasma cytokine (IL-1β, IL-1ra, IL-6, IL-10, and TNF-α) responses and prooxidant-antioxidant balance measured by TBARS and TAC. Acute post-exercise hot water immersion had no significant effects on any of the measured biomarkers. Acute post-exercise immersion in 34°C water (control group) induced a significant increase in IL-6 ($p < 0.01$). Post-exercise hot water immersion over six consecutive days significantly increased resting plasma IL-1β concentration.

Chapter five: Validity of Genius™ 2 and Braun Pro 4000 Thermoscan tympanic thermometers to monitor body core temperature during post-exercise hot water immersion

Abstract

Background: Concern has been raised about the safety of the head-out 40°C water immersion protocol, given that this heating method creates a condition of uncompensable heat stress where the body cannot maintain a thermal steady state. Therefore, body temperature monitoring during this intervention can safeguard the users against the potential risk of developing dangerous hyperthermia and associated heat stroke. However, the thermometric methods that are proven valid and reliable are often restricted to laboratory settings.

Objective: This study examined whether commercially available tympanic thermometers could be a suitable substitute for the expensive ingestible telemetric pills or invasive rectal probes for monitoring core temperature during the post-exercise head-out 40°C water immersion.

Methods: Sixteen male cyclists cycled for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH), after which they were submerged to the neck in a bath of hot water for 40 minutes. Participants' tympanic and core temperatures were measured at rest, after exercise, and every 10 minutes throughout the hot water immersion session. The tympanic temperature was measured by Genius™ 2 and Braun Pro 4000 Thermoscan tympanic thermometers, whereas core temperature was measured via an ingestible telemetric pill. The latter was used as a reference against which the tympanic thermometers were validated.

Results: No statistically significant differences in temperature readings were observed between the telemetric pill and Braun Pro 4000 Thermoscan at any time point during the hot water immersion session, and these temperature readings were significantly correlated at all time points. The overall bias in temperature reading provided by Braun Pro 4000 Thermoscan relative to the telemetric pill was within the acceptable limit (< 0.3°C). Temperatures provided by Genius™2 at the 20-, 30, and 40-minute time points during the hot water immersion period were significantly higher than those of the telemetric pill, and there were no correlations between the devices at the last two time

points. The overall mean bias associated with the Genius^{TM}2 tympanic thermometer was 0.50°C.

Conclusion: The findings from the current study indicate that the Braun Pro 4000 Thermoscan could be a suitable tool for monitoring core temperature during post-exercise head-out 40°C water immersion. Genius^{TM}2 did not pass the validity testing, and thus, it is not recommended for use during hot water immersion.

Keywords: monitoring deep body temperature, tympanic thermometers, post-exercise hot water immersion

5.1. Introduction

Based on the work by Zurawlew et al., (2016), heat acclimation literature promotes taking a hot bath right after exercise as the most practical and easily accessible heat acclimation strategy (Gibson et al., 2020; Heathcote et al., 2018; Kissling et al., 2020; Maloy and Hulsopple, 2021; Pryor et al., 2019; Racinais et al., 2019b). Recently, however, concern has been raised about the safety of hot water immersion (Rodrigues et al., 2020), given that this heating method creates a condition of uncompensable heat stress where the body cannot maintain a thermal steady state. Consequently, the core temperature could be driven to the point of heat stroke. In fact, the medical literature has reported cases of heat stroke resulting from bathing in ~40°C water (Akieda et al., 2008; Lee et al., 2010; Nishikawa et al., 2013).

No ill health effects were observed in any of the laboratory studies that had participants submerged up to the neck in hot (40°C) water for 30-40 minutes immediately after exercise (Gerrett et al., 2021; Klous et al., 2021; Lovell et al., 2008; McIntyre et al., 2021; Waldock et al., 2021; Zurawlew et al., 2016, 2018a, 2018b, 2019). However, one should bear in mind that the risk of heat stroke in those studies was minimised, as all participants were in good physical health, well-hydrated, and their body core temperature was continuously monitored during immersion sessions. On the other hand, in a 'real life' situation, an athlete can commence post-exercise hot water immersion already predisposed to heat stroke. Hypohydration (i.e., body water deficit), a condition typically experienced by athletes after exercise, is one of the factors that could exaggerate an increase in body core temperature during hot water immersion. Body core temperature increases by ~0.2°C for every 1% hypohydration during passive heat exposure (Sawka et al., 1984), and athletes, especially those in endurance sports, commonly experience 2 - 6% hypohydration during exercise (Del Coso et al., 2013; Lee et al., 2010; Tan et al., 2016; Zouhal et al., 2011). Poor sleep and generalised fatigue, conditions also experienced by athletes (Jeukendrup et al., 1992; Urhausen et al., 1998), are other risk factors for heat stroke (Armstrong et al., 1990). Furthermore, current or recent illnesses and various types of drugs can contribute to the development of extremely high body temperature by either impairing the removal of excess heat from the body or increasing body heat production (Kjertakov and Epstein, 2013). While the list of risk factors for heat stroke goes on

(Epstein and Shapiro, 1995; Kjertakov and Epstein, 2013), the risk factors mentioned above are the ones most likely to be experienced by athletes.

From the preceding two paragraphs, it is clear that post-exercise hot water immersion may increase the risk of heat stroke. Therefore, whenever athletes take hot water immersion after exercise, preventive measures need to be in place to safeguard them from heat stroke. In this context, it is important to recognise that visual observation of athletes may not be enough to prevent heat stroke, as the onset of this heat disorder is usually sudden and rapid, and there are no easily recognisable signs that one can use to predict its development (Roberts, 2004). Accordingly, the best way to ensure athletes' safety during hot water immersions is by frequent monitoring of their body core temperature.

To be suitable for monitoring body core temperature in real-life situations, the method must be noninvasive, portable, inexpensive, and sufficiently accurate (Moran and Mendal, 2002). One device that possesses those attributes appears to be the Genius™ 2 tympanic thermometer. Recently, Otani et al., (2020) examined the validity of the Genius™ 2 tympanic thermometer in 8 healthy males during exercise in the heat. Researchers had the participants cycle for 40 minutes at 30°C while continuously measuring their tympanic and core temperature throughout the exercise bout. Tympanic temperature was measured by Genius™ 2 tympanic thermometer, while the core temperature was measured via the rectal route by a rectal probe. The latter method is considered the 'gold standard' for measuring body core temperature (Casa et al., 2007; Gant et al., 2006; Ganio et al., 2009; Hosokawa et al., 2016). The study demonstrated that the Genius™ 2 tympanic thermometer can be used to predict rectal temperature during endurance exercise in the heat, with the difference between tympanic and rectal temperature being only 0.1°C. Those findings suggest that the Genius™ 2 tympanic thermometer might be considered a suitable tool for the detection of dangerous hyperthermia during hot water immersion. There is also evidence that the Braun Pro 4000 tympanic thermometer might be used for the same purpose. Bock et al., (2005) compared tympanic thermometry with pulmonary artery thermometry in twenty-six patients undergoing cardiac surgery. Tympanic temperature was measured using a portable thermometer (IRT 4000, Braun GmbH, Germany), whereas measuring the pulmonary artery temperature served as a reference against which the

tympanic thermometer was validated. The researchers reported that the agreement between the tympanic and pulmonary artery temperatures was 0.08°C. However, whether the agreements between the tympanic and core temperature reported in the studies by Bock et al., (2005) and Otani et al., (2020) would persist during hot water immersion remains to be determined. Therefore, the aim of this study was to examine the validity of the Genius™ 2 and Braun Pro 4000 Thermoscan tympanic thermometers to predict core temperature during post-exercise hot water immersion.

5.2. Methodology and Procedures

5.2.1. Participants

The required sample size was calculated using G*power 3.1.9.2 software based on the following information. To be deemed valid for assessing body core temperature, the maximal allowable imprecision of a given device should be no larger than 0.3°C (Casa et al., 2007; Gant et al., 2006; Ganio et al., 2009; Mogensen et al., 2016; Morrissey et al., 2021). Accordingly, a sample size calculation indicated that to detect a significant difference of 0.3°C between the tympanic and core temperatures with a standard deviation of 0.4°C (Zurawlew et al., 2018b), with $\alpha = 0.05$ and power = 80%, 16 participants would be required. Therefore, sixteen male cyclists with an average age, height, body mass, $\dot{V}O_{2max}$, and PPO of 30.18 ± 6.33 years, 178.1 ± 3.81 cm, 73.94 ± 9.00 kg, 61.66 ± 7.52 $ml \cdot kg^{-1} \cdot min^{-1}$, and 399.37 ± 47.77 watts respectively, were recruited for this study.

5.2.2. Experimental design

Each participant visited the laboratory on two occasions. During the first visit, participants completed a graded exercise test to determine their maximal oxygen consumption ($\dot{V}O_{2max}$). During the second visit, the participants exercised on a bike for 40 minutes at 50% of their peak power output in a cool environment (14°C, 40% RH), after which they were immersed in a bath of hot water for 40 minutes. Participants' tympanic and core temperatures were measured at rest, after exercise, and every 10 minutes throughout the hot water immersion session. The tympanic temperature was measured by Genius™ 2 and Braun Pro 4000 Thermoscan tympanic thermometers, whereas core temperature was measured via an ingestible telemetric pill. The latter was used as a reference against which the tympanic thermometers were validated.

