HEAT ACCLIMATION FOR SPECIAL POPULATIONS

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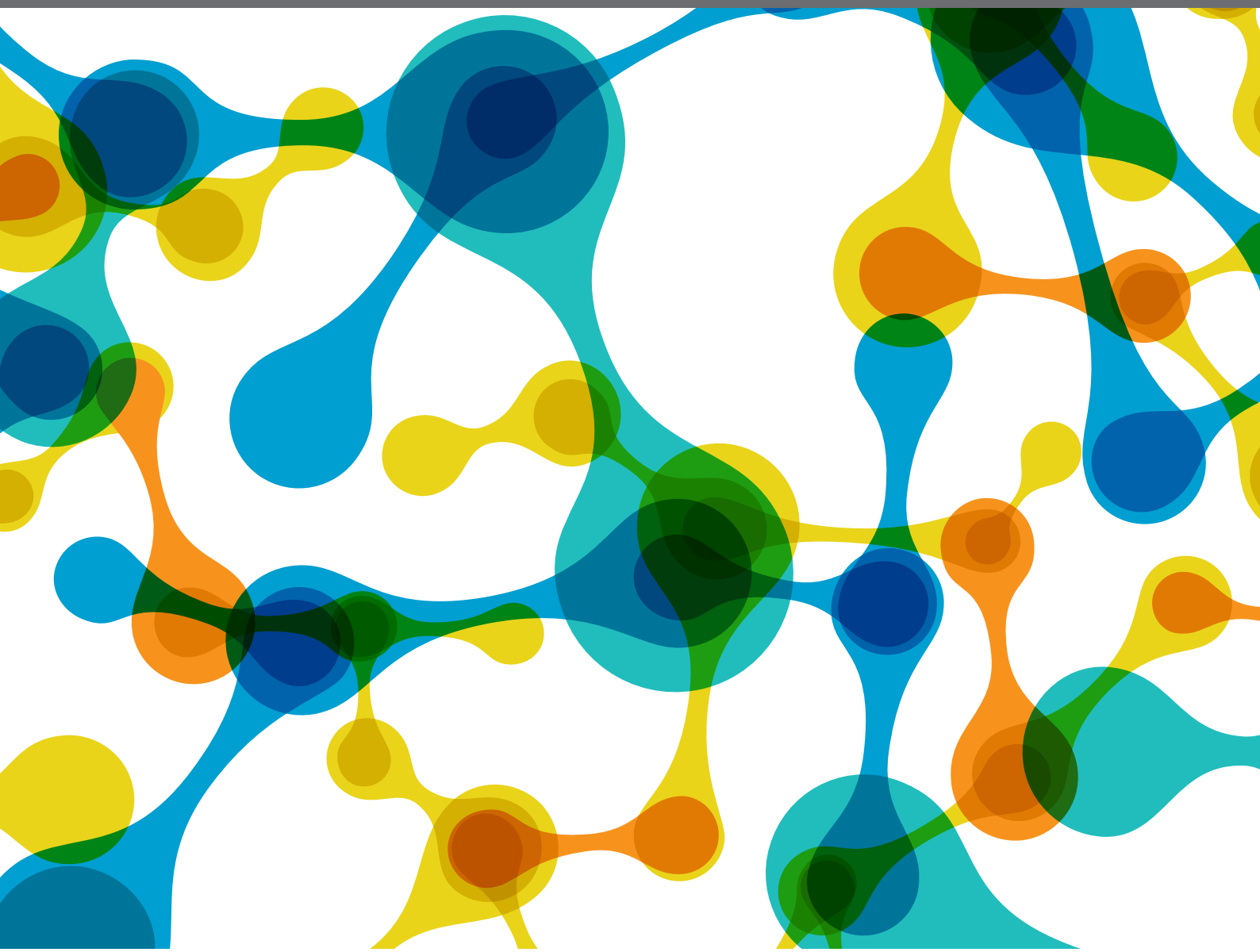

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HEAT ACCLIMATION FOR SPECIAL POPULATIONS

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EDITORIAL
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Editorial: Heat Acclimation for Special Populations

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Keywords: exercise, heat stress, heat training, heat acclimatization, heat therapy

Editorial on the Research Topic

Heat Acclimation for Special Populations

This heat acclimation for special population's Research Topic questions the "one size fits all" approach for heat adaptation and that it may not be appropriate for all populations. Therefore, to highlight these differences we endeavored to collect a set of studies on how heat acclimation may benefit a wide range of special populations who have specific needs.

We have published 12 articles in this Research Topic and defined four main areas of research. (a) an epidemiological approach and the aging process; (b) understanding physiological mechanisms and a novel heat acclimation method; (c) adaptation to the heat for special populations including males, females, military personnel and Paralympic athletes; and (d) the use of heat therapy for special populations. We have summarized the most noteworthy evidence of each study in these research areas.

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AN EPIDEMIOLOGICAL APPROACH AND THE AGING PROCESS

A study conducted by Folkerts et al. investigated how humans adapt or will adapt to heat stress caused by climate change over a long-term interval. They used three commonly used methods over a period of 23 years for older adults (≥65 years), in the Netherlands. The methods used were; minimum mortality temperature (MMT), defined as the mean daily temperature at which the lowest mortality occurs; heat sensitivity, the percentage change in mortality per 1°C above the MMT threshold, or heat attributable fraction, the percentage relative excess mortality above MMT. The key findings suggest that despite differences in the three different methods for determining MMT, the susceptibility of humans to heat decreases over time. This work suggests that future epidemiological research should focus on what factors (e.g., physiological, behavioral, technological, or infrastructural adaptations) influence human adaptation the most. Furthermore, this should lead to the promotion of specific adaptation policies for the general population.

PHYSIOLOGICAL MECHANISMS AND A NOVEL HEAT ACCLIMATION METHOD

Heat acclimation results in physiological adaptations dependent upon the duration of acclimation, type, mode of exercise, and environmental conditions employed. Research by Oberholzer et al. employed prolonged (5.5 weeks, 5 days.wk^{-1}) heat acclimation to determine hematological changes and reported that hemoglobin mass was increased by 3% and this was weakly correlated with plasma volume expansion. The mechanism for the hemoglobin mass increase may therefore be a compensatory response in erythropoiesis, secondary to the plasma volume expansion. However,

a study conducted by Kampmann and Bröde that employed prolonged heat acclimation over a minimum of 3 weeks, walking for 3 h per session showed that energy expenditure (Q_{10} effect) and thermal cardiac reactivity were unaltered by heat acclimation status. Overall, oxygen uptake increased by 7% and heart rate by 39–41 bpm, per degree increase in rectal temperature, which was independent of heat acclimation. Therefore, this has implications for the methods and models employed, using Q_{10} effect and thermal cardiac reactivity, for workplace heat stress assessments.

With more sporting' events taking place in hot environments, and global warming increasing temperatures, novel and practical acclimation methods are being investigated and employed. Heathcote et al. investigated the effects of the length of time delay (10 min, 1, 8 h) between running in temperate conditions and the acute thermoregulatory responses to 30 min of hot water immersion (39°C). Findings recommended undertaking hot water immersion within 10 min of completing training to maximize core temperature and heart rate responses and thus acclimation stimulus.

ADAPTATION TO THE HEAT FOR SPECIAL POPULATIONS INCLUDING MALES, FEMALES, MILITARY PERSONNEL, AND PARALYMPIC ATHLETES

Heat acclimation is widely accepted as the primary intervention one can adopt to optimize performance and work output in the heat (Racinais et al., 2015). In a meta-analysis, Benjamin et al. examined the magnitude of change in maximal oxygen consumption ($\dot{V}O_{2max}$), time to exhaustion, time trial, mean power, and peak power tests following heat acclimation. It was shown that acclimation provided the largest performance enhancement in time to exhaustion tests, followed by time trial, mean power, and peak power tests. The authors identified several factors that had affected the results of these performance tests, such as the method of heat acclimation induction, fitness level of participants and heat index.

Research by Mikkelsen et al. examined whether heat acclimation adaptations improved aerobic capacity and performance in cool conditions by comparing two groups of participants training for 5.5 weeks, one in the heat and the other in cool/control conditions. The authors noted that when tested in cool conditions, participants having been heat acclimated did not improve peak power output or aerobic capacity during atest. However, time trial performance in cool conditions improved in the heat acclimation group, albeit to a similar extent than the cool/control group. They concluded that training in the heat is not superior to training in normal conditions for improving aerobic power or time trial performance in cool conditions.

In a more short-term heat acclimation and sprint performance-oriented paper, Garrett et al. focused on the performance responses of female participants when controlling for menstrual cycle. Results indicated an improvement in mean power during maximal sprinting across the 5-day intervention, in line with reductions in core temperature and heart rate, and an increased plasma volume.

Military personnel face unique situations, such as short-notice deployment to hot operational environments that can present medical, occupational, and logistical challenges. In their review, Parsons et al. characterize the physical challenges that military training and deployment present, consider how heat acclimation can augment military performance in hot environments, and identify potential solutions to optimize the risk-performance paradigm.

With a focus on para-triathletes, Stephenson et al. investigated the effectiveness of mixed, active and passive heat acclimation with a controlled heart rate approach at inducing adaptation relative to able-bodied triathletes. The authors reported that para-triathletes displayed partial heat acclimation (i.e., thermoregulatory adaptations) but the extent to which was greater in the able-bodied cohort.

HEAT THERAPY FOR SPECIAL POPULATIONS

Musculoskeletal injuries disrupt the training, competition and careers of many athletes. Often presented as deconditioning of the cardiovascular, metabolic, and/or muscular systems depending upon the severity of the injury. A review by Ihsan et al. considered an alternative, therapeutic use for heat acclimation that provided evidence for repeated heat exposures embedded into the re-conditioning programs, as part of a *rehabilitation toolbox*, to assist athletes' recovery from injury.

Heat dissipating mechanisms are compromised in individuals with a spinal cord injury, especially in tetraplegia or high-level paraplegia. A review by Zhang and Bishop evaluated the risk of heat injury during training and competition amongst spinal cord injury athletes in order that risk stratification could lead to systematic heat policy to improve knowledge, medical support and protection for these athletes.

A review by Hunt et al. present a compelling perspective on the role of heat therapy and/or heat acclimation that may mediate the upregulation of heat shock proteins (HSP), as an intervention for protecting neurodegeneration in Alzheimer's and Parkinson's disease. The HSP response has been used as a marker of inflammation and heat adaptation. With the pathophysiology of these neurodegenerative diseases linked to loss of protein homeostasis, the authors suggest a heat-induced elevation in HSP from tolerable heat therapy, may improve neuromuscular function, cerebral blood flow, metabolic health, and quality of life. Furthermore, the use heat therapy may lend itself for the treatment of cardiovascular or metabolic diseases.

In summary, as more people travel from cooler to hotter climates, global temperatures increase and more occupations require people to work in hot environments, often at short notice, a better understanding of the individual benefits of heat acclimation is required. The need to understand how heat acclimation can alleviate thermal strain and enhance performance in all humans is essential. Furthermore, it is clear that the "one size fits all" approach may not be appropriate for all populations, as there are often special needs required for heat adaptation. From a sporting perspective, this information is important given the preparations required for the heat and

humidity of the 2021 Olympics in Tokyo, Japan (Gerrett et al., 2019). Importantly, the potential benefits of the use of heat therapy for special populations including the treatment of chronic disease remains largely unexplored and needs a great deal more attention.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Mixed Active and Passive, Heart Rate-Controlled Heat Acclimation Is Effective for Paralympic and Able-Bodied Triathletes

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Purpose: The aims of this study are to explore the effectiveness of mixed active and passive heat acclimation (HA), controlling the relative intensity of exercise by heart rate (HR) in paratriathletes (PARA), and to determine the adaptation differences to able-bodied (AB) triathletes.

Methods: Seven elite paratriathletes and 13 AB triathletes undertook an 8-day HA intervention consisting of five HR-controlled sessions and three passive heat exposures (35°C, 63% relative humidity). On the first and last days of HA, heat stress tests were conducted, whereby thermoregulatory changes were recorded during at a fixed, submaximal workload. The AB group undertook 20 km cycling time trials pre- and post-HA with performance compared to an AB, non-acclimated control group.

Results: During the heat stress test, HA lowered core temperature (PARA: 0.27 ± 0.32°C; AB: 0.28 ± 0.34°C), blood lactate concentration (PARA: 0.23 ± 0.15 mmol l^{-1}; AB: 0.38 ± 0.31 mmol l^{-1}) with concomitant plasma volume expansion (PARA: 12.7 ± 10.6%; AB: 6.2 ± 7.7%; $p \leq 0.047$). In the AB group, a lower skin temperature (0.19 ± 0.44°C) and HR (5 ± 6 bpm) with a greater sweat rate (0.17 ± 0.25 L h^{-1}) were evident post-HA ($p \leq 0.045$), but this was not present for the PARA group ($p \geq 0.177$). The AB group improved their performance by an extent greater than the smallest worthwhile change based on the normal variation present with no HA (4.5 vs. 3.7%).

Conclusions: Paratriathletes are capable of displaying partial HA, albeit not to same extent as AB triathletes. The HA protocol was effective at stimulating thermoregulatory adaptations with performance changes noted in AB triathletes.

Keywords: disability, thermoregulation, isothermic, acclimatization, triathlon, elite

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INTRODUCTION

Competitive sporting events are commonly held in hot and/or humid environments; therefore, strategies are commonly sought to attenuate the deterioration typical of endurance performance in such conditions (Daanen et al., 2018); one such strategy that is commonly used by athletes is heat acclimation (HA). Heat acclimation can invoke myriad positive adaptations, which

improve heat tolerance, including lower: core (T_c) and skin (T_{sk}) temperature; submaximal heart rate (HR); carbohydrate metabolism; and sweat electrolyte content. Additionally, it can lead to an increased sweat rate, plasma volume (PV) expansion, and positive perceptual alterations with a resultant improved performance in the heat (Corbett et al., 2014).

Heat acclimation typically involves daily or alternate days of heat stress over a 5–16-day period, whereby T_c, T_{sk}, and sweat rate are elevated for 1–2 h (Daanen et al., 2018). To provide a constant heat stress across HA, isothermic protocols have been employed, whereby the external workload is manipulated within- and between-HA sessions to maintain a T_c of ~38.5°C (Gibson et al., 2015; Neal et al., 2016; Ruddock et al., 2016). However, these approaches bring the financial burden of measuring T_c *via* ingestible sensors or participant discomfort from rectal temperature assessment. Furthermore, rectal temperature measurement poses a risk of autonomic dysreflexia in athletes with a spinal cord injury (Price and Campbell, 1999). Consequently, controlling HA intensity using HR has been proposed by Périard et al. (2015) as a practical method of maintaining a constant cardiovascular stimulus. Based on evidence that HR is unchanged through isothermic HA (Magalhães et al., 2010; Garrett et al., 2012; Zurawlew et al., 2015; Pethick et al., 2019), this method of controlling the relative intensity would result in a constant thermal load during HA. Initial evidence suggests that this approach may be efficacious in invoking HA in soccer players (Philp et al., 2017).

Despite the efficacy of HA for invoking thermoregulatory adaptations, commonly studied protocols may not be appropriate for elite athletes in preparation for competition, especially in a multi-modal sport such as triathlon. This is due to the protocols typically involving multiple days of exercise in the heat, which does not fit with the weekly training distribution of athletes tapering into competition (Mujika, 2011). As such, passive HA has recently been explored (Stanley et al., 2015; Zurawlew et al., 2015, 2018). Using post-exercise heat exposures, positive adaptations are achievable without excessive physical stress (Stanley et al., 2015; Zurawlew et al., 2015, 2018). Yet, while passive HA can stimulate positive responses, it has been questioned whether it can provide full heat adaptation (Périard et al., 2015; Tyler et al., 2016). As such, Guy et al. (2014) state that for athletes to optimally adapt to the heat, and in a time-efficient manner, protocols may best utilize a combination of active and passive HA.

While HA has been studied in a range of able-bodied (AB) athletes (Lorenzo et al., 2010; Garrett et al., 2012; Stanley et al., 2015; Ruddock et al., 2016), little attention has been paid to Paralympic athletes. These athletes are likely to be at heightened risk for performance decrements in the heat as a consequence of varied impairments in autonomic or behavioral thermoregulatory function (Webborn and Van de Vliet, 2012). In the sole published study of Paralympic athletes and HA, Castle et al. (2013) researched the adaptive potential of target shooters with a spinal cord injury. The athletes performed a 7-day protocol consisting of 20 min moderate intensity arm cranking followed by 40 min passive heat exposure and displayed several thermoregulatory adaptations. While this provided the first evidence of Paralympic athletes' capability to adapt to the heat, even to a relatively modest heat stimulus, it is not known whether HA is also effective in endurance-trained Paralympic athletes. Further, it is not known how adaptations to HA in Paralympic athletes differ from AB individuals.

The aims of this study were to investigate the efficacy of a mixed active and passive HA protocol in the sport of paratriathlon. To negate the issue of potential cost and discomfort associated with isothermic protocols, a controlled relative intensity design was utilized by regulating exercise intensity using HR, which may be more applicable for elite athletes. A further aim was to determine how HA adaptations may differ between Paralympic and AB athletes.

Abbreviations: AB, Able-bodied; AeLT, Aerobic lactate threshold; BLa, Blood lactate concentration; CV, Coefficient of variation; HA, Heat acclimation; HR, Heart rate; PO, Cycling power output; RPE, Rating of perceived exertion; PV, Plasma volume; SD, Standard deviation; T_c, Core temperature; TS, Thermal sensation; T_{sk}, Skin temperature; USG, Urine specific gravity.

MATERIALS AND METHODS

Participants

Twenty-nine (22 males and 7 females) paratriathletes and triathletes were recruited to partake in the present study. From this pool, three separate groups were formed: a group of elite paratriathletes [PARA; n = 7; amputation n = 3, incomplete spinal cord injury n = 1 (wheelchair user), hemiplegia cerebral palsy n = 1, lower leg impairment n = 1, visual impairment n = 1]; an AB HA group (AB-ACC; n = 13); an AB control group (AB-CON; n = 9; **Table 1**). Participants trained at least five times per week. All provided written informed consent and the procedures were approved by the Loughborough University Ethical Advisory Committee (R16-P010). No participants reported being heat acclimated/acclimatized prior to the start of the study.