5.2.3. Measuring tympanic and gastrointestinal temperatures

All measurements were carefully obtained following manufacturers' instructions. Tympanic temperature was measured first in the left and then in the right ear of each participant. One measurement per ear was performed by each tympanic thermometer at each sampling time point. Genius™2 was always set to measure in the ear mode. This device can also obtain a 'core equivalent' temperature by adding 1°C to the tympanic temperature, but the former mode was selected in line with the study by Otani et al., (2020). Braun Pro 4000 Thermoscan has only one mode. The

gastrointestinal temperature was recorded by using a CorTemp™ telemetric system (CorTemp, HQInc, Palmetto, FL, USA) consisting of an ingestible telemetric sensor pill that transmits a radio wave signal to a small data recorder. The telemetric pill was provided to the participants one day before the trial, and they were instructed to swallow it at least 4 hours before reporting to the laboratory the following day. No fluid was allowed during the trial because ingestion of fluid may influence the temperature reading (Wilkinson et al., 2008). All measurements of tympanic and gastrointestinal temperatures were completed within 2 minutes. The order of devices used was as follows: Genius™2, Braun Pro 4000 Thermoscan, and CorTemp™.

5.2.4. Graded exercise test

As per study one.

5.2.5. Exercise and post-exercise hot water immersion session

To ensure adequate hydration (i.e., euhydration), participants were recommended to drink 500 ml of water 2 hours before arriving at the laboratory. Upon arrival at the laboratory, participants were asked to provide a urine sample in a medical-grade collection container to assess hydration status via urine-specific gravity (USG). Urine-specific gravity was measured using a handheld refractometer (URC-NE, Atago, Japan), and the hypohydration threshold was set at USG ≤ 1.020 (Sawka et al. 2007). Upon confirmation of euhydration, participants entered an environmental chamber where the 40-minute cycling session was conducted. The air temperature and relative humidity during the exercise session were maintained at 14°C and 40%, respectively. Once inside the chamber, participants' resting tympanic and gastrointestinal temperatures were measured before they began cycling for 40 minutes at 50% of their peak power output. At the completion of the cycling session, tympanic and gastrointestinal temperatures were measured again, and participants were given 2 minutes to change out of their cycling gear before being immersed up to the neck in an inflatable bath (iCool, Brisbane, Australia) with continuous water recirculation and temperature control. The temperature of the water was maintained at 40°C, and participants were asked to remain submerged for 40 minutes.

5.2.6. Statistical analyses

Statistical analyses were conducted using GraphPad Prism (version 9.5.1, GraphPad Software Inc., La Jolla, CA, USA) or SPSS (version 23, SPSS Inc., Chicago, IL, USA).

Initially, all the data were tested for normality of distribution using the Shapiro-Wilk test. Only gastrointestinal temperature data collected at 20 and 30 minutes during hot water immersion did not pass the testing, and, as such, the relevant data sets from those two time points were analysed by non-parametric test. Paired *t*-test was used to compare mean tympanic temperatures between the left and right ears. A one-way analysis of variance (ANOVA) was used to assess differences in temperature readings between devices at rest, after exercise, and at 10 and 40 minutes during hot water immersion. When a significant F value was obtained through the ANOVA, Tukey's post-hoc test was employed to locate the difference. The Kruskal Wallis test, followed by Dunn's post-hoc test, was applied to the data sets from the remaining two time points of the hot water immersion period. The level of significance was set at $p < 0.05$ for all tests. For uniformity, both parametric and nonparametric data are presented as mean ± standard deviation (SD). The mean bias and limits of agreement between tympanic and gastrointestinal temperatures were explored using the method of Bland and Altman (1986). In line with the previous similar studies (Fenemore et al., 2020; Morán-Navarro et al., 2019), a bias < 0.3°C was considered acceptable and rendered a device valid. The method of Bland and Altman was also used to examine the level of agreement between the tympanic temperatures from the left and right ears. Pearson's product-moment correlation coefficient (*r*) was used to assess the strength of the association between the tympanic and gastrointestinal temperatures. In line with the guidelines for interpreting correlation coefficients proposed by Cohen (1992), correlations were considered weak at $r = 0.10$-0.29, moderate at $r = 0.30$-0.49, and strong at $r = 0.50$-1.0.

5.3. Results

All sixteen participants completed the 40-minute post-exercise hot water immersion session. T-tests found no significant ($p > 0.05$) difference between the mean left and right ear measurements for either Genius™2 or Braun Pro 4000 (Table 5.1). Furthermore, the observed overall mean difference between the left and right ears of -0.01°C for Genius™2 and 0.03°C for Braun Pro 4000 (Figure 5.1) was negligible. Therefore, the results of only one (left) ear were used to perform a statistical comparison with the results of the ingestible telemetric pill.

Table 5.1. Tympanic temperature measured in the left and right ears by Genius™2 and Braun Pro 4000 before (at rest) and after the exercise session and at 10-minute intervals throughout the hot water immersion.

	Genius™2		Braun Pro 4000	
	Left ear	Right ear	Left ear	Right ear
Rest	36.64 ± 0.70	36.79 ± 0.75	36.26 ± 0.42	36.32 ± 0.42
End-exercise	36.33 ± 0.62	36.40 ± 0.43	36.21 ± 0.79	36.09 ± 0.40
10 minutes	37.98 ± 0.44	37.85 ± 0.55	37.27 ± 0.54	37.21 ± 0.57
20 minutes	38.91 ± 0.52	39.05 ± 0.69	38.21 ± 0.49	38.24 ± 0.39
30 minutes	39.67 ± 0.69	39.59 ± 0.81	38.71 ± 0.40	38.71 ± 0.37
40 minutes	39.87 ± 0.67	39.84 ± 1.05	39.11 ± 0.28	38.99 ± 0.51
Mean ± SD	**38.23 ± 1.51**	**38.25 ± 1.52**	**37.63 ± 1.23**	**37.59 ± 1.21**

Data presented as mean ± SD

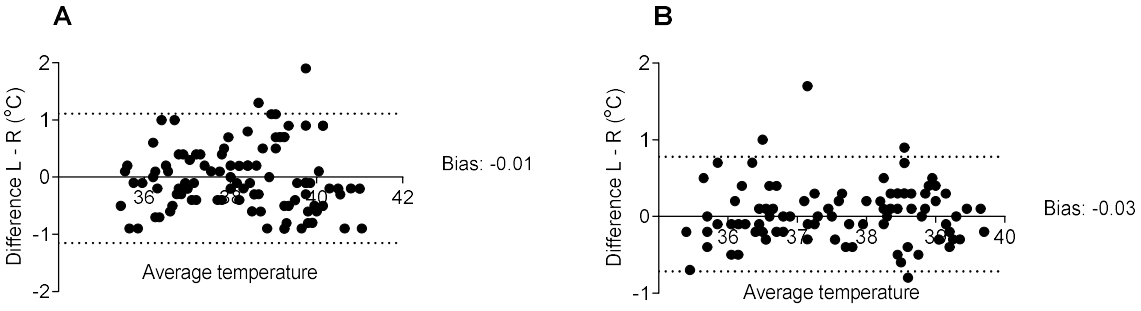

Figure 5.1. Bland-Altman plot indicating the mean bias (solid line) and 95% limits of agreement (dotted lines) in temperature between the left (L) minus the right ear (R) measured with Genious™2 (A) and Braun Pro 4000 (B) thermometers.

The mean values of temperatures measured by telemetric pill, Genius™2, and Braun Pro 4000 at rest, after exercise, and at 10-minute time points throughout the immersion

are shown in Figure 5.2. At rest, the temperature reading of the telemetric pill (37.21 ± 0.35°C) was significantly higher than the readings of both Genius^{TM}2 (36.64 ± 0.70°C, p = 0.01) and Braun Pro 4000 (36.26 ± 0.43°C, p < 0.01) tympanic thermometers. Likewise, a significantly higher post-exercise temperature was recorded by the telemetric pill (37.88 ± 0.56°C) compared to Genius^{TM}2 (36.33 ± 0.62°C, p < 0.01) and Braun Pro 4000 (36.21 ± 0.79°C, p < 0.01) tympanic thermometers. During the hot water immersion, temperatures rose progressively peaking at 39.39 ± 0.19°C, 39.87 ± 0.67°C, and 39.10 ± 0.30°C for the telemetric pill, Genius^{TM}2, and Braun Pro 4000, respectively. The overall increase in gastrointestinal temperature was highly correlated with the increase in tympanic temperatures (Figure 5.3). Despite the difference prior to immersion, no significant (p > 0.05) differences in temperature readings were observed between the telemetric pill and Braun Pro 4000 at any time point during the immersion session, and these temperature readings were significantly correlated at all time points (Figure 5.4). Although there was no significant (p > 0.05) difference in the temperature readings between the telemetric pill and Genius^{TM}2 at the 10-minute time point, the values of the latter device were significantly (p < 0.05) higher during the rest of the immersion period. As a result, the correlations between the temperature readings of the Genius^{TM}2 and intestinal pill were poor at 30- and 40-minute time points during the immersion (Figure 5.5). Furthermore, compared to Braun Pro 4000, Genius^{TM}2 recorded significantly (p < 0.05) higher tympanic temperature at each time point during the immersion.

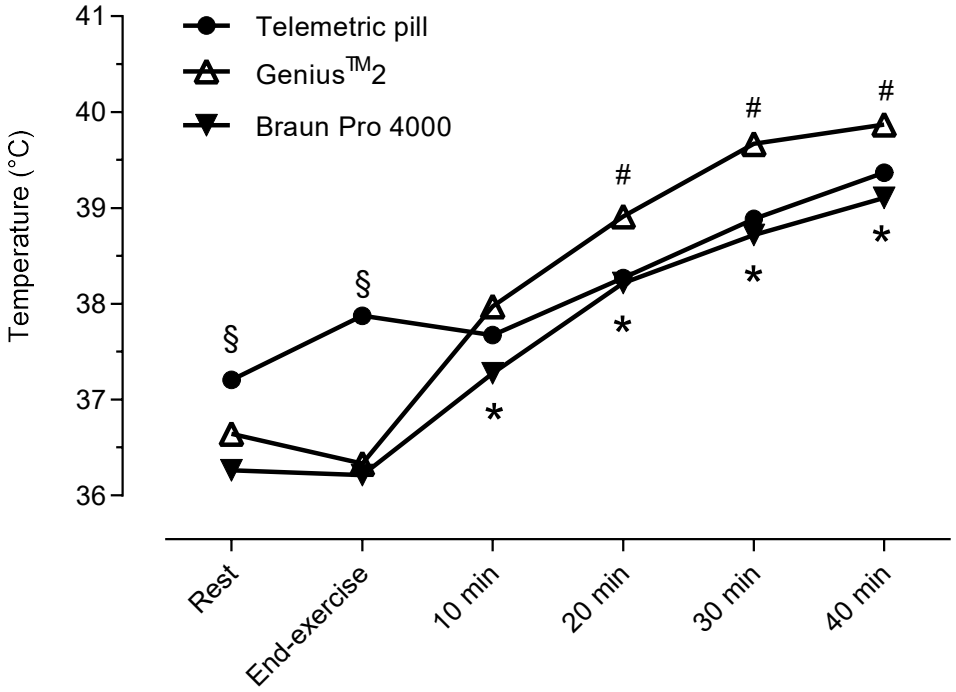

Figure 5.2. Telemetric pill and tympanic temperatures measured at rest, after the exercise session, and at 10-minute intervals throughout the post-exercise hot water immersion period. Data presented as mean. § indicates a significant difference between the telemetric pill and both tympanic thermometers. # indicates a significant difference between the Genious™2 thermometer and telemetric pill. * indicates a significant difference between the Braun Pro 4000 and Genius™2 thermometers.

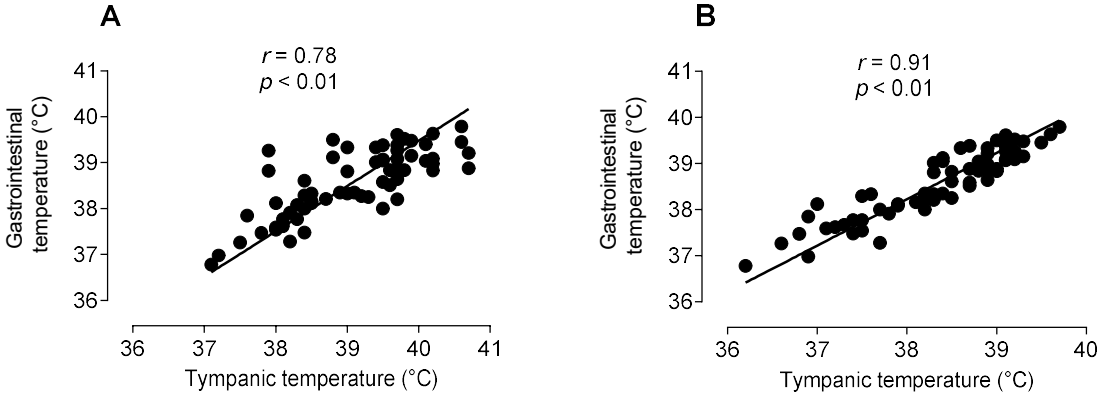

Figure 5.3. Relationship between the overall increase in gastrointestinal temperature and tympanic temperatures, measured by Genius™2 (A) and Branu Pro 4000 (B) thermometers, during the hot water immersion.

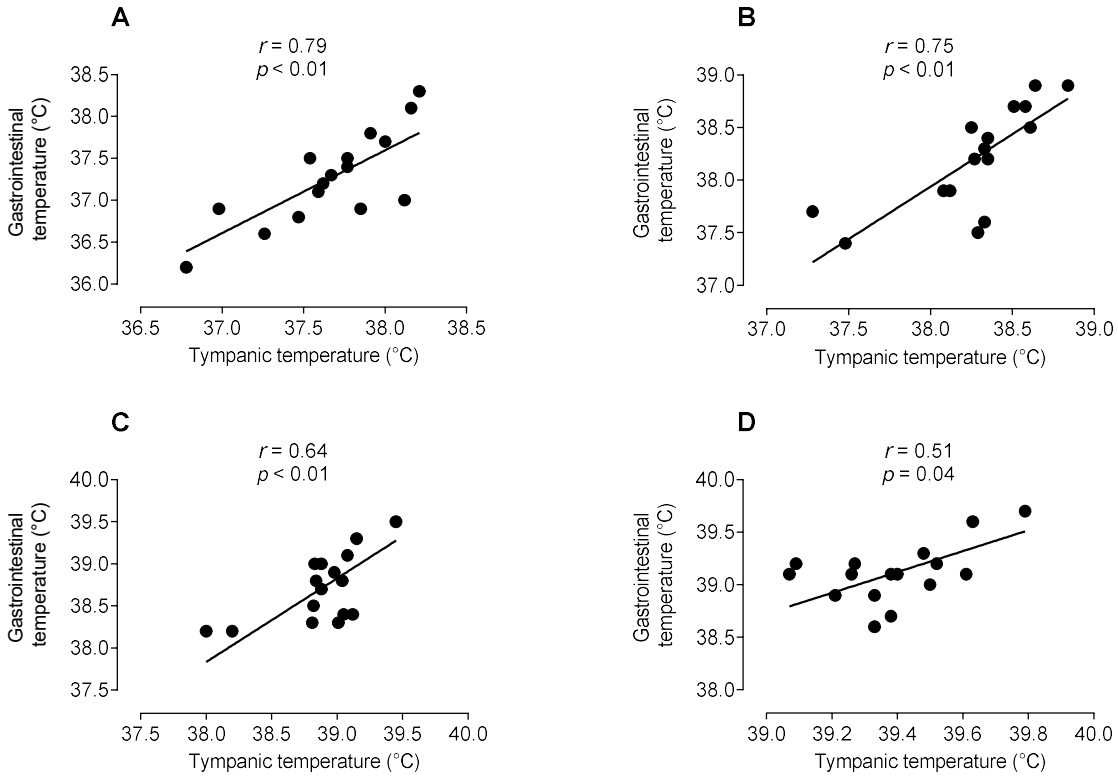

Figure 5.4. Relationship between the gastrointestinal temperature and tympanic temperature, measured by Braun Pro 4000 thermometer, at 10- (A), 20- (B), 30-(C), and 40-minute (D) time points during the immersion session.

The level of agreement between the tympanic and gastrointestinal temperatures was initially calculated for each time point separately (Figures 5.6 and 5.7). As can be seen in these figures, the mean difference between the tympanic thermometers and telemetric pill noted after the exercise session, in general, reduces as the gastrointestinal temperature increases during the hot water immersion. An acceptable mean bias (< 0.3°C) in temperature reading was observed at the last time point of the immersion period for both tympanic thermometers and at the 20- and 30-minute time points for the Braun Pro 4000 thermometer. The overall mean difference between the tympanic and gastrointestinal temperatures during the hot water immersion is shown in Figure 5.8. The mean bias of -0.22°C for the Braun Pro 4000 tympanic thermometer indicates that this device is suitable for predicting body core temperature during hot water immersion. In contrast, the overall mean bias (0.50°C) associated with the Genius^{TM}2 tympanic thermometer suggests that this device is unsuitable for monitoring core temperature during hot water immersion. Although, as already