TABLE 1 | Participant characteristics for the paratriathlon (PARA), able-bodied acclimation (AB-ACC), and able-bodied control (AB-CON) groups.

Parameter	PARA (four males, three females)	AB-ACC (nine males, four females)	AB-CON (eight males)
Age (y)	31 ± 9*	25 ± 7	21 ± 2
Body mass (kg)	67.8 ± 9.0	69.3 ± 9.4	70.0 ± 6.9
Cycling $\dot{V}O_{2peak}$ (ml·kg^{-1}·min^{-1})	57.7 ± 7.6	61.5 ± 6.4	62.7 ± 8.1
MAP (W)	324 ± 73	340 ± 74	379 ± 45
AeLT (W)	181 ± 48	187 ± 42	192 ± 28

*$\dot{V}O_{2peak}$, peak rate of oxygen uptake; MAP, maximal aerobic power output; AeLT, aerobic lactate threshold power output.*Significantly greater than AB-CON (p = 0.039).*

Study Design

Both the PARA and AB-ACC groups undertook an 8-day HA period. Due to the nature of the paratriathletes' pre-competition routine, it was not possible to gain a direct performance measure in this group. However, this was undertaken in the AB-ACC group pre- and post-HA. The AB-CON group solely undertook the performance trials with no HA to determine natural variation in performance (**Figure 1**).

During the study period, all participants were instructed to maintain their usual training routine. All testing was performed in the same geographical location with an average outdoor environmental temperature of 13.6 ± 6.2°C during the study period. Participants were free to drink *ad libitum* during all visits, but fluid intake was restricted to water. Participants were instructed to abstain from alcohol for 48 h before every trial while standardizing food, fluid, sodium, and caffeine intake. During every trial in the heat, participants were instructed to keep clothing consistent. All trials in the heat were conducted in an environmental chamber (Weiss Gallenkamp, Loughborough, United Kingdom; 35.1 ± 0.4°C, 63.4 ± 4.1% relative humidity) with a fan producing an airflow of 2.0 m s^{-1} at the body (5,400 FW, Kestrel Meters, Minneapolis, MN, United States). All trials were scheduled at the same time of day to limit the cofounding effect of circadian rhythm variation (Winget et al., 1985). Prior to all trials, participants first provided a urine sample for the determination of urine specific gravity (USG) by refractometer (PCE-032, PCE Instruments UK Ltd., Southampton, United Kingdom) before nude body mass was recorded *via* electronic scales (Adam Equipment Co. Ltd., Milton Keynes, United Kingdom). Participants with a USG ≥ 1.020 were advised to increase fluid intake pre-trial *via* ingesting 250–500 ml of water 30 min pre-trial. After the trials, nude body mass was again recorded after towel drying. Fluid intake was calculated from drinks bottle mass changes, and sweat loss was calculated from fluid intake and body mass changes. Sweat gain was calculated from sweat loss and T_c, where available.

Graded Exercise Tests

For all participants, the first trial consisted of a submaximal cycling graded exercise test for the determination of individuals' aerobic lactate threshold (AeLT) with a maximal graded exercise test for the determination of maximum HR, maximum aerobic power output (PO), and peak rate of oxygen uptake. This was the only visit conducted in temperate ambient conditions. During both tests, participants cycled on the Cyclus 2 ergometer (RBM elektronik-automation GmbH, Leipzig, Germany), using their own bicycle, at incremental POs. AeLT was determined from athletes' blood lactate concentration (BLa; Biosen C-Line, EKF Diagnostics, Magdeburg, Germany) and oxygen uptake (Metalyzer® 3B, Cortex Biophysik GmbH, Leipzig, Germany) using the methods of Beaver et al. (1985). HR was recorded continuously (Polar RS400, Polar, Kempele, Finland). Maximum HR, maximum aerobic PO, and peak rate of oxygen uptake were defined as the highest value recorded during any 5, 60, or 30 s epoch of the maximal test, respectively.

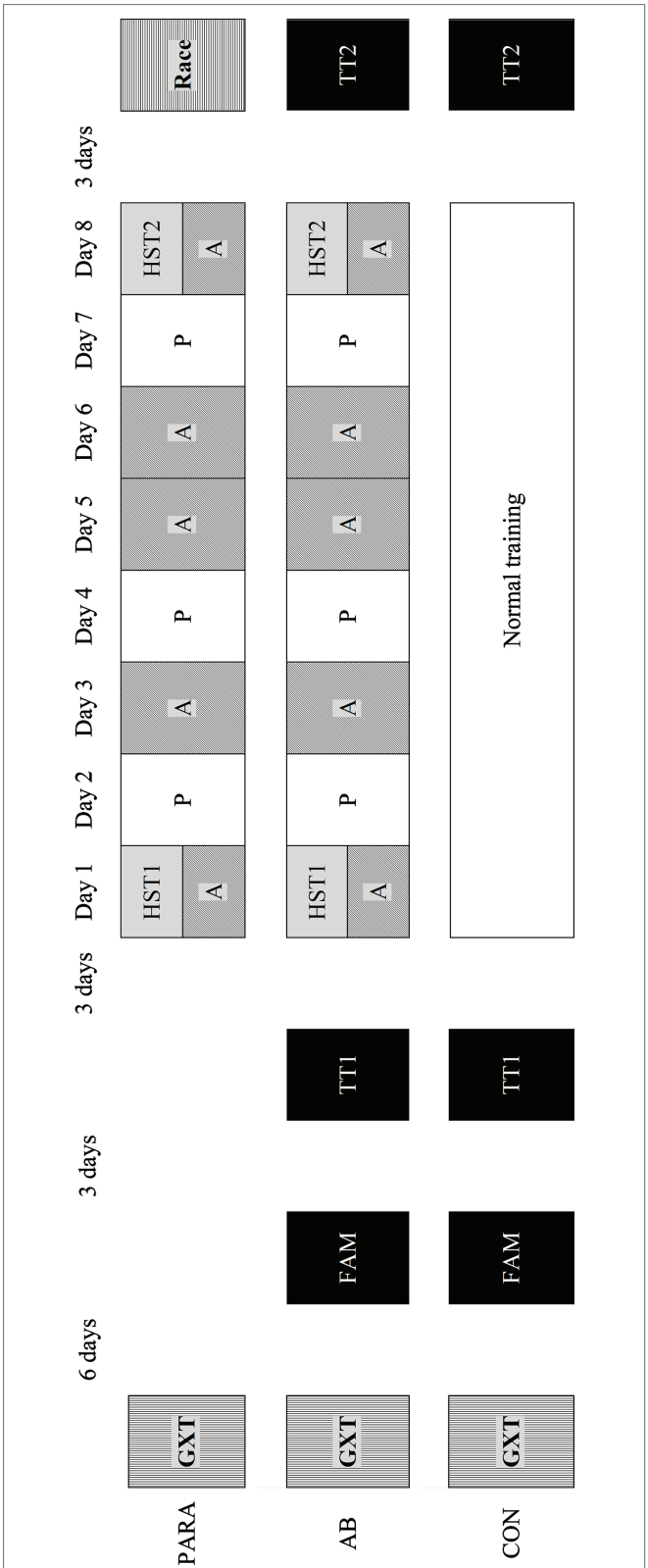

FIGURE 1 | Schematic of the study protocol. PARA, paratriathlon group; AB-ACC, able-bodied acclimation group; AB-CON, able-bodied control group; GXT, graded exercise test; FAM, familiarization; TT, performance trial; HST, heat stress test; A, active heat acclimation; P, passive acclimation.

Familiarization and Performance Trials

The performance trial consisted of a simulated 20 km cycling time trial in the heat. Six days after the graded exercise tests, participants were familiarized to the performance trial. The familiarization, pre-acclimation performance trial (TT1), and post-acclimation performance trial (TT2) were all performed on the Cyclus 2 ergometer. Warm-up for all performance trials was standardized to 10 min cycling at AeLT, followed by 5 min passive recovery. Participants were instructed to perform the test in the shortest amount of time possible with no encouragement given. During all trials, participants were blinded to all measures except distance covered. HR was recorded throughout, while capillary BLa was assessed pre-trial and every 5 km. Similarly, thermal sensation (TS) (Hardy, 1970) and rating of perceived exertion (RPE) (Borg, 1998) were collected every 5 km.

Heat Stress Tests

Participants were first instructed to rest in a seated position of 10 min for the provision of fingertip capillary blood samples, as performed elsewhere (Castle et al., 2013; Stanley et al., 2015; Ruddock et al., 2016). In duplicate, samples were collected in hematocrit tubes (Hawksley, Sussex, United Kingdom) for the determination of hematocrit, while 20 µl samples were collected in capillary tubes (EKF Diagnostics) for the measurement of hemoglobin. After blood samples were collected, participants were fitted with temperature loggers (DS1922L Thermochron iButton®, Maxim Integrated Products, Inc., Sunnyvale, CA, United States) using surgical tape to measure T_{sk} at four sites (*pectoralis major* muscle belly, lateral head of *triceps brachii*, *rectus femoris* muscle belly, and lateral head of the *gastrocnemius*) (Ramanathan, 1964), on the right side of the body, at a recording rate of 30 s. Subsequently, sweat patches (Tegaderm +Pad, 3 M, St. Paul, MN, United States) were placed at four sites (forearm, chest, upper back, and thigh), after cleaning with deionized water, for the collection of localized sweat on the left side of the body. The heat stress test (HST) consisted of 10 min standardized fixed intensity cycling at an intensity equating to 80% AeLT before immediately starting 40 min fixed intensity cycling at participants' AeLT. During the HST, T_c was recorded at 5-min intervals *via* ingestible T_c sensor (CorTemp, HQ Inc., Palmetto, FL, United States) taken ~6 h pre-trial. HR was recorded throughout, while BLa, TS, and RPE were collected pre-trial and at 10-min intervals. After the completion of the HSTs, participants exited the chamber and were instructed to rest in a seated position during which time T_{sk} loggers were removed and sweat patches were cleaned with deionized water and placed in collection tubes (Salivette®, Sarstedt, Nümbrecht, Germany) for later analysis. Capillary blood samples were then repeated as previously.

Active Heat Acclimation

Participants were permitted a self-selected 5 min (days 1 and 8) or 15 min (days 3, 5, and 6) warm-up. Participants were provided with an individualized 5 beat min^{-1} HR zone equating to ~80% maximum HR. This intensity was chosen, based on preliminary testing and previous data (Gibson et al., 2015; Pethick et al., 2019), to elicit a T_c of ~38.5°C. Active HA sessions lasted for 45 min (days 1 and 8) or 90 min (days 3, 5, and 6). During all sessions, participants were instructed to manipulate their cycling PO, *via* the Cyclus 2, to maintain a HR within the predetermined zone. During all sessions, cycling PO was recorded and stored on the Cyclus 2 before later export and analysis. HR was recorded continuously, while BLa, TS, and RPE were collected pre-trial and at 15-min intervals.

Passive Heat Acclimation

Passive HA sessions were performed on days 2, 4, and 7 and were structured to align with triathletes' typical weekly running frequency when tapering for competition (Mujika, 2011). Participants were instructed to undertake their normal run training, or to run for 30 min at a moderate intensity (RPE of 13) before entering the chamber. Participants then rested in the heat for 60 min. During passive HA sessions, HR was recorded every 10 min, while T_{sk} was assessed *via* an insulted skin thermistor (Squirrel SQ2010, Grant Instruments Ltd., Shepreth, United Kingdom) placed at the seventh cervical vertebra (Taylor et al., 2014). Finally, TS was noted pre-trial and at 20-min intervals.

Analytical Methods

Hematocrit

Hematocrit tubes were centrifuged (Haematospin 1,400, Hawksley) at 11,800 revolution min^{-1} for 5 min before being assessed *via* a tube reader (Hawksley). As samples were collected in duplicate, the mean value is presented. The coefficient of variation (CV) for duplicate samples was 1.3%.

Hemoglobin

A 20 µl blood samples were combined with 5 ml Drabkin's solution with the absorbance of the resultant mixture read *via* a zeroed spectrophotometer (Cecil series 1,000, Cecil Instruments Ltd., Cambridge, United Kingdom) at 540 nm. The mean absorbance value of the duplicate samples was subsequently translated into hemoglobin concentration. The CV for duplicate samples was 2.1%.

Plasma Volume Changes

Changes in PV were calculated from hematocrit and hemoglobin using the equation of Dill and Costill (1974).

Sweat Composition

Sweat samples at all four sites were first diluted by a 1:200 ratio in deionized water before being analyzed for sodium concentration *via* flame photometry (Model 410c, Sherwood Scientific Ltd., Cambridge, United Kingdom). All individuals' samples were analyzed in the same batch.

Statistical Analyses

All statistical analyses were conducted using IBM SPSS Statistics 23.0 software (IBM, Armonk, NY, United States). Statistical significance was set at $p < 0.05$. Data were checked for normal distribution using the Shapiro-Wilk test, where normal distribution was not present and non-parametric test were employed. Where

sphericity could not be assumed, the Greenhouse-Geisser correction was used. Differences in participants' physical and physiological characteristics between groups were assessed *via* the Kruskal-Wallis test. To determine the likelihood of a learning effect, PO between the familiarization trial and TT1 was compared *via* paired sample *t* test. Data from the AB-CON group were used to derive the CV in PO during the performance tests without HA. From this, the smallest worthwhile change in PO was calculated (Malcata and Hopkins, 2014). PO and HR were averaged over 5 km segments during TTs, while T_c, T_{sk}, HR, and PO were averaged over 5 min segments during HSTs, active HA, and passive HA where appropriate. Changes in PO, HR, T_c, T_{sk}, BLa, RPE, and TS between TT1 and TT2, HST1 and HST2, active HA sessions, and between passive HA sessions were assessed *via* two-way analysis of variance or the Friedman test within groups. Changes in pre-trial USG, fluid intake, sweat rate, sweat gain, and sweat sodium concentration were evaluated by paired sample *t* test, one-way analysis of variance or Wilcoxon's signed rank test. PV changes were assessed against a fixed zero by one-sample *t* test. Bonferroni, Mann-Whitney *U*, or Wilcoxon's signed rank *post hoc* tests were used to identify any significant differences where appropriate. Data are presented as mean ± standard deviation (SD) where appropriate.

RESULTS

Participant Characteristics

The PARA group were older than AB-CON (p = 0.009), but there were no significant differences in body mass, peak rate of oxygen uptake, maximum aerobic PO, or AeLT between groups ($p \geq 0.352$; **Table 1**).

Performance Tests

There was no significant difference in PO between the familiarization trial and TT1 for either group ($p \geq 0.378$). The CV in PO for AB-CON was 3.7% (TT1: 217 ± 42 W, 33.5 ± 2.4 min; TT2: 219 ± 38 W, 33.4 ± 2.1 min; **Figure 2**); therefore, the smallest worthwhile change in PO for TT2 was 4.3%. The average change in PO for AB was 4.5% (TT1: 199 ± 44 W, 35.3 ± 3.7 min; TT2: 207 ± 45 W, 35.0 ± 3.7 min), indicating a small meaningful improvement in average PO during the performance tests. Eight of the 12 participants in the AB-ACC group experienced an improvement in performance exceeding the smallest worthwhile change. There was no significant trial or trial by time effect for HR or BLa for either group ($p \geq 0.164$). TS was significantly lower during TT2 for the AB-ACC group (p = 0.013); there was no significant change in the AB-CON group (p = 0.090), nor was there an effect of trial on RPE in either group ($p \geq 0.388$). Fluid intake (TT1: 0.63 ± 0.19 L, TT2: 0.76 ± 0.25 L) and sweat loss (TT1: 1.08 ± 0.25 L, TT2: 1.13 ± 0.25 L) were not significantly different across trials for AB-CON ($p \geq 0.139$). Fluid intake (TT1: 0.44 ± 0.22 L, TT2: 0.56 ± 0.30 L) and sweat loss (TT1: 1.08 ± 0.22, TT2: 1.21 ± 0.28) were significantly greater during TT2 for AB-ACC ($p \leq 0.031$). There was no significant difference in pre-trial USG for either group ($p \geq 0.266$).

Heat Stress Tests

There was a significant PV expansion for PARA (12.7 ± 10.6%; p = 0.019). However, there was no effect of HA on sweat rate, sweat gain, fluid intake, sweat sodium concentration, or pre-trial USG ($p \geq 0.066$) (**Table 2**). For AB-ACC, there was a significant effect of trial on sweat rate, sweat gain, and fluid intake ($p \leq 0.045$) and PV change post-HA (6.2 ± 7.7%; p = 0.013)

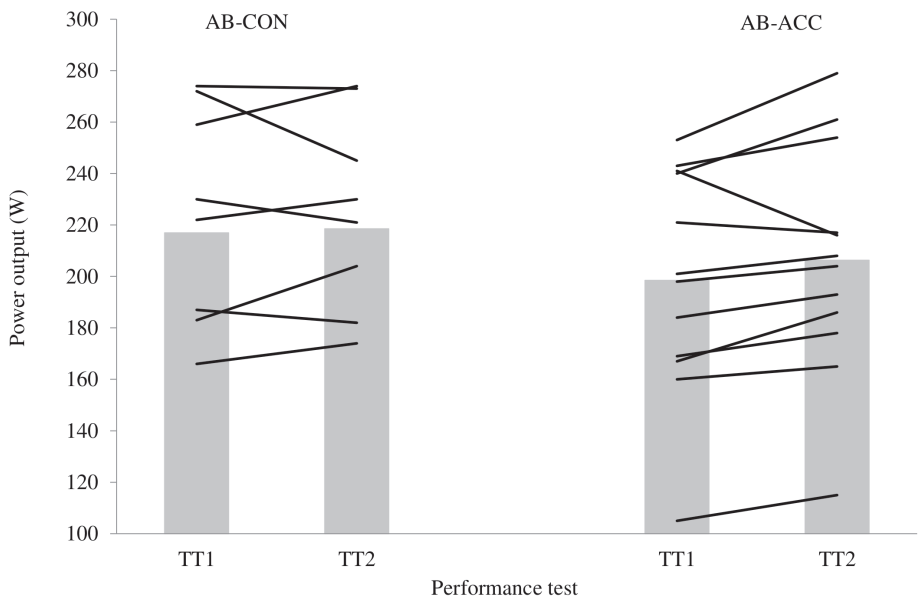

FIGURE 2 | Performance trial (20 km cycling time trial in 35°C, 60% relative humidity) average power output for the control group (AB-CON) and able-bodied acclimation group (AB-ACC). Bars are group mean, and lines are individual participants.

with no significant change in sweat sodium concentration or pre-trial USG ($p \geq 0.678$) (**Table 2**). For both groups, T_c was significantly lower in HST2 ($p < 0.001$) (**Figure 3A**). For PARA, there was a significant trial by time interaction, whereby T_c was lower at 40, 45, and 50 min during HST2 than HST1 ($p \leq 0.049$). There was, however, no change in

TABLE 2 | Pre-trial urine specific gravity (USG), sweat rate, sweat gain, fluid intake and sweat sodium (Na^+) concentration during heat stress tests pre- (HST1) and post- (HST2) heat acclimation in paratriathlon (PARA) and able-bodied (AB-ACC) groups (mean ± SD).

	PARA		AB-ACC	
	HST1	HST2	HST1	HST2
Sweat rate ($L \cdot h^{-1}$)	1.36 ± 0.73	1.49 ± 0.57	1.35 ± 0.44	1.52 ± 0.37*
Sweat gain ($L \cdot h^{-1} \cdot °C^{-1}$)	1.32 ± 0.53	3.33 ± 2.37	0.74 ± 0.28	1.19 ± 0.90*
Fluid intake ($L \cdot h^{-1}$)	0.88 ± 0.40	0.72 ± 0.16	0.82 ± 0.43	1.04 ± 0.55*
Average sweat Na^+ ($mmol \cdot L^{-1}$)	37.1 ± 7.1	40.7 ± 12.5	49.2 ± 16.1	48.1 ± 20.6
Pre-trial USG	1.019 ± 0.004	1.022 ± 0.007	1.014 ± 0.008	1.015 ± 0.008

**Significantly different to HST1 ($p \leq 0.045$).*

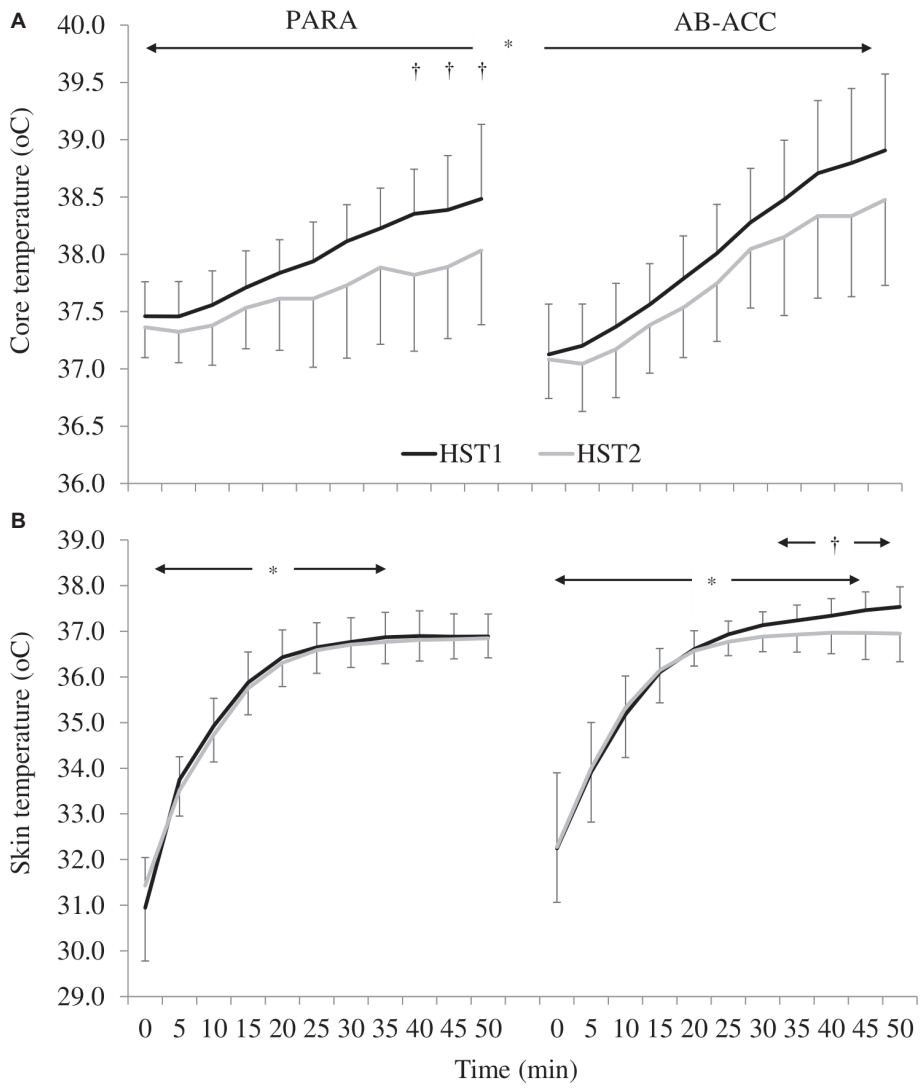

FIGURE 3 | (A) Changes in core temperature across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean ± SD). *Significantly lower during HST2 and increasing over time ($p < 0.001$). †Significantly lower during HST2 ($p \leq 0.049$). **(B)** Changes in skin temperature across HST for the PARA and AB-ACC group (mean ± SD). *Significant increase over time until 35 min (PARA; $p \leq 0.044$) or 45 min (AB-ACC; $p \leq 0.036$). †Significantly lower during HST2 ($p < 0.001$).

resting T_c in either group ($p \geq 0.367$). There was no significant change in T_{sk} for PARA ($p = 0.177$), but T_{sk} was lower during HST2 for AB-ACC ($p < 0.001$); *post hoc* analyses revealed T_{sk} was lower from 30 min during HST2 (**Figure 3B**). There was no significant change in HR for PARA ($p = 0.878$), but for AB-ACC, HR was lower during HST2 ($p = 0.008$) with values lower from 15 min onward ($p \leq 0.045$; **Figure 4**). For both groups, BLa (**Figure 5**), TS, and RPE were significantly lower during HST2 ($p < 0.032$).

Active Heat Acclimation

There was no significant difference in PO between days 1 and 8 (45 min) for PARA ($p = 0.522$), but for AB-ACC, it was higher on day 8 ($p = 0.048$). Comparing days 3, 5, and 6 (90 min), there was no meaningful difference in PO between trials for PARA ($p = 0.483$). However, for AB-ACC, *post hoc* analyses revealed PO was higher on days 5 and 6 than day 3 ($p \leq 0.021$) and was greater on day 6 than days 3 and 5 from 65 min onward ($p \leq 0.044$) (**Figure 6**). There was no significant difference in HR, BLa, sweat rate, fluid intake, or RPE between active HA sessions for either group ($p \geq 0.068$). For AB-ACC, TS was lower on day 5 than days 3 and 6 ($p \leq 0.026$). There was no change in TS over time for PARA ($p \geq 0.450$; **Table 3**).

Passive Heat Acclimation

There was no change in HR or T_{sk} across trials for either group ($p \geq 0.224$; **Table 4**). TS was greater at 60 min than 0 and 20 min ($p \leq 0.032$) and was greater on day 2 than days 4 and 7 in PARA ($p \leq 0.003$). For AB-ACC, HR was

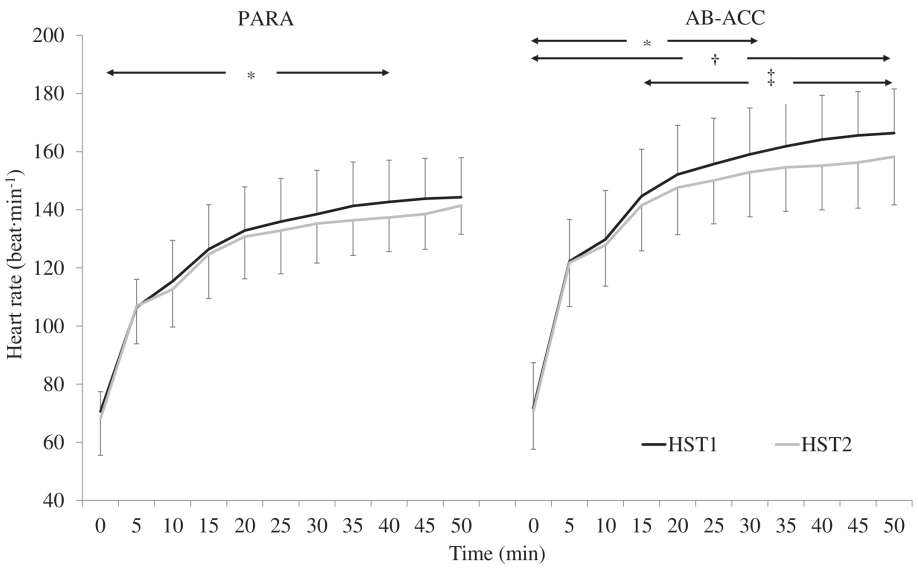

FIGURE 4 | Changes in heart rate across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean ± SD). *Significant increase over time until 40 min (PARA; $p \leq 0.028$) and 30 min (AB-ACC; $p \leq 0.031$). †Significantly lower during HST2 ($p = 0.008$). ‡Significantly lower during HST2 ($p \leq 0.045$).

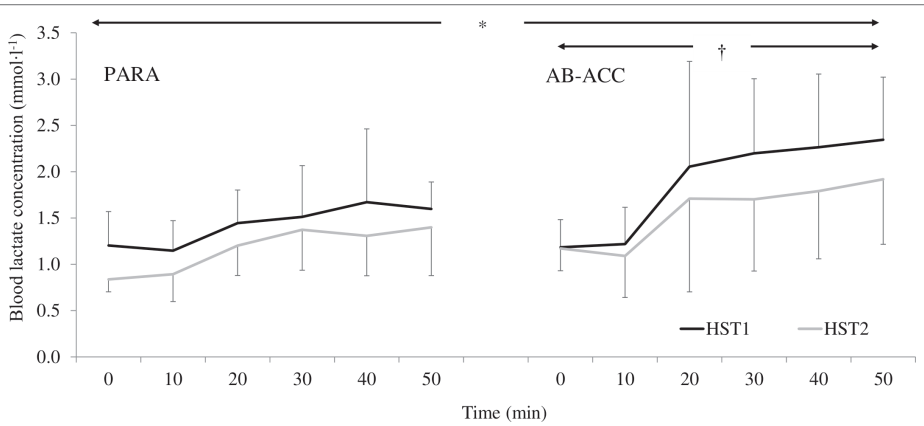

FIGURE 5 | Changes in blood lactate concentration across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean ± SD). *Significantly lower during HST2 ($p < 0.001$). †Significant increase over time ($p \leq 0.037$).

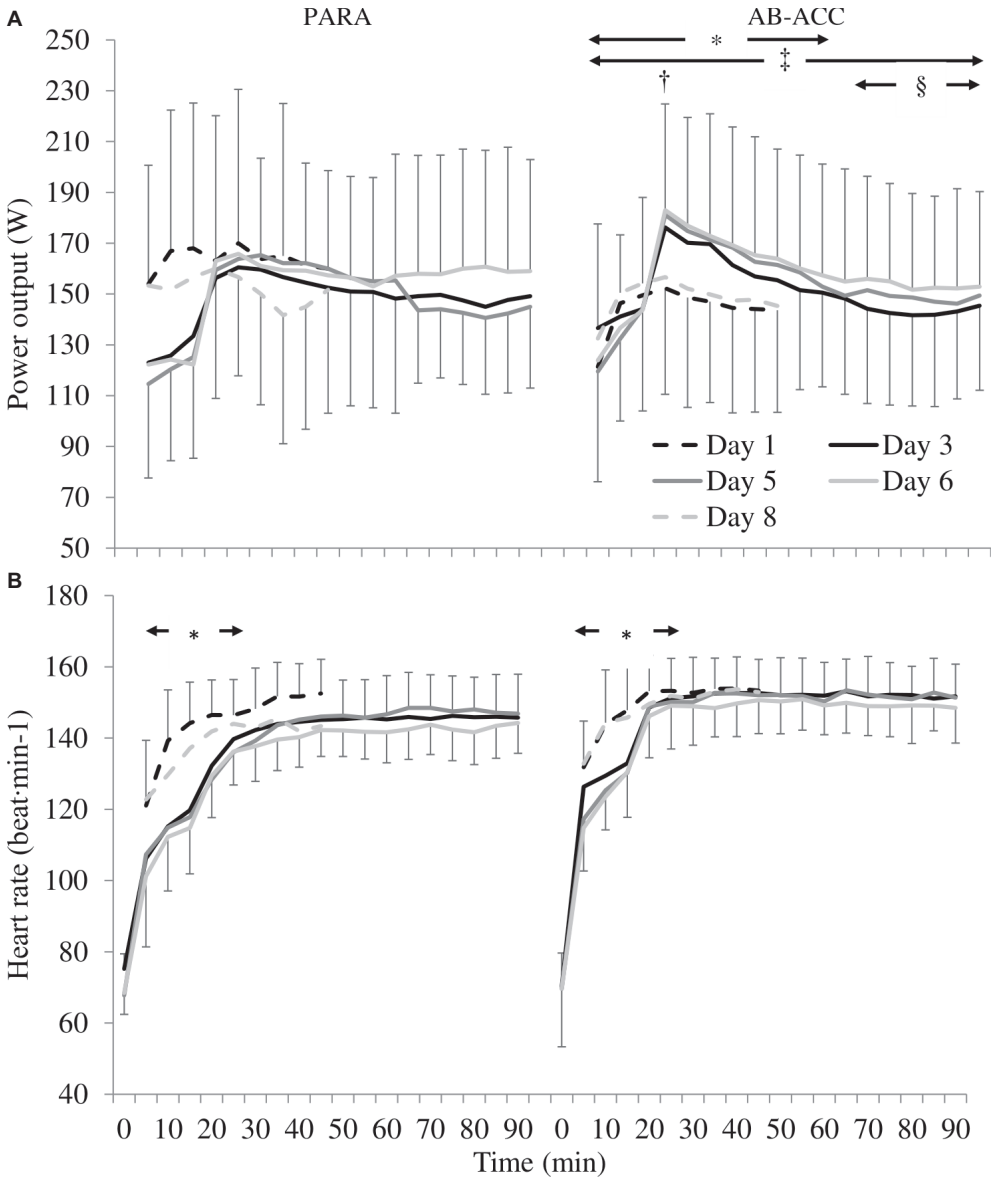

FIGURE 6 | (A) Power output changes between and within active heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (mean ± SD). *Significant difference between days 1 and 8 ($p = 0.048$). †Significantly greater at 20 min than 5 min during days 1 and 8 ($p = 0.010$). ‡Significantly greater than day 3 ($p \leq 0.021$). §Significantly greater than day 6 ($p \leq 0.044$). **(B)** Heart rate changes between and within active heat acclimation sessions for PARA and AB-ACC groups (mean ± SD). *Significant increase up to 20 min ($p \leq 0.040$).

greater at 0 min than all other time points ($p \leq 0.038$), and T_{sk} was lower at 0 min than 10 min ($p = 0.032$).

DISCUSSION

This is the first study to investigate the efficacy of HA in a multi-impairment, Paralympic endurance sport. Utilizing a novel, mixed active and passive HA protocol controlling relative intensity through HR, paratriathletes can display positive thermoregulatory adaptations. These responses were a reduction in exercising T_c, BLa, RPE, and TS with concomitant PV expansion. Furthermore, additional adaptations were noted including T_{sk}, HR, and sudomotor changes during the submaximal HST in a physiologically matched AB group. Finally, there was a small direct performance benefit from the employed HA protocol as AB-ACC athletes improved their PO during a 20 km cycling TT in the heat (4.5%) to an extent greater than the variation noted in a non-acclimated AB-CON group (3.7%).

Medium-term HA (8–14-day heat exposure) has been commonly studied in the literature with typical adaptations recently documented in the meta-analysis of Tyler et al. (2016). This approach has been shown to result in a decrease in exercising: T_c (0.17°C); HR (15 beat min^{-1}), T_{sk} (0.73°C), BLa

TABLE 3 | Blood lactate concentration (BLa), thermal sensation (TS), rating of perceived exertion (RPE), sweat rate, and fluid intake changes between active heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (daily mean ± SD).

	Day 1	Day 3	Day 5	Day 6	Day 8
PARA					
BLa (mmol·L^{-1})	1.75 ± 0.87	1.54 ± 0.51	1.60 ± 0.69	1.49 ± 0.56	1.63 ± 0.89
TS (AU)	6 ± 1	5 ± 2	5 ± 2	5 ± 2	6 ± 1
RPE (AU)	13 ± 1	12 ± 2	12 ± 1	12 ± 1	13 ± 1
Sweat rate (L·h^{-1})	1.40 ± 0.50	1.29 ± 0.52	1.19 ± 0.47	1.33 ± 0.52	1.57 ± 0.45
Fluid intake (L·h^{-1})	1.38 ± 0.90	1.28 ± 0.62	1.30 ± 0.50	1.29 ± 0.56	1.65 ± 0.51
AB-ACC					
BLa (mmol·L^{-1})	1.28 ± 0.27	1.44 ± 0.52	1.47 ± 0.62	1.37 ± 0.52	1.78 ± 0.91
TS (AU)	7 ± 1	6 ± 2	6 ± 2*	6 ± 2	6 ± 2
RPE (AU)	14 ± 1	13 ± 2	13 ± 2	13 ± 2	13 ± 2
Sweat rate (L·h^{-1})	1.31 ± 0.45	1.22 ± 0.40	1.32 ± 0.36	1.35 ± 0.39	1.27 ± 0.81
Fluid intake (L·h^{-1})	1.46 ± 0.77	1.22 ± 0.54	1.31 ± 0.57	1.45 ± 0.64	1.54 ± 0.48

**TS significantly lower on day 5 than days 3 and 6 in AB-ACC ($p \leq 0.026$).*

TABLE 4 | Heart rate (HR), skin temperature (T_{sk}), and thermal sensation (TS) between passive heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (daily mean ± SD).