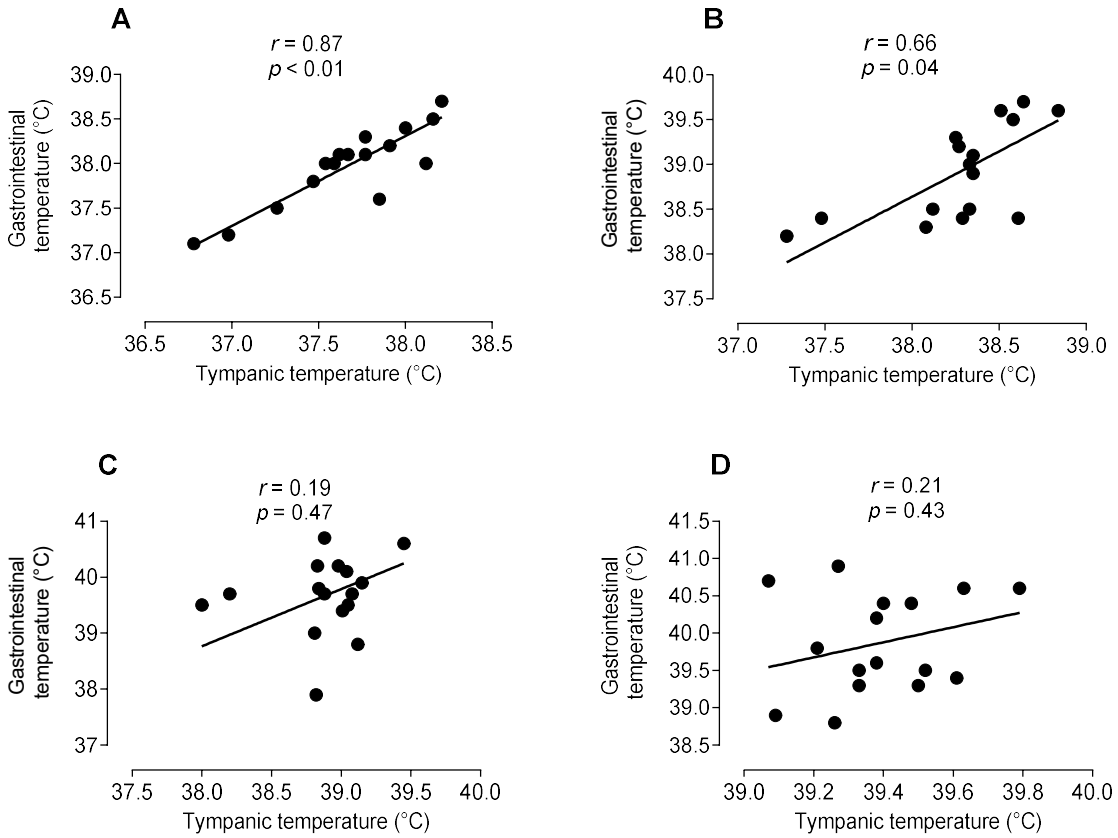

Figure 5.5. Relationship between the gastrointestinal temperature and tympanic temperature, measured by Genius^{TM}2 thermometer, at 10- (A), 20- (B), 30-(C), and 40-minute (D) time points during the immersion session.

mentioned, Genius^{TM}2 provided a temperature reading within < 0.3°C of our criterion standard (i.e., ingestible telemetric pill) when the gastrointestinal temperature was highest (at the end of the hot water immersion), the corresponding limits of agreement were too wide (Figure 5.6. F) to suggest that this device could be a screening tool for such a high degree of hyperthermia.

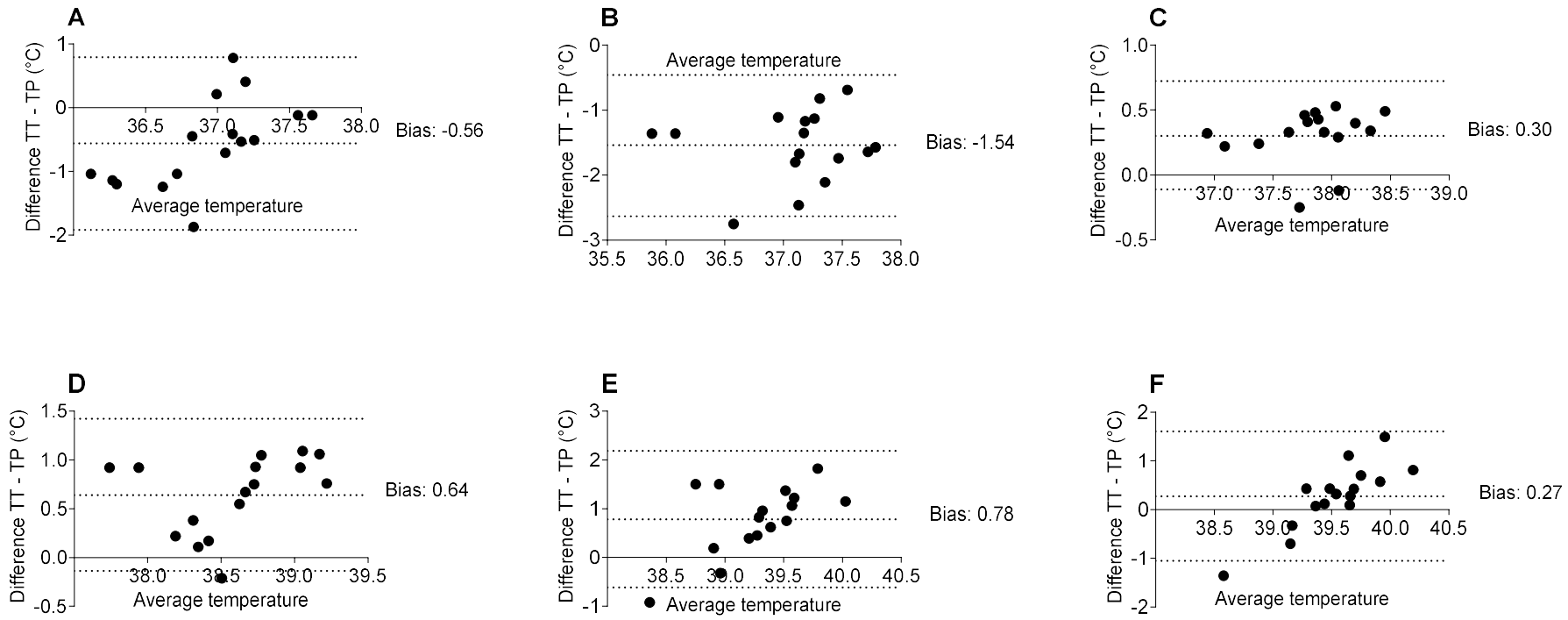

Figure 5.6. Bland-Altman plot indicating the mean bias (solid line) and 95% limits of agreement (dotted lines) in temperature between the Genious^{TM}2 tympanic thermometer (TT) minus the ingestible telemetric pill (TP) before (A) and after (B) the exercise session and at 10- (C), 20- (D), 30-(E), and 40-minute (F) time points during the hot water immersion session.

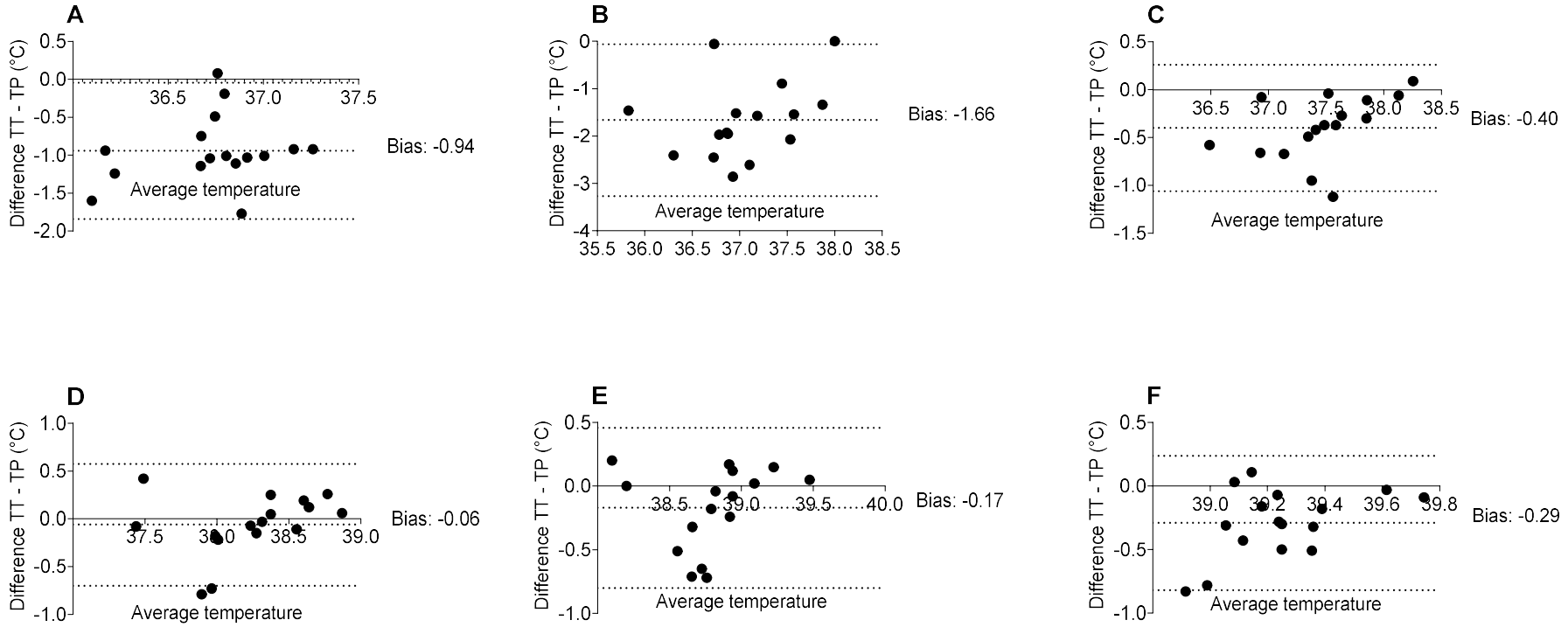

Figure 5.7. Bland-Altman plot indicating the mean bias (solid line) and 95% limits of agreement (dotted lines) in temperature between the Braun Pro 4000 tympanic thermometer (TT) minus the ingestible telemetric pill (TP) before (A) and after (B) the exercise session and at 10- (C), 20- (D), 30-(E), and 40-minute (F) time points during the hot water immersion session.

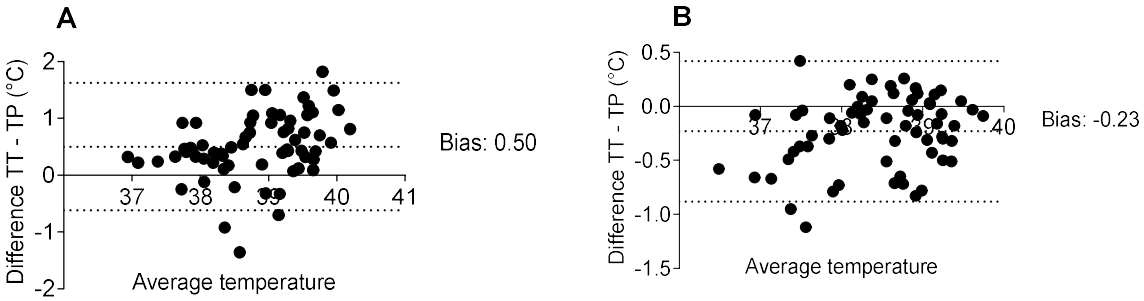

Figure 5.8. Bland-Altman plot indicating the overall mean bias (solid line) and 95% limits of agreement (dotted lines) in temperature between tympanic thermometers (Genius^{TM}2 [A] and Braun Pro 4000 [B]) minus ingestible telemetric pill (TP) during the hot water immersion session.

5.4. Discussion

This study examined the validity of two commercially available tympanic thermometers (Genius^{TM}2 and Braun Pro 4000) for monitoring body core temperature during post-exercise head-out 40°C water immersion. As expected, the hot water immersion protocol successfully induced an increase in gastrointestinal temperature above 39°C, which allowed assessment of the performance of the tympanic thermometers in response to the intended hyperthermic scenario. The key positive findings in this study are that 1) there were no significant ($p > 0.05$) differences between the temperature readings of the Braun Pro 4000 thermometer and telemetric pill at any time point during the hot water immersion; 2) there was a strong overall correlation ($r = 0.91$, $p < 0.01$) between the increase in gastrointestinal temperature and increase in tympanic temperature measured by Braun Pro 4000; and 3) the overall mean difference between the temperature reading of Braun Pro 4000 and that of the telemetric pill during the hot water immersion period was -0.22°C. These findings indicate that the Braun Pro 4000 tympanic thermometer is a valid device for monitoring core temperature during the post-exercise head-out 40°C water immersion protocol. Unfortunately, that is not the case with the Genius^{TM}2 tympanic thermometer since the temperature readings of this device were significantly higher than those of the telemetric pill during the hot water immersion (with the exception at the 10-minute time point) and the overall mean difference between the Genius^{TM}2 and telemetric pill during the hot water immersion period was 0.50°C.

The Genius tympanic thermometer is one of the most commonly studied brands of tympanic thermometers as an alternative method for monitoring deep body temperature in both health and disease (Barnett et al., 2011; Brogan et al.,1993; Easton et al., 2007; Giuliano et al., 2000; Hansen et al., 1996; Haugan et al., 2013; Imamura et al., 1998; Kocoglu et al., 2002; Mangat et al., 2010; Otani et al., 2020; Roth et al., 1996; Robinson et al., 1998; van Staaij et al., 2003). In the context of exercise-induced hyperthermia, the original model of the Genius thermometers did not pass the validity testing (Hansen et al., 1996; Easton et al., 2007; Roth et al., 1996). However, a recent study reported favourable findings while investigating an improved model (Genius^{TM}2) of this thermometer (Otani et al., 2020). Their participants (8 healthy males) completed three exercise trials at 70% peak power output until exhaustion in an environmental chamber. The air temperature (30°C) and relative

humidity (50%) were kept constant throughout the study, but each trial was conducted at different solar radiation including 0, 250, and 500 W/m^2. Both tympanic and rectal temperatures were measured at rest and every 6 minutes during exercise. Genius^{TM}2 was set to tympanic mode, and the mean values of two consecutive temperature readings were used for analyses. The time to exhaustion for the 0, 250, and 500 W/m^2 trials was 46 ± 10 minutes, 43 ± 10 minutes, and 30 ± 7 minutes, respectively. The researchers found no statistically significant difference between the rectal and tympanic temperatures, neither at rest nor during exercise. The agreements between tympanic and rectal temperatures during exercise were –0.11°C, –0.13°C, and –0.03°C in the 0, 250, and 500 W/m^2 trials, respectively. These findings justified the decision for Gemius^{TM}2 to be one of the tympanic thermometers examined in the current study.

Although the main focus of this study was on the performance of the tympanic thermometers during the post-exercise hot water immersion, considering the findings of Otani et al., (2020), one cannot ignore the fact that the temperatures recorded by Genius^{TM}2 before and after the 40-minute exercise session at 14°C were significantly ($p < 0.05$) lower than those of the telemetric pill. It should be noted, though, that the mean difference between the tympanic and deep body temperatures at rest in the present study (-0.5°C) was the same as that reported by Otani et al., (2020) before the trial under solar radiation of 500 W/m^2 (-0.5°C). A striking difference between the data collected in response to exercise in the present and the former study is the level of agreement between the tympanic and deep body temperatures (-1.5°C vs. -0.1°C). Since the thermometers in both studies were set to tympanic mode, the inconsistency cannot be attributed to that factor. It is possible that the tympanic temperature in the current study was affected by the cool (14°C) air in the environmental chamber, causing the tympanic temperature to be 1.5°C lower than the gastrointestinal temperature following the exercise session. The former notion is supported by the evidence that tympanic temperature is significantly reduced during exposure to cool air (Briner, 1996; Keatinge and Sloan, 1975; Teunissen et al., 2011). Indeed, studying 11 male and 7 female normothermic volunteers, Keatinge and Sloan (1975) reported that 20 minutes of passive exposure to 9°C decreased tympanic temperature by 2.3°C although esophageal temperature was virtually unchanged. In another study on seven fit normothermic participants (5 males and 2 females), resting for 10 minutes at 10°C

had no effects on esophageal or rectal temperatures, but it dropped the tympanic temperature by ~1°C (Teunissen et al., 2011). A study that measured tympanic and rectal temperatures in thirty runners who presented to the medical tent after a marathon race at 10°C found that tympanic temperature was lower than rectal temperature by at least 1°C in twenty runners (Briner, 1996). There is also evidence that the decrease in tympanic temperature resulting from exposure to cool air can be prevented by insulating the external ear canal with cotton wool (Teunissen et al., 2011), but it was beyond the scope of the present study to investigate the potential effect of covering the ear on tympanic temperature during the exercise session.

In the context of the discussion above, observations of significantly lower tympanic temperature compared to deep body temperature during exercise are not uncommon even when trials were conducted in hot conditions (Deschamps et al., 1992; Casa et al., 2007; Coso et al., 2008; Easton et al., 2007; Ganio et al., 2009). In such instances, tympanic versus deep body temperature lag has been attributed to selective brain cooling. The latter process, which takes place during hyperthermia as a physiological defence against cerebral overheating, was originally described in animals (Nagasaka et al., 1998). In these species, selective brain cooling during hyperthermia is enabled by panting and the presence of carotid rete. Panting in animals is a powerful heat loss mechanism, and it also cools the arterial blood destined for the brain (Johnsen et al., 1987). The role of carotid rete in the process is crucial since it enables arterial blood to be cooled right before it enters the brain (Jessen, 2001). While humans do not possess carotid rete and do not pant (except during maximal exercises using large muscle groups), mechanisms that selectively cool the human brain when the body experiences hyperthermia have been proposed (Cabanac, 1993; Nagasaka et al., 1998). According to Cabanac (1993), sweating-induced heat loss from the head and heat loss from the upper airways are sufficient to cause selective brain cooling during hyperthermia in humans. However, some researchers refute the concept of selective brain cooling in humans and suggest that the disparity between tympanic and deep body temperatures during hyperthermia results from contamination of tympanic temperature by skin temperature (Brengelmann, 1993). Although the researcher has provided evidence to support his idea, there are instances where the gap between the tympanic and deep body temperatures in hyperthermic humans is hard to explain if one ignores the possibility of the existence

of selective brain cooling in humans. For instance, Brinnel and Cabanac (1987) measured tympanic and core temperatures simultaneously in comatose patients from 33 to 42°C and reported that both temperatures increased similarly up until 38°C, from which point the increase in tympanic temperature was lower than rectal temperature.