	PARA			AB-ACC		
	Day 2	Day 4	Day 7	Day 2	Day 4	Day 7
HR (beat·min^{-1})	74 ± 8	75 ± 8	75 ± 9	78 ± 13	77 ± 11	78 ± 14
T_{sk} (°C)	35.27 ± 1.07	35.21 ± 0.88	35.13 ± 0.66	34.76 ± 1.22	35.00 ± 0.87	35.08 ± 0.71
TS (AU)	4 ± 1	3 ± 1	3 ± 1	4 ± 2	4 ± 2	4 ± 2

TS greater on day 2 than days 4 and 7 in PARA ($p \leq 0.003$).

(~0.9 mmol·l^{-1}), TS, and RPE (~10%); with increased whole-body sweat rates (30.0%) and PV expansion (4.3%; Tyler et al., 2016). Thus, this study presents thermoregulatory adaptations for both groups that are within the commonly reported range for similar duration HA, while the greater T_c (~0.28°C) and PV (6.2–12.7%) adaptations here may be a result of the study design ensuring a consistent thermal impulse and relative exercise intensity. Finally, medium-term HA also results in a median 4% improvement in TT performance (Tyler et al., 2016), similar to the 4.5% improvement noted in the AB-ACC group. A noteworthy finding of the current study is the greater magnitude of PV adaptation for PARA relative to AB-ACC. The exact reason behind this response is unknown as this suggests a greater thermal impulse in PARA, which could not be confirmed due to a lack of T_c measurement during HA. Furthermore, HA typically results in a 0.18°C reduction in resting T_c (Tyler et al., 2016); but no reduction was seen presently. This is likely due to a lack of rigid control pre-trial to accurately detect changes in truly resting T_c.

As noted earlier, the literature to date concerning HA in Paralympic athletes has been confined to one study in target shooters with a spinal cord injury (Castle et al., 2013), despite acknowledgments of the need for greater evidence (Price, 2015; Casadio et al., 2017). The authors stated that the five participants displayed partial HA through a decrease in resting and exercising aural temperature, end-exercise HR, TS, and RPE. These responses were proposed to be mediated by a 1.5 ± 0.6% PV expansion. There were, however, no notable sweat responses, which were credited to the nature of athletes' autonomic impairments. In the present study, very similar HA adaptations were noted; however, the extent of PV was greater and more varied. This is most likely attributed to the disparate athlete impairments, the HA protocol presenting a greater thermal stimulus, and the incongruent athletic backgrounds. Thus, the present data provide support to the notion that Paralympic athletes are capable of partial HA although not to the same extent as AB athletes undergoing the same protocol. While the current study contained a range of physical impairments in the PARA group, the small sample size did not permit meaningful comparisons between impairments. As such, we cannot differentiate impairment-specific adaptations from individual variability. Nonetheless, analysis of individual data suggests those with presumably greatest thermoregulatory impairment (an athlete with spinal cord injury) can display beneficial adaptions to HA with improvements in T_c, HR, BLa, and PV noted. As such, there is currently no reason why all Paralympic endurance athletes should not undergo HA before competition in the heat.

A unique feature of this work was that differences in HA responses between AB and Paralympic athletes were present. It is worth noting, however, that the small and heterogeneous sample size in the PARA group may have prevented some statistically significant findings. For example, improvements in HR and whole-body sweat rates during exercise were noted; however, these did not reach the predefined threshold of significance. Alternatively, the nature of physical impairments in the PARA group may have restricted any notable adaptation.

For instance, the reduced body surface area for heat dissipation of those with an amputation may be the limiting factor in any T_{sk} change, rather than PV and capacity for skin blood flow. Further, limited sweating responses as a result of reduced body surface area, skin grafts, or disrupted afferent input likely reduce the maximum achievable sweating capacity, thus creating a "ceiling" effect. Nonetheless, improvements in exercising T_c, BLa, and perceptual measures demonstrated an enhanced thermoregulatory capacity, which was likely mediated by a significant PV expansion post-HA. While not directly assessed, it may be assumed that this would result in a direct improvement in endurance performance in hot environments as athletes display a diminished heat storage and reliance upon carbohydrate metabolism. Of note, while the PARA was older, this was not considered a major concern since the previous work has shown age that does not affect thermoregulatory or performance changes post-HA (Tyler et al., 2016).

The use of HR to regulate HA training intensity was based on the recommendations of Périard et al. (2015) to provide a constant cardiovascular, and presumed thermoregulatory, stimulus for continued adaptation. This is supported by previous work showing a constant HR across isothermic HA (Magalhães et al., 2010; Garrett et al., 2012; Zurawlew et al., 2015; Pethick et al., 2019). While isothermic protocols have been commonly utilized in the literature to provide a constant thermal stimulus (Tyler et al., 2016), the utility of such approaches has been questioned in the applied sport setting. Therefore, the use of HR provides a feasible means for regulating HA intensity for elite athletes. Participants in the present study were able to maintain their individualized HR zone within and between HA sessions, and as the target HR was relative to their maximum HR, the relative intensity of exercise was the same between groups. Furthermore, due to a lack of change in BLa, RPE, and TS between active HA, participants maintained a constant relative intensity each day. Moreover, the AB-ACC group was able to produce a greater external workload in later HA sessions suggesting that the group displayed thermoregulatory adaptations that permitted a higher workload for a given relative intensity. This is akin to isothermic HA, whereby greater workloads are produced for a set T_c (Magalhães et al., 2010; Garrett et al., 2012; Pethick et al., 2019). However, no significant change in PO was noted for the PARA group. This may be indicative of the greater individual variation in this group with regard to their physical impairments, exercising workload, and capacity for adaptation.

The current protocol was chosen to provide an optimal yet time efficient HA stimulus when considering elite athletes' pre-competition training schedules (Guy et al., 2014; Casadio et al., 2017). A combined HA approach may present a pragmatic solution to overcome the demands of pre-competition schedules while potentiating a stimulus for heat adaptation (Ruddock et al., 2016), although this is severely understudied. However, Ruddock et al. (2016) utilized this study design in their case study of a soccer referee. The authors showed that the participant displayed increases in whole-body sweat rate, PV, and repeated sprint performance while decreasing exercising tympanic temperature and HR. In the present study, passive heat exposure was in the form of post-exercise rest in a climatic chamber. This permitted athletes the capability to maintain running session density and intensity while continuing daily heat exposure as athletes entered the heat with a presumed prior elevated T_c, T_{sk}, and sweat rate. While T_{sk} did not reach the levels reported by Zurawlew et al. (2015) (equilibrating at 40°C water temperature) during passive heating, it was greater than typical resting temperatures, and the reported TS further indicated some thermal strain. Indeed, as beneficial adaptations were noted in the present study for both AB-ACC and PARA, this adds support for the use of mixed active and passive HA for elite athletes, pre-competition. However, it is unknown if a solely active or passive approach would have induced disparate adaptations.

The timings of this study meant that the PARA group undertook their HA sessions in preparation for a competitive race in the heat. Therefore, it was neither feasible nor appropriate to include a direct performance test for this group. However, this was included in the AB-ACC and AB-CON groups. The AB-ACC group displayed a small improvement in average PO, thus providing evidence for a direct performance benefit of the HA protocol. Furthermore, the AB-ACC group displayed physiological responses during TT2 indicative of an enhanced thermoregulatory capacity. Specifically, a greater sweat rate and fluid intake was noted, while participants produced a greater PO for a similar HR and lower TS, whereas no significant changes were noted for the AB-CON group. It can therefore be assumed that these thermoregulatory adaptations, among others, permitted the improvement in performance.

Here, the lack of a control group engaging in exercise in thermoneutral conditions prevented the ability to state that changes in thermoregulatory variables were due solely to HA. Nonetheless, due to the relatively low HA intensity and the high cardiorespiratory fitness levels of both PARA and AB-ACC groups, it is unlikely any non-HA-specific training adaptation occurred (Lorenzo et al., 2010). However, there is the potential that HA resulted in a positive phenotypic adaptation in athletes' AeLT and/or maximum rate of oxygen uptake. This has been shown by previous research (Lorenzo et al., 2010; Corbett et al., 2014; Tyler et al., 2016), although not all (Chalmers et al., 2014; Neal et al., 2016), and may have reduced the metabolic heat production during HST2. As post-HA graded exercise tests were not conducted and gas exchange variables were not measured during HSTs, this could not be confirmed. Furthermore, it is noteworthy that T_c was not recorded across exercise HA. Although both groups maintained a constant relative intensity, assuming a constant thermal stimulus, this was not confirmed. Lastly, the current study did not control for menstrual cycle phase in female participants, which may have influenced T_c (Casadio et al., 2017).

CONCLUSIONS

A mixed active and passive HA protocol, controlling exercise intensity by HR, induced positive thermoregulatory adaptations in paratriathletes and AB triathletes. Both groups displayed reductions in T_c, BLa, RPE, and TS during a submaximal

HST with significant PV expansion. Furthermore, the AB-ACC group presented additional adaptations including reduced HR and T_{sk} with an elevated sweat rate. This is the first evidence of differences in thermoregulatory variable changes to the same protocol between Paralympic and AB athletes matched for physiological variables. The HR-controlled exercise HA resulted in a constant relative intensity between HA sessions with the AB-ACC group capable of producing a greater PO for a set intensity over the study period. Finally, there was evidence of a direct performance benefit as the AB group improved their PO during a 20 km TT in the heat to a greater extent than the natural variation shown in a non-acclimated, matched cohort.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Participants Sub-Committee Loughborough University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BS and VG-T designed the study. BS collected and analyzed the study data. BS and KT undertook the statistical analyses. BS wrote the manuscript, which was edited by KT and VG-T.

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Hematological Adaptations to Prolonged Heat Acclimation in Endurance-Trained Males

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Heat acclimation is associated with plasma volume (PV) expansion that occurs within the first week of exposure. However, prolonged effects on hemoglobin mass (Hb_{mass}) are unclear as intervention periods in previous studies have not allowed sufficient time for erythropoiesis to manifest. Therefore, Hb_{mass}, intravascular volumes, and blood volume (BV)-regulating hormones were assessed with 5½ weeks of exercise-heat acclimation (HEAT) or matched training in cold conditions (CON) in 21 male cyclists [(mean ± SD) age: 38 ± 9 years, body weight: 80.4 ± 7.9 kg, VO_{2peak}: 59.1 ± 5.2 ml/min/kg]. HEAT (n = 12) consisted of 1 h cycling at 60% VO_{2peak} in 40°C for 5 days/week in addition to regular training, whereas CON (n = 9) trained exclusively in cold conditions (<15°C). Before and after the intervention, Hb_{mass} and intravascular volumes were assessed by carbon monoxide rebreathing, while reticulocyte count and BV-regulating hormones were measured before, after 2 weeks and post intervention. Total training volume during the intervention was similar (p = 0.282) between HEAT (509 ± 173 min/week) and CON (576 ± 143 min/week). PV increased (p = 0.004) in both groups, by 303 ± 345 ml in HEAT and 188 ± 286 ml in CON. There was also a main effect of time (p = 0.038) for Hb_{mass} with +34 ± 36 g in HEAT and +2 ± 33 g in CON and a tendency toward a higher increase in Hb_{mass} in HEAT compared to CON (time × group interaction: p = 0.061). The Hb_{mass} changes were weakly correlated to alterations in PV (r = 0.493, p = 0.023). Reticulocyte count and BV-regulating hormones remained unchanged for both groups. In conclusion, Hb_{mass} was slightly increased following prolonged training in the heat and although the mechanistic link remains to be revealed, the increase could represent a compensatory response in erythropoiesis secondary to PV expansion.

Keywords: hemoglobin mass, blood volume, critmeter, hematocrit, vasopressin, erythropoietin

INTRODUCTION

Natural heat acclimatization as well as laboratory-based heat acclimation translates into plasma volume (PV) expansion within the first few days of exposure (Périard et al., 2016). Longer intervention periods are typically required for the corresponding expansion in red blood cell volume (RBCV) and total hemoglobin mass (Hb_{mass}) (Siebenmann et al., 2017b; Montero and Lundby, 2018) but are still desirable due to the potential for elevating arterial O_2 delivery and improving endurance performance (Ekblom et al., 1972; Montero et al., 2015). Previous studies have, however, employed relatively short heat acclimation protocols leaving limited time for erythropoiesis to compensate for the hemodilution accompanying the initial PV expansion (Patterson et al., 2004; Keiser et al., 2015; McCleave et al., 2017; Rendell et al., 2017). Therefore, we tested whether exercise training in the heat, i.e., exercise-heat acclimation performed over a period of 5½ weeks, elicits higher Hb_{mass}.

RBCV and Hb_{mass} expand in response to conventional endurance training (ET) which manifests after 4–6 weeks of ET in untrained individuals (Montero et al., 2015, 2017). In endurance athletes with high Hb_{mass}, on the other hand, this effect is blunted throughout the season or after intense training periods (Gore et al., 1997; Prommer et al., 2008) and additional environmental or cardiovascular stressors may be required to prompt Hb_{mass} expansion further in such athletes. Therefore, hypoxic exposure or altitude training are strategies that are commonly employed by athletes, although their use is highly debated (Lundby and Robach, 2016; Bejder and Nordsborg, 2018). Prolonged exercise-heat acclimation is an alternative approach that potentially increases Hb_{mass} which however remains to be explored.

A potential mechanism underlying an expansion in Hb_{mass} may relate to the early PV expansion concomitant to exercise-heat acclimation as the reduced hematocrit, and thus arterial O_2 content, triggers the release of erythropoietin (EPO) from the kidney (Adamson, 1968; Montero and Lundby, 2019). Indeed, the kidney has been proposed to act as a "critmeter," regulating hematocrit by adjusting RBCV and PV mediated by EPO (Donnelly, 2001). Also, increased PV after 2 weeks of ET coincides with elevated EPO while RBCV remains unaffected which supports that a reduced hematocrit due to a sole expansion in PV may regulate erythropoiesis (Montero et al., 2017). It is also noteworthy that key PV-regulating hormones, e.g., vasopressin and angiotensin II exert direct effects on erythropoiesis (Engel and Pagel, 1995; Kim et al., 2014; Montero and Lundby, 2018). Thus, both an expansion in PV but also the changes in PV-regulating hormones could ultimately affect Hb_{mass}. We therefore conducted the present study to test the hypothesis that exercise-heat acclimation for 5½ weeks would stimulate erythropoiesis and increase total Hb_{mass} in endurance-trained individuals and aimed at identifying some of the potential underlying hormonal and hematological mechanisms.

MATERIALS AND METHODS

The presented data were obtained as part of a large study exploring the effects of prolonged exercise-heat acclimation on performance and the underlying hematological mechanisms. For performance data, the reader is referred to the accompanying paper submitted in this issue (Mikkelsen et al., 2019, submitted). The study protocol was approved by the ethical committee of the Capital Region of Denmark (H-17036662) and conformed to the Declaration of Helsinki.

Participants

Twenty-one healthy, endurance-trained, male cyclists provided oral and written consent for participation and were included in this study (**Table 1**). All participants conducted their regular cycling training during the preceding 3 months in cold temperatures outside (winter: <15°C) and were thus not heat acclimatized prior to commencement of the intervention.

Study Design

Participants first underwent baseline testing consisting of blood sampling and determination of body composition, peak oxygen uptake (VO_{2peak}), Hb_{mass}, and intravascular volumes. After baseline measurements, participants were age- and VO_{2peak}-matched into two groups which were thereafter randomly assigned as the exercise-heat acclimation (HEAT, n = 12) or the control (CON, n = 9) group. Participants then completed the 5½-week intervention period, where after blood sampling and determination of Hb_{mass} and intravascular volumes was repeated. In addition, blood sampling was conducted after 2 weeks into the intervention period prior to an exercise training session.

Intervention

HEAT conducted 1 h of cycling in a climatic chamber on 5 weekly occasions for 5½ weeks (28 ± 2 sessions in total). Temperature in the climatic chamber corresponded to 35°C in the first week and was augmented by 1°C each week (relative humidity of 30 ± 8%). This gradual increment in temperature provided a constant adaptation stimulus and resulted in a rectal temperature of >38.5°C after 35 ± 8 min of training during

TABLE 1 | Participant characteristics at baseline.

	HEAT (n = 12)	CON (n = 9)
Age (years)	38.8 ± 8.9	37.7 ± 9.3
Body mass (kg)	80.2 ± 6.3	80.6 ± 9.5
Height (cm)	185 ± 3	184 ± 4
Body fat (%)	13.7 ± 4.0	14.7 ± 2.9
VO_{2peak} (L/min)	4.8 ± 0.4	4.6 ± 0.4
VO_{2peak} (ml/min/kg)	60.0 ± 5.1	57.9 ± 5.1
Training volume pre (min/week)	417 ± 105	499 ± 164
Training volume during (min/week)	509 ± 173	576 ± 143
Training volume > 80% HR_{max} pre (min/week)	102 ± 71	102 ± 55
Training volume > 80% HR_{max} during (min/week)	157 ± 90	122 ± 57

HR_{max}, maximal heart rate; VO_{2peak}, peak oxygen uptake.
Pre refers to before the intervention. Data are presented as mean ± SD.

all training sessions. Airflow was provided by a fan only if the participant could not complete the training otherwise and participants were allowed to drink warm water *ad libitum* during the training. CON maintained their regular outdoor training (<15°C) but reported to the laboratory once a week and cycled in cold conditions (<15°C) to maintain familiarization to stationary cycling. All training sessions in the laboratory, i.e., in the climatic chamber for HEAT, consisted of cycling at 60% VO_{2peak} as determined in cold conditions (~15°C) and were conducted on the participants' personal bikes using a stationary Tacx-trainer device (Tacx Neo Smart T2800; Tacx, Netherlands) and associated software (Tacx Trainer software 4; Tacx, Netherlands). Participants in both groups completed a training log to quantify their training volume and intensity (assessed by heart rate) 2 weeks prior to the intervention and 2 weeks into the intervention. Participants were instructed to maintain their training routine throughout the intervention but to subtract the training hours performed in the laboratory from their regular training. This resulted in similar training volumes between HEAT and CON.

Measurements

Body Composition

Baseline body mass and fat percentage were assessed by bioimpedance (InBody 270; InBody, Denmark).

Peak Oxygen Uptake

An incremental exercise test was performed to determine VO_{2peak}. The test was conducted on the participants' personal bikes, which were installed on a stationary Tacx-trainer device (Tacx Neo Smart T2800; Tacx, Netherlands). Following a 10 min warm up with 5 min at 100 W and 5 min at 175 W (80 RPM), workload was increased by 25 W/min until exhaustion. VO_2 and VCO_2 were obtained by breath-by-breath recordings (Jaeger Oxycon Pro; Viasys Healthcare, Germany). The gas analyzers and the flowmeter were calibrated before each test. A plateau in VO_2 despite increased workload and/or attainment of a respiratory exchange ratio (RER) $\geq$ 1.15 served as test validation criteria. VO_{2peak} was defined as the highest observed value over a 30s-period.

Hemoglobin Mass and Intravascular Volumes

Hb_{mass} and intravascular volumes were assessed using the carbon monoxide (CO) rebreathing technique (Siebenmann et al., 2017a). For some of the participants (n = 11), an automated version of the CO rebreathing (OpCO; Detalo Health, Denmark) was used. The same method (manual/automated) was applied for intra-individual pre-post comparisons and the distribution of which technique was used was random among HEAT (n = 7) and CON (n = 4). The procedure was as follows: the participant rested for 20 min in the supine position before each measurement. During this time, the participant drank 500 ml of water and an 18-G venous catheter was placed into an antecubital vein. The participant was then connected to a breathing circuit and breathed 100% O_2 for 4 min. 2 ml of blood were sampled and analyzed immediately in quadruplicates for (1) percent carboxyhemoglobin (%HbCO) and hemoglobin concentration ([Hb]) (ABL835; Radiometer, Denmark) and (2) hematocrit with the microcentrifuge method (4 min at 13,500 RPM). Subsequently, the participant was switched by a sliding valve to a O_2-filled rebreathing circuit and a bolus of 1.5 ml/kg body weight of 99.997% chemically pure CO (CO N47; Strandmøllen, Denmark) was administered to the rebreathing circuit. O_2 was supplied into this circuit on a demand basis. The participant rebreathed the O_2-CO gas mixture for 10 min. A second blood sample was obtained after 10 min of CO rebreathing and analyzed in quadruplicates for %HbCO. The remaining CO volume in the rebreathing circuit was determined as previously specified (Siebenmann et al., 2017a) and was subtracted from the applied CO dose. For the calculation of Hb_{mass}, the absorbed CO dose and the changes in %HbCO from before to after rebreathing were used. Total blood volume (BV), RBCV, and PV were then derived from Hb_{mass}, [Hb], and hematocrit (Burge and Skinner, 1995).

Blood Sampling and Analyses

Venous blood was collected in EDTA-coated tubes for analyses of [Hb] and reticulocyte count (Sysmex XN; Sysmex Europe, Germany) on whole blood. Furthermore, 2 ml of blood was collected in a heparinized syringe (PICO50; Radiometer, Denmark) to analyze blood electrolyte concentration with an automated hemoximeter (ABL835; Radiometer, Denmark). A third blood sample was obtained in a sodium heparin-coated vacutainer. After centrifugation, plasma was collected and stored at −80°C until further analysis. Plasma EPO was determined with an ELISA kit (Human Erythropoietin Quantikine IVD ELISA Kit; R&D Systems, USA) with an intra-assay coefficient of variation (CV) of 2.8–5.2% and inter-assay CV of <1%. Plasma protein and albumin concentrations were measured with an automated analyzer (Cobas 8,000, c702 modul; Roche, Germany) with an intra- and inter-assay CV of <5%. Total protein and albumin were calculated by multiplying the respective concentrations with PV. Plasma copeptin as a more stable proxy for vasopressin was determined using an automated immunofluorescent assay (Thermo Fisher Scientific BRAHMS; Germany) (Alehagen et al., 2011), while pro-ANP was measured with a mid-regional assay on a Kryptor Plus platform (Thermo Fisher Scientific BRAHMS; Germany) (Hunter et al., 2011), both with intra- and inter-assay CV of <6.5%.

Statistical Analyses

All statistical analyses were performed using SPSS 22 (IBM SPSS Statistics, USA). Figures were made using GraphPad Prism 8.0.0 (GraphPad Software; USA). Power calculations before the onset of the study estimated that a sample size of $n \geq 9$ in each group would allow detecting a meaningful change in Hb_{mass}. Prior to analyses, data were evaluated for normality and equal variance and were log-transformed if required. Independent t-test was applied to assess differences in training volume between HEAT and CON. The influence of HEAT on the effects of ET on Hb_{mass}, intravascular volumes and

hematological parameters was assessed with a two-way repeated measures ANOVA. Main effects of time (pre-post ET) as within-subject factor and of group (HEAT-CON) as between-subject factor were determined along with the corresponding interactions. In addition, Pearson's correlation coefficient was computed to assess associations between hematological parameters. Data are expressed as means ± standard deviation (SD). p <0.05 was considered statistically significant.

RESULTS

Heat acclimation in HEAT was verified by improved exercise tolerance in the heat and lowered sweat sodium concentration, while no signs of heat acclimation were observed for CON [see Mikkelsen et al. (2019), submitted, for details].

Hemoglobin Mass and Intravascular Volumes

Hb_{mass} increased in both groups (p = 0.038) but this increase tended (p = 0.061) to be larger in HEAT (+3.2 ± 3.3% from 1,052 ± 97 to 1,085 ± 108 g) than in CON (+0.2 ± 3.2% from 983 ± 137 to 985 ± 141 g) (**Figure 1A**). RBCV increased in both groups (p = 0.006) from 3,136 ± 295 to 3,270 ± 364 ml (+4.2 ± 4.2%) in HEAT and from 2,888 ± 395 to 2,929 ± 453 ml (+1.3 ± 3.3%) in CON (**Figure 1B**). Also, PV increased in both groups (p = 0.004) from 4,091 ± 506 to 4,394 ± 626 ml (+7.6 ± 8.7%) in HEAT and from 4,012 ± 569 to 4,200 ± 471 ml (+5.3 ± 7.5%) in CON (**Figure 1C**). As a result of the elevated RBCV and PV, BV was expanded (p = 0.001) from 7,227 ± 725 to 7,664 ± 876 ml (+6.1 ± 5.9%) in HEAT and from 6,900 ± 884 to 7,130 ± 858 ml (3.5 ± 4.6%) in CON (**Figure 1D**). There was no time × group interaction for RBCV, PV or BV.

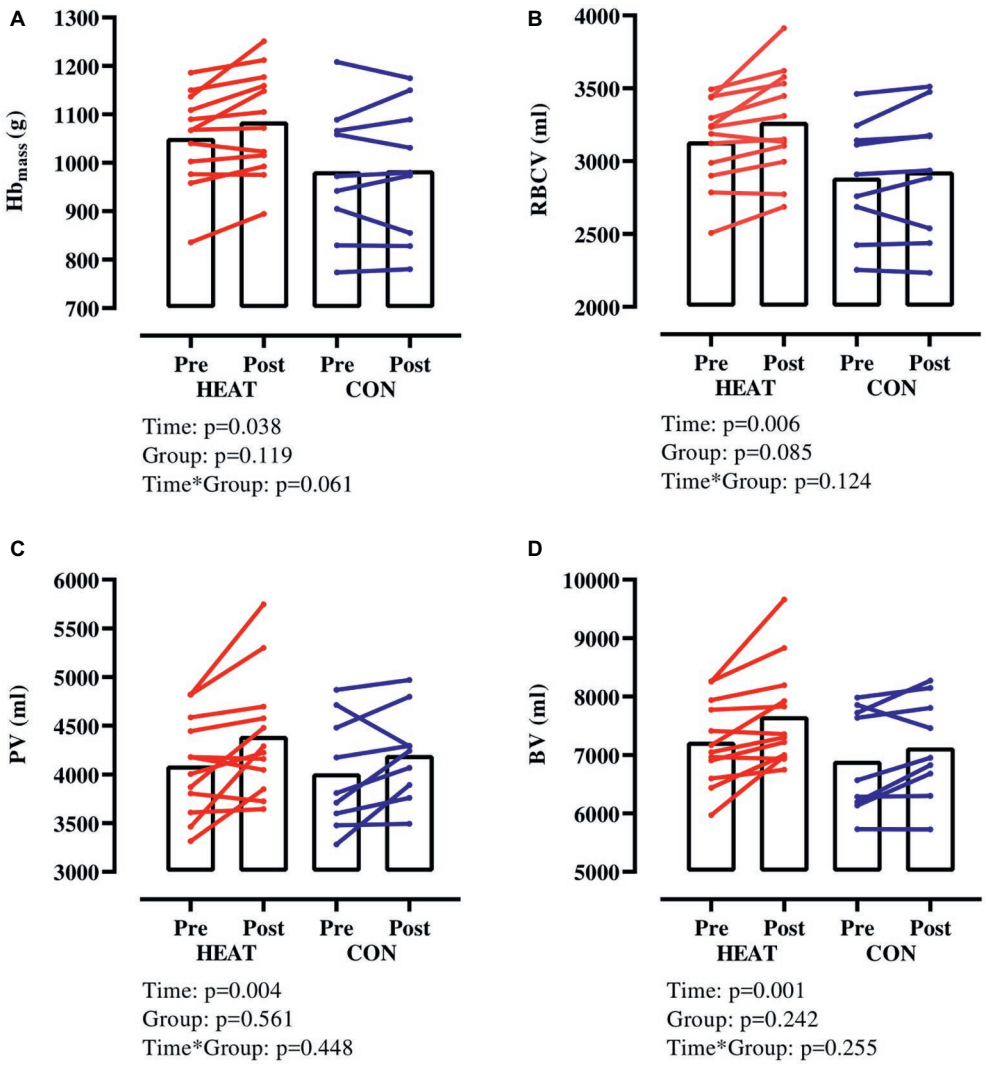

FIGURE 1 | Hb_{mass} and intravascular volumes with exercise-heat acclimation (HEAT) or matched control training (CON). **(A)** hemoglobin mass (Hb_{mass}), **(B)** red blood cell volume (RBCV), **(C)** plasma volume (PV), **(D)** blood volume (BV).

General Hematological Characteristics and Plasma Hormones

Hematocrit, [Hb] and reticulocyte count remained unaffected throughout the intervention in both groups (**Table 2**). There was an effect of time for mean corpuscular hemoglobin concentration (p = 0.015) and for plasma albumin (p = 0.014) and protein concentration (p = 0.028), however, no effect of group or interaction of time × group were detected. Likewise, total albumin and protein content increased in both groups (p = 0.004 and p < 0.001, respectively). Plasma EPO, pro-ANP and copeptin remained unchanged. Furthermore, blood sodium, chloride, calcium and potassium concentrations were unchanged in both groups.

Correlations

We pooled HEAT and CON to examine whether the expansion in PV is correlated to accentuated erythropoiesis and found that changes in PV were weakly associated with altered Hb_{mass} in response to the intervention (**Figure 2A**). Furthermore, hematocrit determined before and after the intervention was negatively associated with plasma EPO at these time points (**Figure 2B**) and similarly, there was a tendency toward a negative association (r = −0.416, p = 0.076) between [Hb] and EPO determined at 2 weeks into the intervention. However, no association of copeptin and pro-ANP with EPO was detected at any time point.

DISCUSSION

The present study provides a detailed picture of the hematological adaptations to prolonged exercise-heat acclimation and we report a 3% increase in Hb_{mass} following heat acclimation corresponding to a change of +34 g (range: −17 to 114 g) for HEAT compared to +2 g (range: −50 to 61 g) for CON. This observation is in agreement with our hypothesis, although we only observed a tendency toward a higher increase in Hb_{mass} after exercise-heat acclimation compared to matched training in cold conditions. The Hb_{mass} expansion was weakly correlated to the overall PV change, indicating that the PV expansion is accompanied by an elevation of total Hb_{mass}. Hence, we suggest that in endurance-trained individuals with high Hb_{mass}, heat imposed on ET may trigger a further erythropoietic stimulus, leading to additional Hb_{mass} expansion.

Studies on the adaptation of Hb_{mass} to heat exposure are rare and equivocal. Although, some report unchanged Hb_{mass} in response to 10 or 21 days of exercise-heat acclimation (McCleave et al., 2017; Rendell et al., 2017), we hypothesized these training durations were insufficient to elicit increased erythropoiesis. A reason for this hypothesis was that higher RBCV and Hb_{mass} is only detected after >4 weeks of conventional ET in untrained individuals (Montero et al., 2017). Indeed, in the present study, 5½ weeks of exercise-heat acclimation elicited a slight expansion of 34 g, whereas Hb_{mass} in CON remained similar with +2 g. This ∼3% Hb_{mass} expansion in HEAT was greater than the typical error of measurement of the CO rebreathing we observe in our laboratory when using the manual method (Siebenmann et al., 2015, 2017a) and when using the automated version (Fagoni et al., 2018). Higher Hb_{mass} has also previously been reported ∼3½ weeks after the initiation of an exercise-heat acclimatization period (Karlsen et al., 2015). Opposite to exercise-heat acclimation as in the present study, participants were residing and training in a natural hot environment thus heat exposure time was substantially longer. However, similar exercise-heat acclimatization has also resulted in unaltered Hb_{mass} (Gore et al., 1997). Hence, whether exercise-heat acclimatization manifests in erythropoietic adaptation remains controversial. Overall, applying exercise-heat acclimation, i.e. laboratory-based intermittent heat exposure appears to trigger an erythropoietic response and may be easier to implement, as it does not involve traveling to hot areas and allows furthermore to carefully control for exposure temperature and humidity.

It is recognized that heat exposure and undergoing exercise-heat acclimation or acclimatization results in an expansion of PV between 3 and 27% within the first days of exposure (Périard et al., 2016). We observed that exercise-heat acclimation may furthermore pose an erythropoietic stimulus. In fact, the

TABLE 2 | Hematological characteristics and plasma hormone concentrations.

	HEAT			CON		
	Pre	Mid	Post	Pre	Mid	Post
[Hb] (g/dl)	14.5 ± 0.9	14.6 ± 1.0	14.3 ± 1.0	14.0 ± 1.0	14.4 ± 0.9	14.2 ± 0.8
Hematocrit (%)	43.5 ± 2.5	—	42.8 ± 3.2	41.9 ± 2.8	—	41.0 ± 2.6
Reticulocytes (10^9/L)	52.0 ± 8.9	59.3 ± 14.8	54.8 ± 16.2	55.3 ± 14.1	60.4 ± 12.8	54.8 ± 16.3
MCHC (g/dl)	33.4 ± 0.9	—	34.1 ± 2.0	33.4 ± 1.3	—	35.3 ± 2.5
Plasma proteins (g/L)	73.2 ± 3.9	75.7 ± 5.3	74.6 ± 2.6	74.4 ± 3.4	78.4 ± 4.9	76.3 ± 2.7
TCP (g)	298 ± 29	—	327 ± 46	297 ± 34	—	321 ± 38
Albumin (g/L)	42.8 ± 2.1	44.5 ± 2.8	42.5 ± 2.0	43.3 ± 3.0	45.0 ± 5.2	43.8 ± 2.9
Total albumin (g)	175 ± 20	—	187 ± 27	173 ± 19	—	184 ± 20
Copeptin (pmol/L)	68.3 ± 26.2	65.0 ± 18.1	78.6 ± 27.4	59.7 ± 24.6	58.4 ± 27.1	65.2 ± 24.7
Pro-ANP (pmol/L)	5.71 ± 2.29	6.20 ± 3.24	5.57 ± 2.45	4.57 ± 1.72	4.30 ± 1.13	5.00 ± 1.82
EPO (mIU/ml)	9.3 ± 2.9	8.7 ± 1.7	9.4 ± 2.2	9.7 ± 2.3	9.4 ± 3.4	9.3 ± 1.8

EPO, erythropoietin; [Hb], hemoglobin concentration; MCHC, mean corpuscular hemoglobin concentration; TCP, total content of protein; Pro-ANP, pro-atrial natriuretic peptide. Before (Pre), after 2 weeks (Mid) and after the intervention (Post). Results represent mean ± SD.

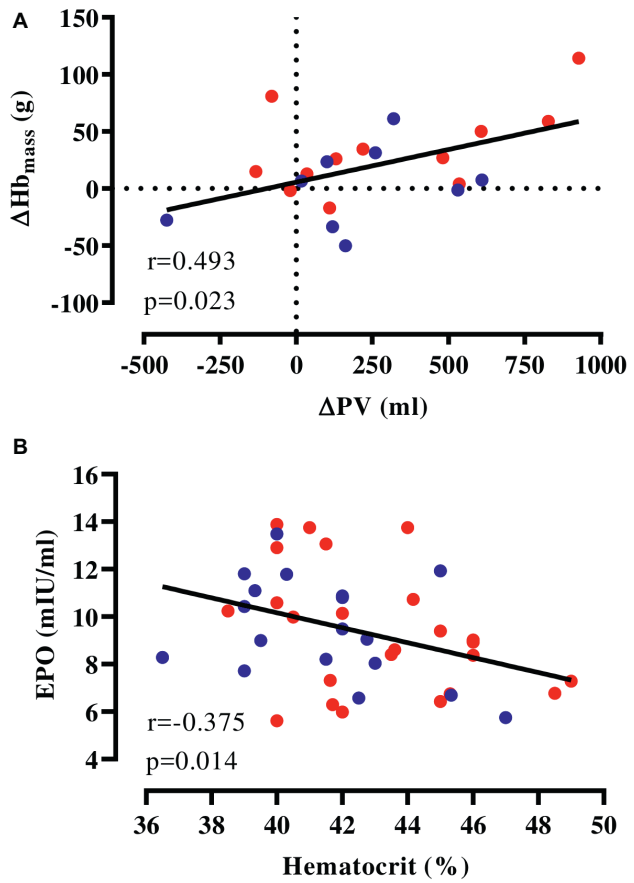

FIGURE 2 | Correlation of hematological parameters. **(A)** Changes in Hb_{mass} and PV with the intervention (HEAT: red, CON: blue), **(B)** absolute EPO and hematocrit (values before and after the intervention pooled). EPO, erythropoietin; Hb_{mass}, hemoglobin mass; PV, plasma volume.

higher Hb_{mass} may be a consequence of the exercise-heat acclimation-induced PV expansion (Montero and Lundby, 2018). The mechanistic basis for this was introduced with the concept of the kidney functioning as a "critmeter" that controls hematocrit by adjusting PV and RBCV, and thus stabilizes arterial O_2 content (Donnelly, 2001). The mediating hormone is the glycoprotein EPO that is released upon renal tissue hypoxia resulting from hemodilution and promotes the production of red blood cells in the hematopoietic bone marrow (Jelkmann, 2011). Indeed, it is observed that the rise in EPO coincides with the expansion in PV after 2 weeks of ET (Montero et al., 2017). In the present study, we found that alterations in Hb_{mass} were weakly correlated to PV changes when participants from HEAT and CON were included in the analysis. Furthermore, participants with low hematocrit possessed higher plasma EPO as previously reported for anemic individuals (Erslev, 1991). Thus, accumulating evidence, including our correlational data, points toward PV fluctuations being a driver of erythropoiesis. It needs to be highlighted, however, that the tendency in higher Hb_{mass} in HEAT was not reflected in any changes in plasma EPO or other BV-regulating hormones measured after 2 weeks and by the end of the intervention and the above hypothesis is only supported by the correlational analyses. Moreover, the [Hb] was unchanged after 2 weeks into the intervention period, indicating either normalized PV at this time point or partial Hb_{mass} expansion already compensating for elevated PV. While the latter appears unlikely (Keiser et al., 2015; Rendell et al., 2017), there is some evidence pointing toward only transient effects of exercise-heat acclimatization on PV (Wyndham et al., 1968). Since CO rebreathing was omitted during the intervention period, we cannot conclude on the precise time course of Hb_{mass} and intravascular volume adaptations to exercise-heat acclimation. In addition, determination of the PV-regulating hormones pro-ANP and copeptin, a proxy measure of vasopressin, did not reveal any alterations.