Considering the concept of selective brain cooling, the finding that tympanic temperature measured by Genius^{TM}2 during the hot water immersion was, on average, 0.5°C higher than gastrointestinal temperature ($p > 0.05$) was somewhat surprising. This is especially true since the degree of hyperthermia induced by the hot water immersion (39.3°C) was higher than the threshold of hyperthermia (38.7°C) above which selective brain cooling is triggered (Kuhnen and Jessen, 1991). Unlike Otani et al., (2020), who validated Genius^{TM}2 during exercise in the heat, the present study showed that hot water immersion has an unfavourable impact on the agreement of deep body temperature and tympanic temperature measured by Genius^{TM}2. The cause(s) of inconsistency in the validity of the Genius^{TM}2 tympanic thermometer to predict deep body temperature under different hyperthermic scenarios is unknown. However, there is a methodological difference between the studies that is worth noting. The current study relied on a single measurement of tympanic temperature, whereas Otani et al., (2020) took two consecutive measurements and used their average value for analyses. In a clinical trial, the latter procedure produced more narrow limits of agreement between tympanic and rectal temperatures compared to measuring ear temperature once (Stavem et al., 1997). Interestingly, the broadest limits of agreement between tympanic and body temperatures (-0.6 to 1.2°C) during the trials in Otani's study are narrower than those observed in the current study during the hot water immersion session (Figure 5.8. A). Yet, the Bland-Altman plots presented in the former study (Stavem et al., 1997) revealed no difference in the mean biases between tympanic and rectal temperatures regardless of whether the tympanic temperature was sampled twice or just once. Nonetheless, whether those findings apply to healthy individuals during exercise in the heat or hot water immersion remains to be tested. It is also interesting that Genius^{TM}2 overestimated the gastrointestinal temperature during the hot water immersion even though the device was set in tympanic mode. Theoretically, if the “core equivalent” mode had been used, the average difference between tympanic and gastrointestinal temperatures would have been 1.5°C since the former temperature is computed by adding 1°C to the tympanic temperature.

Braun is another brand of tympanic thermometers whose products are frequently used when comparing tympanic with deep body temperatures in various populations (Bock et al., 2005; Casa et al., 2007; Duru et al., 2012; Fenemore et al., 2020; Ganio et al., 2009; Hamilton et al., 2013; Keene et al., 2015; Mangat et al., 2010; Mogensen et al., 2018; Morán-Navarro et al., 2018; Morrissey et al., 2021; Teller et al., 2014; Wan et al., 2022). Although the model ThermoScan® 7 IRT6520 was validated for use during exercise in the heat (Fenemore et al., 2020; Morán-Navarro et al., 2018), the idea to examine the Braun Pro 4000 Thermoscan thermometer in the current study came from the observations that this device is used to monitor body temperature during post-exercise hot water immersion in practice (Philip et al., 2022). Also, there was some evidence, though weak, that Braun Pro 4000 Thermoscan might be a suitable substitute for the expensive ingestible telemetric pills or invasive rectal probes for monitoring core temperature during the post-exercise head-out 40°C water immersion hot water immersion (Bock et al., 2005). These researchers compared tympanic thermometry with pulmonary artery thermometry in twenty-six patients undergoing cardiac surgery under ambient temperatures ranging from 18 to 27°C. Tympanic temperature was measured using a portable thermometer (IRT 4000, Braun GmbH, Germany), whereas measuring the pulmonary artery temperature served as a reference against which the tympanic thermometer was validated. It was reported that the agreement between the tympanic and pulmonary artery temperatures was 0.08°C, with 95% confidence intervals between –0.44°C and 0.61°C.

In the current study, the above findings that tympanic temperature measured by Braun Pro 4000 Thermoscan reflects deep body temperature in patients were extended to trained individuals immersed in hot water following exercise. The bias between temperatures in Bock's study is smaller than in the current study (-0.2°C), though the limits of agreement are similar to those in the current study (-0.8°C to 0.4°C). The former could be attributed to the methodological differences between the studies. Indeed, the measurements in Bock's study were conducted under steady-state conditions, whereas the current study compared the temperatures under conditions of a rapid change in deep body temperature. Fenemore et al., (2020) and Morán-Navarro et al., (2018) reported comparable biases (0.1°C and 0.2°C, respectively) to that in this study in studies where Braun ThermoScan® 7 IRT6520 was validated for monitoring deep body temperature during exercise in the heat. In contrast, using

model IRT 4520 of the Braun thermometers, Casa et al., (2009) and Ganio et al., (2007) reported unacceptable biases between rectal and tympanic temperatures during outdoor (-1.0°C) and indoor (-0.67°C) exercise in the heat, respectively. An even larger bias (-2.4°C) than in those two studies was reported by Morrissey et al., (2012), who compared tympanic temperature measured by model Pro 6400 and rectal temperature in 26 heat stroke patients following a long-distance running race. The inconsistency in the findings between the cited studies (Casa et al., 2007; Fenemore et al., 2020; Ganio et al., 2009; Morán-Navarro et al., 2018; Morrissey et al., 2021), including this study, regarding the validity of the Braun tympanic thermometers to predict deep body temperature under hyperthermic conditions could be explained by the fact that different models of thermometers were used across the studies. The findings that Braun Pro 4000 Thermoscan is valid for monitoring deep body temperature during post-exercise hot water immersion would be welcomed by those who use the former method as a means for heat acclimation. However, the findings could also be relevant to other populations since hot water immersion has been promoted as an alternative to physical exercise (Kjertakov et al., 2023; Kjertakov and Petersen, 2022).

Given that the same person collected all the data in this study, the discrepancy between Braun Pro 4000 Thermoscan and Genius^{TM}2 in predicting deep body temperature during hot water immersion cannot be attributed to the operator's technique in measuring ear temperature. Interestingly, the bias between the temperature reading of Braun Pro 4000 Thermoscan and the telemetric pill following the exercise session was similar to that of Genius^{TM}2 and the telemetric pill (Figure 5.8). While the former observation is likely a result of contamination of tympanic temperature with cool air, as previously explained for Genius^{TM}2, it is surprising that the thermometers performed similarly during the exercise session but differently during the hot water immersion session. It is possible that the contamination of tympanic temperature with the cool air during the exercise session masked the potential difference between the thermometers. Regardless, the findings that Genius^{TM}2 and Braun Pro 4000 Thermoscan underestimated gastrointestinal temperature by 1.5°C and 1.6°C, respectively, during exercise at 14°C suggest that these devices should not be used for triage of ill athletes during endurance sporting events in cool conditions. Theoretically, an underestimation of core temperature by ~1.5°C in a heat

stroke victim whose true core temperature is 41°C would lead to a misdiagnosis and potentially fatal outcome due to inappropriate treatment (Kjertakov and Yoram, 2013).

This study has some limitations. One limitation is that the intra- and inter-tester reliability associated with the use of Genius^{TM}2 and Braun Pro 4000 Thermoscan was not determined. However, a study on 70 healthy individuals and 30 febrile patients showed that both thermometers possess high reliability (Mangat et al., 2010). Indeed, the mean difference in repeated measurement performed in the left ear by the same tester was -0.04°C and -0.03°C for Genius^{TM}2 and Braun Pro 4000 Thermoscan, respectively. The mean difference between testers was -0.1°C and -0.04°C for Genius^{TM}2 and Braun Pro 4000 Thermoscan, respectively. Another limitation of this study is that tympanic temperature was sampled only once in each ear. Sampling tympanic temperature at least twice could have allowed some additional analyses. For example, one study found that using the average of two ear measurements vs. a single measurement narrowed the limits of agreement between tympanic and rectal temperatures in 103 patients (Stavem et al., 1997). Another study on 100 patients showed that measuring tympanic temperature twice in both ears and retaining the highest measurement produced the lowest interval between the limits of agreement in Bland-Altman plots exploring the difference between tympanic and rectal temperature (Smitz et al., 2009). It would have been interesting to see whether more favourable outcomes could have been found, particularly for Genius^{TM}2, if the current study had adopted the methodologies of those two studies. Finally, the positive findings of this study might not be applicable beyond the studied age group or sex, although it was shown that age and sex do not influence the correlation between tympanic and rectal temperatures (Stavem et al., 1997).

In conclusion, the current study showed that the Braun Pro 4000 Thermoscan tympanic thermometer could be a suitable substitute for the expensive ingestible telemetric pills or invasive rectal probes for monitoring core temperature during the post-exercise head-out 40°C water immersion. Indeed, no statistically significant difference in temperature readings was found between Braun Pro 4000 Thermoscan and telemetric pill (the reference method) at any time point during the hot water immersion period, and the overall bias in temperature reading provided by Braun Pro 4000 Thermoscan relative to telemetric pill was within the acceptable limit (< 0.3°C).

Unfortunately, the Genius^{TM}2 tympanic thermometer did not pass validity testing since the overall mean difference in temperature reading between this device and the telemetric pill during the hot water immersion period was 0.50°C.

Chapter six: General discussion

The promotion of heat acclimation as a strategy for preparing non-heat acclimated endurance athletes to compete in the heat began in the 1980s (American College of Sports Medicine, 1987; Armstrong, 1988; Sutton and Bar-Or, 1980) and intensified in the 1990s (Armstrong et al., 1996; Armstrong and Maresh, 1991; Lloyd, 1994; Maughan and Shirreffs, 1997; Montain et al., 1996; Sparling and Millard-Stafford, 1999; Terrados and Maughan, 1995) and 2000s (Binkley et al., 2002; Maughan and Shirreffs, 2004; Reilly et al., 2006; Seto et al., 2005; Sparling, 2000; Wendt et al., 2007). The papers published throughout those three decades suggested that successful heat acclimation requires exercising in the heat for 10 to 14 days. Several papers published during the subsequent decade documented that significant endurance performance improvements can be achieved following only five days of exercise in the heat (Garret et al., 2012; James et al., 2017; Racinais et al., 2015b; Wingfield et al., 2016). Although the practical significance of the latter findings for athletes with tight competition schedules is obvious, even the short-term heat acclimation protocol requires access to an environmental chamber or to be able to travel to the competition venue at least one week in advance and complete the heat acclimation process there. Since both options are beyond the reach of many athletes, numerous studies over the past several years were devoted to testing more practical and easily accessible heat acclimation strategies, with the main focus being on hot water immersion (Ashworth et al., 2023; Greenfield et al., 2021; Kissling et al., 2022; McIntyre et al., 2021, 2022; Waldock et al., 2021; Zurawlew et al., 2016, 2018a, 2018b, 2019). However, only two of those studies, which used the six-day post-exercise head-out 40°C water immersion intervention in physically/recreationally active individuals, assessed endurance exercise performance and reported inconsistent findings (McIntyre et al., 2021; Zurawlew et al., 2016). The latter and the lack of data about the ability of the six-day post-exercise head-out 40°C water immersion to improve endurance performance in the heat in previously non-heat acclimated trained athletes was the motive for the main aim of the first study. An additional aim was to examine whether athletes can use this heat acclimation strategy to improve their exercise performance when competing under moderately warm environmental conditions.

As indicated in the third chapter, the hot water immersion intervention after exercise for six days significantly reduced peak heart rate, peak thermal sensation, and peak rating of perceived exertion by 7.37 beats·min^{-1} (p = 0.03), 0.56 AU (p < 0.01), and 1.00 AU (p = 0.02), respectively during the 30-minute continuous test at 27°C. Despite those changes, the exercise performance of the HWI group during the 20 km time trial test under the same conditions showed no significant improvement. A first suggestion for such an outcome could be that the magnitude of reduced cardiovascular and perceptual strain following the intervention in the HWI group was insufficient to improve time trial performance significantly. The findings from the second study (Chapter Four) also suggest that the HWI participants might not have been able to perform at their best due to the increased inflammation. Indeed, the post-intervention resting blood sample showing significantly increased IL-1β level in the HWI group was taken on the day of testing at 27°C. While there are several potential mechanisms by which chronic inflammation may impair exercise performance, one that could have impacted the time trial performance at 27°C in the present study is reduced mitochondrial respiration (Cheng et al., 2020). The importance of mitochondrial respiration for cycling time trial performance is highlighted by Jacobs et al., (2011) in the study where the former variable was identified as the best predictor for the 26 km time trial performance in sixteen highly trained cyclists. Overreaching, muscle weakness, and chronic fatigue are other factors associated with chronic inflammation that could impair exercise performance (Cheng et al., 2020). However, the items in the wellness questionnaire, whose purpose was to signal the development of any of those conditions, were unchanged over time in the HWI group.

Making an inference about the potential impact of the inflammation on the post-intervention time trial at 35°C in the HWI group is difficult since it is unknown how long the chronically increased IL-1β persisted after the day it was detected. It is also difficult to explain the findings that the HWI group showed significantly reduced cardiovascular strain during the continuous test at 27°C but not at 35°C, even though the tests were separated by only 48 hours. If, as theorised in Chapter Three, the significantly decreased peak heart rate during exercise at 27°C was induced by expansion in plasma volume, the expanded plasma volume was likely diminished two days later, nullifying the cardiovascular benefits of the hot water immersion intervention. Support for the proposed behaviour of plasma volume post-intervention in the HWI group

comes from a study that examined the effects of repeated post-exercise sauna bathing on plasma volume in competitive male rowers (Creasy et al., 2003). In that study, plasma volume on day two post-sauna increased by 3.2% but declined by -1.7% on day five. The findings that neither cardiovascular nor thermoregulatory strain was significantly reduced during the continuous test at 35°C in the HWI participants explain the lack of a significant improvement in their time trial performance in the heat. Nevertheless, Chapter Three concludes that a 1.77% reduction in the finishing time of the time trial test in the heat following the post-exercise hot water immersion intervention, likely attributable to reduced perceptual strain, is practically significant. Theoretically, if athletes spend one hour at a core temperature of ≥ 38.5°C during the hot water immersions instead of only 15-20 minutes (Figures 3.2 and 3.3), they should gain significant thermoregulatory benefits and, as a result, greater exercise performance improvement. However, the former requires replacing the current head-out hot water immersion protocol with one that allows participants to maintain the core temperature at the targeted level for one hour. Remaining submerged up to the neck in 40°C water until the core temperature gets to 38.5°C and then moving the trunk up such that the water reaches chest level while keeping the arms outside the bath would reduce the thermal load and should allow maintenance of the core temperature between 38.5 and 39.0°C for one hour. Similar hot water immersion protocols have been described in the literature (Brunt et al., 2016; James et al., 2021; Kissling et al., 2022). Since monitoring core temperature is crucial for effectively implementing the modified hot water immersion protocol, potential users of this protocol will welcome the findings from the third study (Chapter Five) that the Braun Pro 4000 Thermoscan is valid for such a purpose. However, the ergogenic properties of the proposed hot water immersion protocol require examination.

Hailes et al., (2011) showed in fifteen unacclimated recreationally-trained males that a five-day heat acclimation consisting of daily cycling at 38°C until the rectal temperature reached 39.5°C (~40 minutes) induces chronic inflammation. Indeed, of the eighty cytokines measured, resting values of eighteen were significantly increased on the fifth day compared to the first day. In another study, Kaldur et al., (2014) reported that ten days of heat acclimation consisting of 110 minutes of exercise per day at 42°C increased resting oxidative stress. Although the potential clinical consequences associated with the increased inflammation and oxidative stress

following heat acclimation in the study by Hailes et al., (2011) and Kaldur et al., (2014), respectively, remained unknown, both conditions may negatively impact exercise performance and health (Cheng et al., 2020). The latter, and the possibility that the six-day post-exercise hot water immersion intervention might induce chronic inflammation and oxidative stress justified the conduction of the second study.

The second study (Chapter Four) showed that acute post-exercise hot water immersion did not induce significant changes in plasma concentration of either cytokines (TNF-α, IL-1β, IL-1ra, IL-6, IL-10) or TBARS and TAC. Given that increased inflammation and oxidative stress are part of the acute phase immune response (Cannon and Blumberg, 2000), the above findings suggest that acute post-exercise hot water immersion does not pose a challenge to the immune system in well-trained male endurance athletes. However, the functioning of the immune system of the HWI group could have been impacted after six consecutive days of post-exercise hot water immersion due to a disrupted balance between pro- and anti-inflammatory cytokines. If the inflammation induced by repeated post-exercise hot water immersion indeed interferes with the function of the immune system, completing this heat acclimation strategy the week before a race could further increase the risk of minor illnesses associated with heavy exertion during the event (Nieman, 1997). Even a minor illness developed during a cycling stage race could ultimately impair exercise performance. None of the participants in the HWI group reported health issues, but as explained earlier in the text, there are other pathways unrelated to health status by which chronically increased inflammation could have impaired their time trial performance following the six-day intervention (Cheng et al., 2020; Moldoveanu et al., 2001). Unfortunately, it was beyond the scope of the second study to determine the biological significance of increased IL-1β following the post-exercise hot water immersion intervention. Nor does the study design allow us to make inferences about the source of the increase in plasma IL-1β.

Besides the theoretical implications of chronically increased inflammation in the HWI group, it could also be theorised that the former condition developed probably because the recovery time between two sessions was insufficient for the acutely elevated IL-1β level to return to normal before the next session culminating in chronically elevated IL-1β (Smith, 2003). While the finding that IL-1β was unchanged in the HWI group

following the first session does not support such a theory, there is evidence that plasma IL-1β concentration does not increase significantly until after 2 hours of maximal exercise (Suzuki et al., 2002). Thus, there is a chance that the first post-exercise hot water immersion session led to a significant increase in IL-1β after the blood sample was obtained. If the proposed theory is valid, completing hot water immersion sessions on alternative instead of consecutive days should allow enough time for IL-1β levels to normalise between the sessions. It should be mentioned, though, that the effects of the latter approach on exercise performance in the heat are unknown. An alternative recommendation for athletes intending to use this heat acclimation strategy to prepare for sporting events in the heat would be to complete the last hot water immersion session at least one week ahead to minimise the risk of entering the event in a state of inflammation. The available evidence indicates that reduced perceptual strain during exercise in the heat induced by this heat acclimation strategy, and the only benefit of it observed in the HWI group in the first study, is retained for at least two weeks (Zurawlew et al., 2019). However, the performance benefits (1.7%) trained cyclists can gain from this heat acclimation method might not be worth the cost when preparing for short (30-60 minutes) endurance cycling events in the heat since a simple pre-exercise cooling by means of consuming ice drink can improve exercise performance by 6.5% during such an event (Ihsan et al., 2010).