The strong association between endurance performance and Hb_{mass} implies that strategies to stimulate and induce an overall increase in Hb_{mass} are commonly applied by endurance athletes (Gore et al., 1997). A classic procedure is altitude training or "live high-train low", where the hypoxia-induced augmented RBCV is observed to enhance performance (Stray-Gundersen and Levine, 2008), although more recent evidence questions this approach (Bejder and Nordsborg, 2018; Robach et al., 2018). Nonetheless, data showing Hb_{mass} expansion with altitude training in individuals with a similarly high Hb_{mass} as in the present study, report an increase of 5–6% (Robach and Lundby, 2012), which is slightly higher than the +3% observed in the current study with prolonged exercise-heat acclimation. Notably, hypoxia leads to an early contraction in PV (Siebenmann et al., 2017b), whereas exercise-heat acclimation is a training approach that circumvents this reduction in PV.

Eventually, the question arises as to whether the trend in higher Hb_{mass} translated into better endurance performance. It is known that the infusion of packed red blood cells leads to improved VO_{2peak} consequent of increased O_2 transport capacity and facilitated cardiac output (Ekblom et al., 1972). The autologous transfusion of ~135 ml red blood cells is furthermore sufficient to improve time trial performance by ~5% in well-trained men (Bejder et al., 2019). Considering that participants in HEAT in the present study had elevated RBCV by 134 ± 140 ml, some participants may indeed have benefitted from a performance effect in cold conditions. However, while there was an improved time trial performance in HEAT in cold conditions, the same was observed for CON and the intervention did not affect VO_{2peak} [Mikkelsen et al. (2019), submitted in this issue]. Yet, it has been suggested that VO_{2peak} is elevated by ~4 ml/min for each1g rise in Hb_{mass} (Schmidt and Prommer, 2010), which hypothetically would correspond to a mean increase in VO_{2peak} of ~1.75 ml/min/kg (+3%) in HEAT. While this slight increment is of relevance for competing athletes, it is likely that our VO_2 measurement was not sufficiently sensitive to detect this difference (Carter and Jeukendrup, 2002). Taken together, even though Hb_{mass} and thus O_2 transport capacity tended to be higher in HEAT than in CON, this did not manifest in better performance in the cold. Nonetheless, it is worthwhile investigating whether exercise-heat acclimation for even longer periods results in a gradual Hb_{mass} expansion and whether that may ultimately improve endurance performance in the cold.

We acknowledge some limitations to our study. First, we only observed a tendency toward a modifying effect of exercise-heat acclimation on Hb_{mass}. This tendency is likely related to the variation in the two groups and therefore limited statistical power may hinder us from drawing definite conclusions. Nevertheless, the time × group interaction was borderline significant ($p = 0.061$) and the inclusion of a carefully matched control group (VO_{2peak}, age, and training volume) is considered as a major strength of the study design. Secondly, we were not able to pinpoint the time course of erythropoietic adaptation to exercise-heat acclimation, as reticulocyte count and EPO remained unaltered at 2 weeks of exercise-heat acclimation. This is in contrast to conventional ET, where EPO peaks after 2 weeks and thereafter returns to baseline (Montero et al., 2017). However, at altitude, a steep rise in EPO is detected already after 24 h of exposure whereafter it normalizes (Lundby et al., 2014) and it thus may be that the erythropoietic stimulus in the present study occurred earlier. Thirdly, we hypothesized that the expansion in PV is a mechanism underlying the higher Hb_{mass}. However, we only revealed a weak correlation between alterations in PV and Hb_{mass} and we can thus only speculate on a potential association of the PV expansion with the higher Hb_{mass}. Accordingly, there is need for further experimental verification in humans (Montero and Lundby, 2018). Ultimately, even though participants in the present study were endurance-trained, they did not reach the very high Hb_{mass} values of professional endurance athletes (Jelkmann and Lundby, 2011). Given that it appears challenging to augment erythropoiesis in athletes with high initial Hb_{mass} (Robach and Lundby, 2012), it remains to be examined whether prolonged exercise-heat acclimation in professional endurance athletes is feasible and beneficial for Hb_{mass} expansion.

In summary, when endurance-trained individuals were exposed to environmental stress, i.e., heat, during a substantial part of their weekly training, Hb_{mass} tended to be more expanded than with conventional ET. The mechanisms triggering the response remain to be revealed but could involve a compensatory response in erythropoiesis secondary to PV expansion as the higher Hb_{mass} was correlated to the expansion in PV although EPO and BV-regulating hormones remained unchanged.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary files. Some data can be found in the accompanying article (Article DOI: 10.3389/fphys.2019.01372).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Capital Region of Denmark, protocol no.: H-17036662. The patients/participants provided their written informed consent to participate in this study.

DISCLOSURES

The license for the product Tacx Trainer device and software was obtained from the copyright holders.

AUTHOR CONTRIBUTIONS

LN and CL contributed in conception and design of research. LO, CS, CM, NJ, JP, JG, and A-KM performed experiments. LO and CS analyzed data. LO, CS, NM, LN, and CL interpreted results of experiments and drafted the manuscript. LO prepared figures. LO, CS, CM, NJ, JP, NM, JG, A-KM, LN, and CL approved the final version of manuscript and edited and revised the manuscript.

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Prolonged Heat Acclimation and Aerobic Performance in Endurance Trained Athletes

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Heat acclimation (HA) involves physiological adaptations that directly promote exercise performance in hot environments. However, for endurance-athletes it is unclear if adaptations also improve aerobic capacity and performance in cool conditions, partly because previous randomized controlled trial (RCT) studies have been restricted to short intervention periods. Prolonged HA was therefore deployed in the present RCT study including 21 cyclists [38 ± 2 years, 184 ± 1 cm, 80.4 ± 1.7 kg, and maximal oxygen uptake (VO_{2max}) of 58.1 ± 1.2 mL/min/kg; mean ± SE] allocated to either 5½ weeks of training in the heat [HEAT (n = 12)] or cool control [CON (n = 9)]. Training registration, familiarization to test procedures, determination of VO_{2max}, blood volume and 15 km time trial (TT) performance were assessed in cool conditions (14°C) during a 2-week lead-in period, as well as immediately pre and post the intervention. Participants were instructed to maintain total training volume and complete habitual high intensity intervals in normal settings; but HEAT substituted part of cool training with 28 ± 2 sessions in the heat (1 h at 60% VO_{2max} in 40°C; eliciting core temperatures above 39°C in all sessions), while CON completed all training in cool conditions. Acclimation for HEAT was verified by lower sweat sodium [Na^+], reduced steady-state heart rate and improved submaximal exercise endurance in the heat. However, when tested in cool conditions both peak power output and VO_{2max} remained unchanged for HEAT (pre 60.0 ± 1.5 vs. 59.8 ± 1.3 mL O_2/min/kg). TT performance tested in 14°C was improved for HEAT and average power output increased from 298 ± 6 to 315 ± 6 W ($P < 0.05$), but a similar improvement was observed for CON (from 294 ± 11 to 311 ± 10 W). Based on the present findings, we conclude that training in the heat was not superior compared to normal (control) training for improving aerobic power or TT performance in cool conditions.

Keywords: cycling time trial, maximal oxygen uptake, exercise, peak power output, cycling efficiency

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INTRODUCTION

It is well documented that natural heat acclimatization as well as laboratory-based heat acclimation (HA) improve exercise performance in hot environments (see Daanen et al., 2018 for review and Lorenzo et al., 2010; Karlsen et al., 2015a,b; Racinais et al., 2015 for specific studies). In contrast, if HA also leads to physiological adaptations that will improve exercise performance in cool conditions remains controversial (Corbett et al., 2014;

Minson and Cotter, 2016; Nybo and Lundby, 2016). On one hand, Scoon et al. (2007), Karlsen et al. (2015b), and Keiser et al. (2015) report no effect of HA (or similar performance effects as reported for a matched control group training in cool settings) on exercise endurance performance in cool conditions. On the other hand, a few randomized controlled trials (RCTs) studies have reported beneficial effects (Lorenzo et al., 2010; McCleave et al., 2017; Rendell et al., 2017) on VO_{2max}, time trial (TT), and lactate threshold. Furthermore, studies that did not include a control group in the study design also report that training in the heat may benefit aerobic performance in cool setting (Hue et al., 2007; Buchheit et al., 2011, 2013; Racinais et al., 2014; Neal et al., 2016a,b). The difference in findings from the above studies may, to some extent, relate to the heterogeneity of the studies, as they differ in the participants' training status, the conditions undertaken by the control group and, in particular, the duration of the intervention period. To conclude if HA may translate into improved aerobic performance in cool conditions, it is not sufficient to merely demonstrate improved performance in untrained or recreationally active adults, as improvements in performance could relate to a standard training effect rather than environmental stress. Also, for athletes, the improvement accomplished by training in the heat would need to be superior compared to control training that includes high-intensity intervals (Levine and Stray-Gundersen, 1997).

The proposed ergogenic effect of HA for subsequent performances in cool conditions has been attributed to a combination of hematological, cardiovascular, and skeletal muscle adaptations (Corbett et al., 2014). One mechanism of particular interest has been the expansion of plasma volume (and general increase of extra-cellular volume; see Patterson et al., 2004) that could translate into increased hemoglobin mass (Hb_{mass}) and higher capacity for systemic oxygen delivery (Scoon et al., 2007); although the erythropoietic effect was not observed in the study by Patterson et al. (2004). Diverse effects on Hb_{mass} as well as plasma and blood volume across available HA studies may relate to relative short intervention periods deployed in previous studies allowing limited time for erythropoiesis to occur. In that context, significant effects of environmental interventions, e.g., altitude exposure is typically considered to require several weeks depending on the strength of the stimuli (Rasmussen et al., 2013; Siebenmann et al., 2015). Therefore, to determine whether HA positively enhances erythropoiesis in trained subjects, HA studies with sufficient duration are warranted.

In addition to central hemodynamic responses, peripheral adaptations of relevance for performance have been proposed to be enhanced by heat training (Coyle, 1999; Bassett and Howley, 2000). For example, improved gross efficiency (GE) following heat training has been observed in some studies (Shvartz et al., 1977; Sawka et al., 1983) but not in others (Karlsen et al., 2015b; Rendell et al., 2017). While enhanced exercise efficiency has been proposed to involve changes in skeletal muscle recruitment patterns in response to heating (Shvartz et al., 1977; Sawka et al., 1983; Corbett et al., 2014), the improved efficiency could merely relate to acquaintance with the experimental testing as the studies reporting this effect have not controlled for familiarization effects.

Therefore, the present study was conducted to evaluate the efficacy of prolonged HA (induced by training in the heat) compared to continued normal training (in settings with low thermal stress) with focus on the potential for improving exercise performance and aerobic capacity in cool conditions. Specifically, a long-term ($5^1/_2$ weeks) laboratory-based heat-training period was employed to ensure adequate time for any potential erythropoietic effect to occur. We included a group of endurance trained male cyclists that, following familiarization and a controlled lead-in phase, were randomly allocated to a heat training (HEAT) or control (CON) group. It was hypothesized that long-term heat training would improve aerobic capacity, peak power output and prolonged exercise performance, as determined by a 15-km time-trial (TT). We evaluated if potential performance effects involved improved exercise efficiency, thermoregulatory factors related to sudomotor adaptations and hematological adaptations leading to plasma and blood volume expansion. The present paper is focused on the overall performance effects (TT, peak power, and aerobic capacity), while we refer to the accompanying publication by Oberholzer et al. (2019, submitted to the special issue) for details on the hematological adaptations and in-depth analyses of mechanisms involved.

MATERIALS AND METHODS

Participants

Twenty-four well-trained, sub-elite male cyclists [38 ± 9 years, 184 ± 4 cm, 80.4 ± 8.0 kg, and maximal oxygen uptake (VO_{2max}) of 58.1 ± 5.3 mL/min/kg; Mean ± SD] with at least 3 years of cycling experience were initially recruited (see **Table 1** for group-specific overview of baseline descriptive data). Participants had conducted their usual off-season training (environmental temperatures <15°C) leading up to the study and were thus assumed to be only partly heat acclimated due to training status (Armstrong and Maresh, 1991). Following pre-intervention testing, participants were block-allocated into two performance-, VO_{2max}-, and age-matched groups (n = 12) that subsequently were randomly designated as either the heat training (HEAT) or control (CON) group, however, due to personal reasons unrelated to the study, three subjects withdrew before commencement of the intervention, resulting in 12 and 9 participants in the HEAT and CON completing the study, respectively. Data from drop-outs were excluded from the analysis. Before providing their written consent to participate, subjects were informed of potential risks and discomforts associated with the experimental procedures. The study was conducted in accordance with the Helsinki declaration and approved by the ethics committee of the Capital Region of Denmark (protocol: H-17036662).

Study Overview

An overview of the study protocol is displayed in **Figure 1**. Upon enrolment into the study, participants first completed one familiarization session, completing a 30 min preload, followed by a 15 km TT. During the following 2 weeks, participants were monitored in a lead in phase, with registration of weekly training

TABLE 1 | Baseline descriptive data of participants and training characteristics before (during lead-in phase) and during the intervention for the heat training group (HEAT) and control group (CON).

	Heat (n = 12)	Con (n = 9)
Age (years)	39 ± 9	38 ± 9
Height (cm)	185 ± 3	183 ± 5
Body mass (kg)	80.2 ± 6.3	80.5 ± 9.5
Body fat percentage (%)	13.7 ± 3.9	14.7 ± 2.9
VO_{2max} (L/min)	4.8 ± 0.4	4.6 ± 0.4
VO_{2max}/kg (mL/min/kg)	60.0 ± 5.1	57.9 ± 5.1
iPPO (W)	409 ± 20	408 ± 33
iPPO/kg (W/kg)	5.1 ± 0.5	5.1 ± 0.6
Training volume (min/week – lead in)	417 ± 105	499 ± 164
Training volume (min/week – during)	509 ± 173*	576 ± 143*
Intense training (min/week > 80% HR_{max})	102 ± 71	102 ± 55
Intense training during intervention (min/week)	157 ± 90*	122 ± 57*

**Denotes a main effect of time compared to lead in ($P < 0.05$). All other comparisons were not different ($P > 0.05$). Values are mean ± SD.*

volume – both total and high intensity. On three subsequent sessions, baseline assessment of VO_{2max}, cycling efficiency and TT performance, as well as hematological parameters (see accompanying paper by Oberholzer et al., 2019 for details) were conducted. Participants were then allocated to their respective groups and trained in a 5½ week period (see "Intervention Period" section for more detail). Following the training period, a post-test battery identical to the pre-intervention battery was conducted. All performance testing and training was conducted at the same time of day (within 2 h) using the participants' personal bikes installed in a stationary Tacx-trainer device (Tacx Neo Smart T2800, Wassenaar, Netherlands) and associated software (Tacx Trainer software 4, Wassenaar, Netherlands). For each subject the same personal bike and Tacx-trainer were used during both pre- and post-intervention testing to circumvent any equipment differences. Pre-intervention performance testing was conducted during a 2-week period preceding the intervention (hematology within 2 days) and post-intervention testing within 6 days of the intervention's conclusion. A recovery period lasting a minimum of 24 h separated all performance tests to preclude residual fatigue confounding the results. Subjects were instructed to abstain from performing any exhaustive exercise the day leading up to a performance test and to refrain from consumption of caffeine for 12 h and alcohol for 24 h prior to testing.

Testing

Time Trial

Endurance performance was evaluated through the fastest possible completion of a simulated 15 km TT (mean slope of 0.1%, ∼600 m of uphill cycling) preceded by a 30-min preload at 60% of VO_{2max}. The TT and preload were separated by 5 min of passive rest. Subjects had visual access to real-time information regarding heart rate (HR), distance completed/remaining, speed, cadence, and power output, but were blinded to elapsed time. The TT was conducted in temperate ambient conditions (14 ± 0.2°C, RH: 54 ± 3%; Galloway and Maughan, 1997) with airflow of ∼3 m/s directed toward the subjects' frontal surface [WBGT of ∼10°C (Reed Heat Index checker 8778, Reed Instruments, United States)]. Provision of a maximal effort was facilitated by verbal encouragement throughout the test in addition to a prize being rewarded for the best performance.

VO_{2max}, Cycling Efficiency, Incremental Peak Power Output, and Anthropometry

Upon arrival to the laboratory and prior to performing any exercise, body mass and fat percentage were quantified on an electronic bio-impedance scale (InBody 270, InBody, Denmark). Cycling efficiency, VO_{2max}, and incremental peak power output (iPPO) were assessed through completion of an incremental cycling test to volitional exhaustion. Following warm-up stages consisting of 5 min at 100 W and 5 min at 175 W (80 RPM), respectively, the work load was increased by 25 W/min, terminating when the subject was incapable of maintaining a pre-defined and self-selected cadence despite strong verbal encouragement. Breath by breath recordings of VO_2 and VCO_2 were obtained throughout the test [Jaeger Oxycon Pro, Viasys Healthcare, Germany (calibrated for room humidity, flow, and O_2/CO_2 concentration prior to each test)] and subsequently interpolated to 5 s mean values. Values ≥4 standard deviations from the local mean were discarded. A plateau in VO_2 despite increased work load and/or attainment of a respiratory exchange ratio (RER) ≥1.15 served as test validation criteria. VO_{2max} was defined as the highest observed value over a 30-s period and iPPO as the last completed work stage (W) plus the fraction (s) of the last non-completed stage [iPPO = (Last completed work stage (W)) + (25 W/60 × $t(s)$)].

Intervention Period

Participants in the HEAT group underwent 60-min heat training sessions in a climatic chamber on 5 weekly occasions

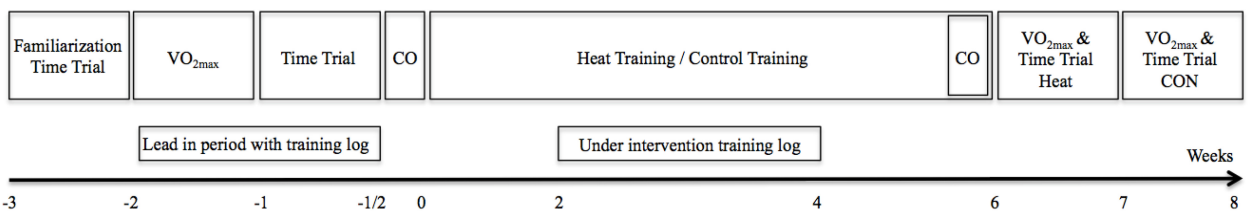

FIGURE 1 | Study overview and time course. CO, carbon monoxide rebreathing procedure.

(28 ± 2 total sessions), while subjects in CON reported to the laboratory and trained once a week in cool conditions (∼15°C) to minimize group differences in the level of familiarization to stationary cycling and completed all other training and habitual intervals in cool settings. Both groups performed this part of the training at a constant intensity corresponding to 60% of VO_{2max} (204 ± 3 W). For HEAT, ambient temperature was set at 35°C the first week (3 days) and subsequently increased by one degree each week ending at 40°C (RH: 30 ± 2%), in order to accommodate for the relative decrease in intensity as HA was induced (Daanen et al., 2018). Rectal core temperature (T_{core}) was elevated to ≥38.5°C after 35 ± 8 min of training and end T_{core} was 39.6 ± 0.4°C in all training sessions. Subjects were encouraged to undertake each training session without airflow for as long as subjectively tolerated but were provided with individually adjusted airflow when requested (ventilation with a floor fan of ∼1–3 m/s) to facilitate evaporation and provide some perceptual benefit to ensure that the exercise component could be completed. HR, T_{core}, and sweat Na^+ were quantified during the first and last weekly training session. Warm water was ingested *ad libitum* during training to avoid fluid consumption acting as a heat sink. Body mass was measured before and after each training session and subjects were instructed to replenish 150% of lost fluid during the following hours to re-establish euhydration. During the entirety of the intervention period, outside environmental temperature did not exceed 15°C.

Confirmation of Acclimation Status Testing

To confirm acclimation status, a sub group of six participants from HEAT underwent a heat tolerance test (HTT) on day 1, 14, and 28. In order to avoid any partial HA, none of the participants from the CON group completed HTT testing. The HTT was conducted as a time to exhaustion (TTE) test under standardized 40°C at 60% VO_{2max}, with no access to fan or other cooling (see "Measurement" section for details). Additionally, all participants in HEAT, were monitored for HR, T_{core}, and changes in sweat Na^+ concentration, in the beginning and end of all weeks of training.

Measurements

Heart Rate and Core Temperature

Heart Rate was assessed using participants' personal HR monitors (Garmin edge 500/520/820/1000/1030, Garmin Ltd., United States), was provided as a continuous feedback tool during TT, and was logged for acclimation status. T_{core} was recorded by a flexible rectal probe (Ellab, Denmark) self-inserted ∼10 cm beyond the anal sphincter. Both HR and T_{core} were measured during the first and the last HA training of all weeks of training as well during the HTT. Values were manually logged every 10 min and at TTE.

Sweat Rate

To calculate sweat rate (adjusted for fluid consumption) and to account for the effect of body mass during the TT, body mass (towel dried while wearing cycling shorts) was measured (InBody 270, InBody, Denmark) prior to the preload and following the TT. Sweat was obtained for Na^+ content analysis (ABL 800 Flex, Radiometer, Denmark) by absorbent pads (Tegaderm +Pad, 3M, Denmark) placed on the upper back at the level of the scapulae, after thorough cleansing of the skin with demineralized water.

Training and Training Quantification

Training quantification was carried out during a 2-week lead in phase prior to the intervention and a 2-week period during the intervention, to quantify the impact of the intervention on participants' habitual training procedures. Participants were instructed to fill out a training log containing information regarding total weekly training volume and total high-intensity training volume, the latter defined as training at HR above 80% of maximum (Karlsen et al., 2015b). Both groups were instructed to preserve their usual interval training routines alongside the intervention and to subtract the training associated with the intervention from their habitual training, to maintain total training volume as reported in their initial training log reported in the lead in phase.

Cycling Efficiency

Gross efficiency was calculated as the ratio of external mechanical work (W) to energy expenditure (EE). EE was calculated from steady state VO_2 (confirmed in each participant by visual inspection of the VO_2-time curve), RER and corresponding VO_2 values obtained during the last 90 s of exercise at 100 (GE100) and 175 W (GE175), respectively, completed with fixed cadence of 80 RPM:

$$GE = \left(\frac{\frac{watt}{1000} * 60}{\left(\left(\left(\frac{1-RER}{0,3} \right) * 19,3 \right) + \left(\left(\frac{RER-0,7}{0,3} \right) * 21,1 \right) \right) * \left(\frac{VO_2 \frac{ml}{min}}{1000} \right)} \right)$$

Statistical Analyses

All data are expressed as mean values with standard error unless otherwise stated. Pre-intervention group characteristics, performance results, and training logs were assessed using a student's independent *T*-test to confirm homogeneity between groups. The changes pre- to post-intervention values between the heat training and control group for TT power output and completion time, relative, and absolute VO_{2max}, iPPO, GE100, GE175, training time, and training time above 80% HR max were assessed with a two-way mixed-measures ANOVA, with the repeated factor of time point (two levels: Pre and Post) and the independent factor of intervention (two levels: HEAT and CON). Adaptations (T_{core}, HR, sweat rate, and sweat Na^+) in the HEAT group during the intervention period were evaluated with a paired samples *T*-test. When applicable, *post hoc* testing was carried out using a Holm–Sidak test. The probability of making a Type 1 error in all tests was maintained at 5%. All statistical analyses were carried out using GraphPad Prism (version 7.0, GraphPad Software, La Jolla, CA, United States).

RESULTS

Heat Training and Acclimation Effects

In the HEAT group T_{core} increased during each training session in the heat and was above 39°C at the end of all sessions but decreased from the initial to the last training session (39.9 ± 0.4 vs. 39.4 ± 0.3°C, $P < 0.05$; see the weekly progression in **Table 2**). Similarly, end-training HR declined during the first 2 weeks of training in the heat and was significantly lowered from the first to the last training session in the heat (169 ± 10.5 vs. 155 ± 17 BPM, $P < 0.05$). In addition, sweat Na^+ was reduced by 46 ± 14%, from 91 ± 5 mmol/L during the first week to 69 ± 8 mmol/L ($P < 0.01$), in the third week of training in HEAT, but did not decrease thereafter (see **Table 2**). Sweat rate during the cool TT testing did not significantly change from Pre to Post in either group (HEAT: 0.83 ± 0.03 vs. 0.93 ± 0.06 L/h; CON: 0.78 ± 0.03 vs. 0.78 ± 0.07 L/h, $P > 0.05$). The sub group of six participants, who underwent the HTT at 40°C, extended their TTE by 25.6 min (Day 1: 38.7 ± 2.4 vs. day 28: 64.3 ± 2.9 min, $P < 0.001$), from the first to the last test.

Time Trial in Cool Conditions

There was a main effect of time for average TT power output (+5.8 ± 0.9%, $P < 0.001$, see **Figure 2**), resulting from significant increases in both groups (HEAT: +6.0 ± 1.1%, $P < 0.05$; CON: +5.5 ± 1.6%, $P < 0.05$). Accordingly, TT performance time significantly decreased in both HEAT (by 37.4 ± 8.6 s, $P < 0.05$) and CON (37.1 ± 10.1 s, $P < 0.05$), with similar improvements across groups (i.e., there was no group × time interaction effect).

VO_{2max}, Incremental Peak Power Output, and Cycling Efficiency

There was no change from pre to post in VO_{2max} in either group [HEAT: 4.8 ± 0.1 vs. 4.8 ± 0.1 L/min; CON: 4.6 ± 0.1 vs. 4.7 ± 0.1 L/min ($P > 0.05$, **Figure 2**)], and since there was no change in body weight from pre to post, relative VO_{2max} (mL O_2/min/kg) also remained similar for both groups [HEAT: 60.0 ± 1.5 vs. 59.8 ± 1.2 mL/min/kg; CON 57.9 ± 1.7 vs. 59.4 ± 2 mL/min/kg ($P > 0.05$, see **Figure 3**)].

There was no main effect or significant effect of HA on iPPO [HEAT: 409 ± 6 vs. 422 ± 7 W; CON: 408 ± 11 vs. 411 ± 9 W ($P > 0.05$, **Figure 3**)], and cycling efficiency, evaluated as GE at 100 W (HEAT: 15.4 ± 0.3 vs. 15.2 ± 0.2%; CON: 14.8 ± 0.5 vs. 15.2 ± 0.4%) or 175 W (HEAT: 19.7 ± 0.3 vs. 19.4 ± 0.2%; CON: 19.3 ± 0.5 vs. 19.8 ± 0.5%), did not display any changes in either group ($P > 0.05$) (**Figure 3**).

Training and Training Quantification

Compared to the lead-in period, there was a main effect of time ($P < 0.05$) for weekly training volume, resulting from a non-significant increase in both groups (HEAT: +13 ± 7%; CON: +11 ± 9%, $P > 0.05$). Also, the weekly training volume above 80% of maximum heart rate increased for HEAT from 102 ± 21 to 157 ± 27 min/week and for CON from 102 ± 18 to 122 ± 19 min/week (both $P < 0.05$ from pre to post, but not significantly different across groups).

Hematological Parameters

Main effects of time were detected for both BV and PV (both $P < 0.05$, but no time × intervention interaction). For BV with a 5.7 ± 1.5% increase from pre to post in the HEAT group ($P < 0.01$) and a 3.2 ± 1.6% increase for CON ($P < 0.05$) with no significant differences between groups ($P > 0.05$).

From PRE to POST, PV increased by 6.5 ± 2.2% in the HEAT group ($P < 0.01$) and there was a 4.5 ± 2.4% increase in CON ($P < 0.05$), with the overall response not different across groups. As previously mentioned, we refer to the accompanying paper (Oberholzer et al., 2019) for detailed description of the hematological parameters and involved mechanisms.

DISCUSSION

The prolonged HA period employed in present study was associated with significant sudomotor adaptions (with the reduction in sweat [Na^+] leveling off after 2–3 weeks of training in the heat), improved exercise endurance in hot environmental settings (i.e., increased TTE at fixed submaximal workload in 40°C), plasma volume expansion and an elevation of total blood volume. However, when transfer effects to endurance performances in cool conditions were tested post HA (in settings with low environmental heat load; i.e., below 15°C), the heat-training group did not increase peak aerobic power, improve submaximal exercise efficiency or VO_{2max}, and TT performance effects were similar compared to the matched control group. We refer to the accompanying paper (Oberholzer et al., 2019) for detailed discussion of potential benefits and mechanisms involved in the hematological response observed for the HEAT group, but from the present measures of performance and aerobic

TABLE 2 | Sweat sodium concentration, end-training HR, and rectal temperatures (n = 12) during the intervention for the heat training group (HEAT).

	Sweat [Na^+] (mmol l^{-1})	End-exercise HR (bpm)	Start rectal temperature (°C)	End-training rectal temperature (°C)
First heat session	93 ± 5	167 ± 5	37.7 ± 0.2	39.9 ± 0.1
End of week 1	74 ± 5*	160 ± 4	37.2 ± 0.3*	39.7 ± 0.1
End of week 2	71 ± 6*	160 ± 3*	37.2 ± 0.2*	39.7 ± 0.1
End of week 3	69 ± 7*	155 ± 4*	–	39.4 ± 0.1*
End of week 4	72 ± 7*	156 ± 4*	37.0 ± 0.3*	39.3 ± 0.1*
End of week 5	79 ± 6*	156 ± 4*	–	39.4 ± 0.1*

*Denotes a significant reduction compared to first training session ($P < 0.05$). Values are mean ± SE.

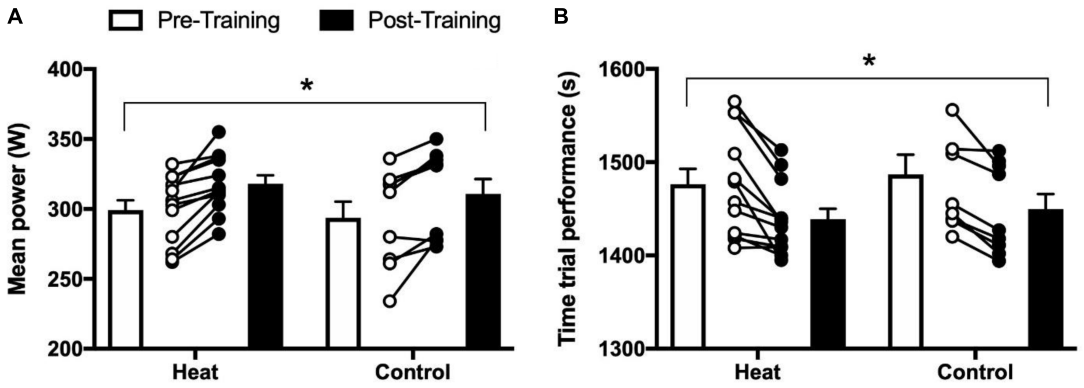

FIGURE 2 | (A) Mean power output in watts and **(B)** time trial performance in seconds, during a 15 km time trial in cool/temperate conditions, for the intervention group (HEAT) and a control group. Open bars and circles illustrate average and individual measurements, respectively, before intervention, black bars and circles illustrate average and individual measurements, respectively, after intervention. *Denotes a significant main effect of time ($P < 0.05$). Values are shown as mean ± SE.

power in a population of endurance-trained cyclists, it appears that the potential physiological advantage of a slightly increased Hb-mass was outweighed by the concurrent hemodilution.

To characterize HA status and the gradual heat adaptation, we measured sweat [Na^+], resting and exercise T_{core} as well as HR responses at the beginning and end of every week of training, and hematological responses were measured at the start and end of the study. End exercise T_{core} and HR decreased during the intervention period, despite an increase in ambient temperature, and PV expanded by ∼7%, as expected following HA (Périard et al., 2016). Further, in the subgroup of six participants who were tested at day 1, 14, and 28 under standardized ∼40°C conditions, TTE was increased from 38.7 min to 64.3 min. For all subject, the total sweat rate or sweat Na^+ measured during the TT in cool conditions did not increase (from pre to post). This apparent lack of adaptation may be explained by testing in compensable conditions where sweating is dictated by the evaporative requirements (Ravanelli et al., 2018) and due to rapid alterations in the onset and decay of sweat sodium Na^+ (Williams et al., 1967; Armstrong and Maresh, 1991). Taken together with the lowered resting rectal temperature, these measures provide strong evidence that the participants were successfully acclimated following the prolonged heat training period.

In terms of exercise performance as well as the muscular and cardiovascular adaptations required to improve performance, the present findings are in contrast with previous investigations reporting beneficial effects of heat training for aerobic performance in cool or temperate conditions (Sawka et al., 1985; Buchheit et al., 2011; Neal et al., 2016a; Rendell et al., 2017). One explanation for this discrepancy could be the lack of a control group in these studies or failure to control for training quality in the lead-in phase as well as the during the intervention period. One study (Sawka et al., 1985) showed an increase in both VO_{2max} and iPPO after nine consecutive days of HA (2 h per day at 49°C, 20% RH), while others report improved TT, iPPO or intermittent exercise performance (Buchheit et al., 2011; Neal et al., 2016a; Rendell et al., 2017). If the present study had not included a control group, our findings would be in line with some of these observations, as both the HEAT and CON group improved their TT performance, likely resulting from improved fractional VO_{2max} utilization (Bassett and Howley, 2000), as cycling efficiency and VO_{2max} remained unaffected. Supporting our findings, but with a shorter intervention period, Karlsen et al. (2015b) demonstrated that compared to a matched control group, there was no difference in TT performance, VO_{2max} and iPPO in trained cyclists, after 2 weeks of a heat training camp in hot dry environment (all training in heat group conducted outdoors in Qatar). Collectively, these findings suggest that many of the previous observations suggesting that a period with training in hot conditions improves exercise performance in temperate conditions relates to the physical training or additional training load, rather than the environmental heat stress *per se*.