Accurate assessment of body core temperature in situations imposing a risk for developing dangerous hyperthermia, such as the post-exercise head-out 40°C water immersion, is vital for preventing potentially fatal heat stroke (Moran and Mendal, 2002). Although both ingestible telemetric pills and rectal probes allow accurate measurement of core temperature under thermally challenging conditions (Lim et al., 2008), these thermometric methods are most often restricted to laboratory settings. The invasive nature of rectal probes and the high cost of ingestible telemetric pills limit their use in the field. Given that, it is not surprising that numerous studies attempted to identify a thermometric device that is sufficiently accurate but, at the same time, noninvasive, portable, and inexpensive. Tympanic thermometers appear to be the most studied devices, and a few reports confirmed their validity to monitor core temperature during exercise in the heat (Fenemor et al., 2020; Morán-Navarro et al., 2019; Otani et al., 2020). However, since the applicability of those findings is likely limited to the studied interventions, the usefulness of tympanic thermometers to

monitor core temperature during post-exercise head-out 40°C water immersion needs to be confirmed before these devices are accepted as viable screening tools for hyperthermia during the latter scenario. That gap in the literature stimulated the conducting of the third study.

The decision for Genius^{TM}2 to be one of the tympanic thermometers examined in the third study (Chapter Five) was based on evidence that this device is valid for monitoring core temperature during exercise in the heat (Otani et al., 2020). On the other hand, the idea to examine the Braun Pro 4000 Thermoscan thermometer came from the observations that this device is used to monitor body temperature during post-exercise hot water immersion in practice (Philip et al., 2022). The third study found no statistically significant difference in temperature readings between Braun Pro 4000 Thermoscan and telemetric pill (the reference method) at any time point during the hot water immersion period, and the overall bias in temperature reading provided by Braun Pro 4000 Thermoscan relative to telemetric pill was within the acceptable limit (< 0.3°C). Accordingly, it was concluded that the Braun Pro 4000 Thermoscan tympanic thermometer could be a suitable substitute for the expensive ingestible telemetric pills or invasive rectal probes for monitoring core temperature during the post-exercise hot water immersion. Unfortunately, the temperature readings of the Genius^{TM}2 tympanic thermometer were significantly higher than those of the telemetric pill during the hot water immersion (with the exception at the 10-minute time point), and the overall mean difference between the devices during the hot water immersion period was 0.50°C. The latter findings suggest that the evidence that a tympanic thermometer is valid for monitoring core temperature during exercise in the heat does not guarantee that the same device will be valid for use during different hyperthermic scenarios such as hot water immersion.

Limitations

The primary limitation of this PhD project is the lack of female participants. The initial plan was to include a female group, but the onset of the COVID-19 pandemic forced us to deviate from this plan. The regional lockdown during the pandemic disrupted the recruitment process and postponed the timely start of the studies. Subsequently, there was not enough time left to recruit female participants. The limitation of the first study is the adoption of a parallel-group design rather than a cross-over design. The latter

design is considered to be more powerful than the former. Another limitation of the first study is that the order of the testing was not randomised i.e., testing in a hot condition was always preceded by testing in a warm condition. This design was adopted from the study by Zurawlew et al., (2016), where testing in a thermoneutral condition was always followed by testing in a hot condition.

Directions for further research

In a study on ten physically active males, Zurawlew et al., (2016) found significantly improved 5 km running time trial performance (4.9%, p = 0.01) following six post-exercise hot water immersion sessions. Findings from the third study (Chapter Four) showed practically (1.77%), though not statistically significant (p = 0.06), improvement in 20 km cycling time trial performance in the heat in well-trained male cyclists using the same heat acclimation strategy as Zurawlew et al., (2016). However, the findings from these studies are not directly applicable to females due to considerable sex differences in responses to short-term heat acclimation (Mee et al., 2015). Since exercise performance outcomes of this heat acclimation strategy in females are unknown, future studies need to address that question. The effects of the six-day post-exercise hot water immersion intervention on inflammatory responses and prooxidant-antioxidant balance in females also require examination. Another finding from the project that might need confirmation in females is the validity of the Braun Pro 4000 Thermoscan thermometer for monitoring core temperature during the post-exercise hot (40°C) water immersion. Furthermore, several more tympanic thermometers await to be tested for monitoring core temperature during hot water immersion. Perhaps the first to be examined is Braun ThermoScan® 7 IRT 6520, as this device has been validated for use during exercise in the heat (Fenemore et al., 2020; Morán-Navarro et al., 2018).

Conclusion

The findings from the first study (Chapter Three) indicate that six post-exercise hot water immersion sessions reduce cardiovascular and perceptual strain during exercise at 27°C and reduce only perceptual strain during moderate-intensity exercise at 35°C. Although the post-exercise hot water immersion intervention did not significantly improve the 20 km time trial performance either at 27°C or 35°C, the reduction in the completion time of the latter test by 1.77% in the HWI group can be

considered practically significant. The second study (Chapter Four) showed that acute post-exercise hot water immersion had no significant effect on any measured biomarkers. Acute post-exercise immersion in water at 34°C induced a significant increase in IL-6. Furthermore, it was found that post-exercise hot water immersion over six consecutive days significantly increased resting plasma IL-1β concentration, which could be a cause for concern, knowing what consequences chronic inflammation could have on athletes' exercise performance and health. Finally, the third study (Chapter Five) found that the Braun Pro 4000 Thermoscan could be suitable for monitoring core temperature during post-exercise head-out 40°C water immersion hot water immersion.

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Appendix

Table 3.2. Summary of the self-reported wellness data collected right before the testing sessions in both groups. Data expressed as mean ± SD.

	Pre-intervention				Post-intervention				ANOVA's *p* values		
	HWI		CON		HWI		CON				
	27°C	35°C	27°C	35°C	27°C	35°C	27°C	35°C	Time	Group	T x G
Sleep quality	4.0 ± 0.8	3.5 ± 0.7	4.1 ± 0.6	4.1 ± 0.6	3.5 ± 0.7	3.7 ± 0.7	3.7 ± 1.2	3.2 ± 0.9	0.69	0.25	0.31
Muscle soreness	2.8 ± 1.2	3.7 ± 1.2	3.0 ± 0.8	2.7 ± 0.7	2.7 ± 2.0	3.0 ± 1.5	2.7 ± 1.4	3.0 ± 1.2	0.54	0.64	0.76
Fatigue	3.8 ± 1.9	4.1 ± 1.9	3.2 ± 2.2	3.5 ± 2.1	3.1 ± 2.0	4.0 ± 1.8	4.0 ± 2.2	4.1 ± 2.1	0.68	0.90	0.58
General health	8.2 ± 1.3	8.7 ± 1.3	8.0 ± 1.5	8.1 ± 1.3	7.8 ± 0.8	7.2 ± 2.0	7.1 ± 1.6	6.5 ± 1.6	0.29	0.09	0.52
Mood	8.2 ± 1.4	7.8 ± 1.2	7.1 ± 2.1	6.7 ± 1.3	7.4 ± 0.7	7.8 ± 1.2	7.4 ± 1.2	7.1 ± 1.5	0.10	0.95	0.44
Physical readiness	8.0 ± 0.8	7.4 ± 1.2	8.2 ± 2.0	7.8 ± 0.8	7.1 ± 0.6	7.2 ± 0.4	7.2 ± 2.5	7.0 ± 2.3	0.76	0.21	0.84
Mental readiness	9.0 ± 1.0	7.5 ± 1.3	8.5 ± 1.2	8.2 ± 1.7	7.2 ± 1.6	7.4 ± 1.2	7.4 ± 1.9	7.0 ± 1.8	0.33	0.13	0.26

Abbreviations: T x G – time and group interaction.

Table 3.3. Participants' hydration status assessed via urine specific gravity (USG) before the testing sessions. Data expressed as mean.

	Pre-intervention				Post-intervention				ANOVA's *p* values		
	HWI		CON		HWI		CON				
	27°C	35°C	27°C	35°C	27°C	35°C	27°C	35°C	Time	Group	T x G
USG	1.012	1.012	1.013	1.010	1.007*	1.013	1.014	1.013**	0.08	0.28	0.00

Abbreviations: T x G – time and group interaction.

*Significantly different from the post-intervention testing at 35°C in the HWI group, $p < 0.05$.

*Significantly different from the post-intervention testing at 27°C in the CON group, $p < 0.05$.

**Significantly different from the pre-intervention testing at 35°C in the CON group, $p < 0.05$.