Supporting this notion, previous studies reporting beneficial effects of heat training for subsequent performance in temperate conditions employed untrained individuals (Nadel et al., 1974; Shvartz et al., 1977; Sawka et al., 1983, 1985; King et al., 1985; Young et al., 1985; Takeno et al., 2001). Of particular relevance, many of these studies reported improved exercise economy following heat training (Shvartz et al., 1977; Sawka et al., 1983; King et al., 1985; Young et al., 1985). Improvements in exercise economy are likely explained by alterations in skeletal muscle fiber type composition and enhanced muscular strength (Corbett et al., 2014). In the present study, which employed a sub-elite cycling population, exercise economy was unchanged by 6 weeks of training in either the control or heat training group. Likewise, Keiser et al. (2015) observed no improvements in either TT performance or VO_{2max} in well trained cyclist in a laboratory-based acclimation study with a cross-over design. This finding further suggests that previous improvements in exercise performance with heat training had more to do with training untrained participants, rather than the direct effect of environmental heat stress.

One exception to the above studies (Lorenzo et al., 2010), reported increases in both TT performance and VO_{2max}, compared to a control group, using a sample of endurance trained participants. Both groups performed their usual training

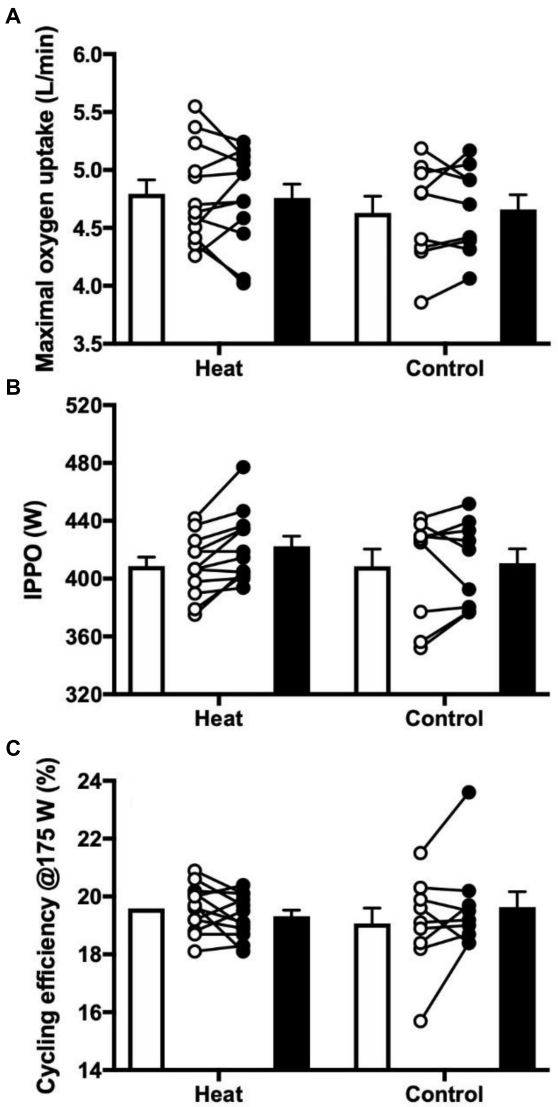

FIGURE 3 | (A) Maximal oxygen uptake, **(B)** incremental peak power output in watts, and **(C)** cycling efficiency in percent at 175 W during an incremental test to exhaustion in cool/temperate conditions (~13°C). Open bars and circles illustrate average and individual measurements, respectively, before intervention, black bars and circles illustrate average and individual measurements, respectively, after intervention. Values are shown as mean ± SE.

outdoors in cool temperate conditions, while adding HA or CON training at low intensity (50% VO_{2max}) in the laboratory; however, additional training was not controlled for in either HEAT or CON. In contrast to the study by Lorenzo et al. (2010), both Keiser et al. (2015) with laboratory-based acclimation and Karlsen et al. (2015b) with natural acclimation (all training in outdoor hot environment) report no superior effect of training in the heat compared to control and the overall effect may depend on the "quality" of the participant habitual training (e.g., if the regularly include high intensity training). Since elite endurance athletes in general will optimize and include high quality, and specifically intense training in preparation for competitions, we find it relevant to ensure that high intensity training is included both in the lead-in phase and during the intervention, as it will clearly influence the potential to develop or maintain VO_{2max} in endurance trained individuals (Gormley et al., 2008). How the overall training impact or load (considering both volume and intensity) is quantified and subsequently matched across groups (in studies where superimposed environmental heat stress elevates HR for a given power output) may always be a matter of debate. In the present study we secured that both groups maintained high-intensity intervals in cool settings and although there was an increased weekly time with HR above 80% of maximum compared to the lead-in period, there was no significant differences across groups; neither for total training volume nor time with HR above 80% of maximum during the lead-in period or the intervention phase of the study. Also, HEAT graduate improved heat tolerance and raised cool TT power output during the post-testing indicating that overload (accumulated fatigue) was not limiting their performance or physiological adaptation to training.

Considering that systemic oxygen delivery and hence VO_{2max} depends on both cardiac output and arterial oxygen content (Ekblom, 1986; Bassett and Howley, 2000), it is likely that beneficial effects of an increased blood volume on cardiac filling and cardiac output may be outweighed by lower arterial oxygen content per liter blood induced by the lower [Hb] associated with plasma volume expansion. It should also be considered that the total training volume and weekly volume with HR above 80% of maximum HR increased for both groups during the intervention period. Further, a perfect match between groups (when aiming at maintaining similar volume, intensity and still optimized training) is an issue in studies with superimposed environmental stress, or as intended in the present study, with part of the training substituted by training in the heat. Thus, training quality is a multifaceted matter that may not be adequately quantified by the total volume and/or relative HR intensity. Some participants in the present study indicated that the physiological strain associated with the intervention compromised their ability to uphold habitual high-intensity interval training procedures, and it is well-established that training intensity is imperative toward development and maintenance of VO_{2max} in endurance trained individuals (Gormley et al., 2008). However, considering that HEAT by both measures of training quality (total volume and HR above 80% of maximum) was exposed to similar training load as CON and that they in fact improved TT performance, it is unlikely to be the cause for the unchanged peak power or effects on VO_{2max}.

McCleave et al. (2017) reported improvements in 3 km TT, but it should be mentioned that their running TT performance was identical in the heat training and control group in the tests conducted immediately following the 3 week intervention, but superior in the heat group following additional 3 weeks of return to normal training practice. Potentially, timing of the follow-up testing may be important and performance could be optimal in the post acclimatization period, when PV returns toward normal. However, that relies on the premise that total red blood cell mass remain elevated (i.e., red blood cells follows expected life time of

∼120 days; see Kurata et al., 1993) and the normalized PV results in advantageous hemoglobin up-concentration (Convertino et al., 1980). Additionally, heat training purportedly improves exercise performance through both thermal and non-thermal adaptations (Corbett et al., 2014). Performance testing in the present study was conducted in ∼10°C wet bulb globe temperature, and therefore likely did not meet the threshold required to impose a thermal limitation (Junge et al., 2016). However, exercise in environmental conditions on the warmer side could meet the threshold, or rather range, of thermal conditions where improved thermoregulatory capacity induced by HA would be ergogenic. Also, the performance tests applied in the present study (incremental peak power test and the 15 km TT including the preload period) had duration of less than 1 h and potential effects of initiating exercise with increased PV and higher total body water during prolonged physical activities (ultra-sports) cannot be excluded.

CONCLUSION

Overall, we conclude that training in the heat was not superior compared to normal (control) training for improving aerobic power or TT performance in cool conditions. However, during a competitive season athletes may be exposed to varying environmental conditions, and although HEAT was not superior compared to CON for improving endurance in cool settings, it is noteworthy that the replacement of a substantial part of overall training volume with heat training did not compromise the effect of training toward temperate exercise performance. Implementation of heat training could therefore be advantageous as part of an integrated pre-season training preparation but the timing of a specific heat training camp may depend the specific competition schedule, considering that the benefit from HA may decay in term of benefitting performance in the heat, whereas for performance in cool settings, a period with return to training without superimposed heat stress may be beneficial.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material. Some data can be found in the accompanying article (doi: 10.3389/fphys.2019.01379).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Capital Region of Denmark (protocol: H-17036662). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors completed the experimental study [testing and analyses (physiological and statistics)], and drafted the proof of manuscript. CM, NJ, CL, and LN contributed to the design, ethical approval, and development of test/study protocol. LN and CL managed the project.

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How Does a Delay Between Temperate Running Exercise and Hot-Water Immersion Alter the Acute Thermoregulatory Response and Heat-Load?

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Hot-water immersion following exercise in a temperate environment can elicit heat acclimation in endurance-trained individuals. However, a delay between exercise cessation and immersion is likely a common occurrence in practice. Precisely how such a delay potentially alters hot-water immersion mediated acute physiological responses (e.g., total heat-load) remains unexplored. Such data would aid in optimizing prescription of post-exercise hot-water immersion in cool environments, relative to heat acclimation goals. Twelve male recreational runners (mean ± SD; age: 38 ± 13 years, height: 180 ± 7 cm, body mass: 81 ± 13.7 kg, body fat: 13.9 ± 3.5%) completed three separate 40-min treadmill runs (18°C), followed by either a 10 min (10M), 1 h (1H), or 8 h (8H) delay, prior to a 30-min hot-water immersion (39°C), with a randomized crossover design. Core and skin temperatures, heart rate, sweat, and perceptual responses were measured across the trials. Mean core temperature during immersion was significantly lower in 1H (37.39 ± 0.30°C) compared to 10M (37.83 ± 0.24°C; $p = 0.0032$) and 8H (37.74 ± 0.19°C; $p = 0.0140$). Mean skin temperature was significantly higher in 8H (32.70 ± 0.41°C) compared to 10M (31.93 ± 0.60°C; $p = 0.0042$) at the end of the hot-water immersion. Mean and maximal heart rates were also higher during immersion in 10M compared to 1H and 8H ($p < 0.05$), despite no significant differences in the sweat or perceptual responses. The shortest delay between exercise and immersion (10M) provoked the greatest heat-load during immersion. However, performing the hot-water immersion in the afternoon (8H), which coincided with peak circadian body temperature, provided a larger heat-load stimulus than the 1 h delay (1H).

Keywords: heat acclimation, heat stress, hot bath, passive heating, endurance athletes

INTRODUCTION

Exercise in a hot environment increases thermoregulatory and physiological strain (Cheuvront et al., 2010) and unpleasant thermal perceptions (Kamon et al., 1974; Stevens et al., 2018), which contribute to deteriorated performances (Guy et al., 2015). Considering that many major sporting events are held under hot and humid conditions, including the upcoming Tokyo 2020 Summer Olympic Games (Kakamu et al., 2017), endurance athletes are recommended to employ heat acclimatization (training in natural heat) or heat acclimation (training in artificial heat) strategies (both abbreviated to HA) to negate heat-mediated performance decrements and possibly provide some protection against exertional heat illnesses (Racinais et al., 2015; Kakamu et al., 2017). Factors central for HA are increased sweating, and elevated core temperature (Tc) and skin temperature responses (Wendt et al., 2007; Périard et al., 2015; Neal et al., 2016). As such, acute HA training sessions aim to maximize these responses.

A mean performance improvement from HA programs of 7 ± 7% has been demonstrated across 27 datasets, where 24/27 reported an improvement >1% (Tyler et al., 2016). Strategies to implement HA into a program prior to a major competition contingent to travel circumstances are also available (Saunders et al., 2019). As such, the positive benefits on performance and recommendations for implementation of HA are clear, yet evidence-based active HA protocols (typically involving specialized facilities and/or relocation) may be logistically difficult to incorporate into complex training programs and the schedules of elite athletes (Casadio et al., 2017). The training sessions themselves can also be onerous, generally involving exercise in the heat for 30–100 min, preferably on consecutive days, for a minimum duration of 1 week (Houmard et al., 1990; Périard et al., 2015; Casadio et al., 2017). Despite the ergogenic potential, during the International Association of Athletics Federations (IAAF) World Athletics Championships in Beijing 2015, where hot and humid conditions were predicted, only 15% of athletes engaged in HA prior to competition (Périard et al., 2017), suggesting that implementing HA may be challenging for some athletes.

In response to these challenges, alternative HA strategies have been investigated, including post-exercise sauna bathing (Scoon et al., 2007) and post-exercise hot-water immersion [HWI; (Zurawlew et al., 2016)]. A total of 16 original investigations have been performed on the topic to-date (Heathcote et al., 2018); the majority demonstrating beneficial hallmark physiological adaptations of heat acclimation (including lowered resting and exercising Tc and heart rate, and increased plasma volume), and importantly, these adaptations were demonstrated in both recreationally active and endurance-trained individuals (Zurawlew et al., 2018a). Further, the use of post-exercise sauna (12 × 30 min exposures) improved running time to exhaustion by 32% in competitive runners/triathletes (Scoon et al., 2007) and post-exercise HWI (6 × 40 min exposures) improved 5 km running performance time in the heat by 4.9% in recreationally active individuals (Zurawlew et al., 2016).

Post-exercise HWI therefore presents a practical HA strategy for athletes residing in cooler climates, compared to expensive alternatives requiring artificial heat chambers and/or relocation. Passive heating has typically been applied immediately after exercise training when used for HA purposes (Scoon et al., 2007; Stanley et al., 2015; Zurawlew et al., 2016), with exercise conducted in laboratory settings, enabling easy access to heating facilities. Practically however, the ability to commence HWI immediately after exercise could be challenging for athletes who lack such facilities near training locations. Indeed, a delay of up to 1 h between training and HWI could easily occur when considering the activities that may prevent immediate immersion (e.g., debrief with coach, stretching, travel, bath preparation, etc.). In other circumstances, athletes may have other commitments during the day, which could delay HWI until the afternoon/evening. Precisely how such a delay and the observed Tc circadian oscillation across a day interact to potentially alter HWI-mediated physiological responses (e.g., total heat-load) remains relatively unexplored. Therefore, the aim of this study was to assess the acute physiological responses central to thermoregulatory strain (Tc, skin temperatures, heart rate, and sweat rate) when post-exercise HWI (30 min; 39°C) was delayed for 10 min (10M), 1 h (1H), or 8 h (8H) following a temperate treadmill run (18°C). It was hypothesized that both 1 and 8 h delay between exercise and HWI would reduce the thermo-physiological strain (e.g., heat-load) of the HA session.

MATERIALS AND METHODS

Participants

Twelve male, recreational [i.e., performance level one-two (De Pauw et al., 2013)] long distance runners (mean ± SD; age: 38 ± 13 years, height: 180 ± 7 cm, body mass: 81 ± 13.7 kg, body fat: 13.9 ± 3.5%) volunteered for the study. Females were excluded due to the confounding influence of menstrual cycle mediated Tc fluctuations (Wendt et al., 2007; Mee et al., 2017). Inclusion criteria stipulated that the participants had performed a 10 km time trial within 6 months prior to the study in ≤50 min (mean time: 47 ± 3 min, range: 42–49 min). Exclusion criteria included any contraindications to exercise as per the Exercise and Sports Science Australia adult pre-exercise screening tool, previous diagnosis of low blood pressure, history of heat illness, or use of prescribed medication during the time of the study. Approval for the project was granted by the Human Research Ethics Committee at Southern Cross University (Approval number: ECN-17-121), and written informed consent was obtained before commencing any testing procedures.

Experimental Design

Participants completed a 40-min submaximal treadmill run (Trackmaster TMX425 CP, Carrollton, Texas, USA) before a 30-min bout of HWI, on three separate occasions, 7–10 days apart. With a randomized crossover design, each trial involved a different time delay between exercise and HWI, including 10 min (10M), 1 h (1H), and 8 h (8H). A schematic of the experimental design is illustrated in **Figure 1**. During 8H, participants were permitted to leave the laboratory after the run and conduct their normal daily activities but were instructed to avoid any physical activity (all participants confirmed that they complied with these instructions). Participants were required to avoid alcohol and

caffeine during testing days and to ensure adequate hydration by ingesting 500 ml of water 2 h prior to arrival. Data collection was completed during winter to minimize natural HA. The data collection took place in the Northern Rivers Region of NSW, Australia. The participants generally arrived to the laboratory wearing a tracksuit and they all ran in shorts and a short sleeve top.

Exercise Protocol

A 40-min treadmill run [climate controlled laboratory; 18.0 ± 0.9°C; relative humidity (RH) 64.5 ± 4.7%] commenced in the morning (between 06:00 and 07:30; time held consistent after first laboratory visit), to control for circadian variation of internal body temperature (Słomko and Zalewski, 2016). A pedestal fan set at a wind speed of 10 $km \cdot h^{-1}$ was placed 2.5 m in front of the treadmill to replicate the convective cooling of running outdoors. During the first trial, running speeds were self-selected *via* rating of perceived exertion (RPE) (Borg, 1998). Participants were instructed to run for 10 min at "light" intensity (RPE = 11), 20 min at "hard" intensity (RPE = 15), and further 10 min at "light" intensity. The treadmill speeds were recorded and replicated in subsequent trials so that each participant ran at the same speeds in all trials. Participants consumed water at 33°C *ad libitum* during the run. This temperature was chosen to minimize any cooling effect from the fluid on the ingested capsule while remaining palatable.

Hot-Water Immersion

The HWI strategy used was 30 min at 38.9 ± 0.1°C to the level of the waist wearing shorts. This was implemented using a bathtub (2.3 m long × 1.1 m wide × 0.5 m high) in a bathroom (8 m^2; 24.2 ± 2.3°C, 76.3 ± 8.1% RH), with water temperature and flow maintained using a two-tap mechanism. Consumption of fluids during immersion was not permitted. The strategy used was based on piloting that determined 39°C was the highest temperature that was safe for the participants to complete with the specified depth and duration in the environment available (i.e., a small room with high humidity; representing the average bathroom). We note that this HWI strategy is less aggressive than that investigated previously (i.e., 40°C for 40 min to the level of the neck), which was too demanding for 6/10 participants to complete on the first exposure in the previous study (Zurawlew et al., 2016). Hence, the HWI strategy presented here is designed to be safe and achievable for the first exposure, and the demands of the HWI (i.e., increased temperature, depth, and/or duration) may be increased toward 40°C for 40 min to the level of the neck in subsequent exposures over time as appropriate for the individual.

Immersion termination criteria was set according to ethical requirements (i.e., reaching a Tc >39.4°C, rapid increase in heart rate, light headedness or reporting a thermal comfort rating that reached "very uncomfortable"); however, all participants completed the full 30-min protocol.

Measurements

Before each initial experimental trial, participants underwent anthropometrical measurements including body mass by an electronic scale (Charder MS3200, Taichung City 412, Taiwan),

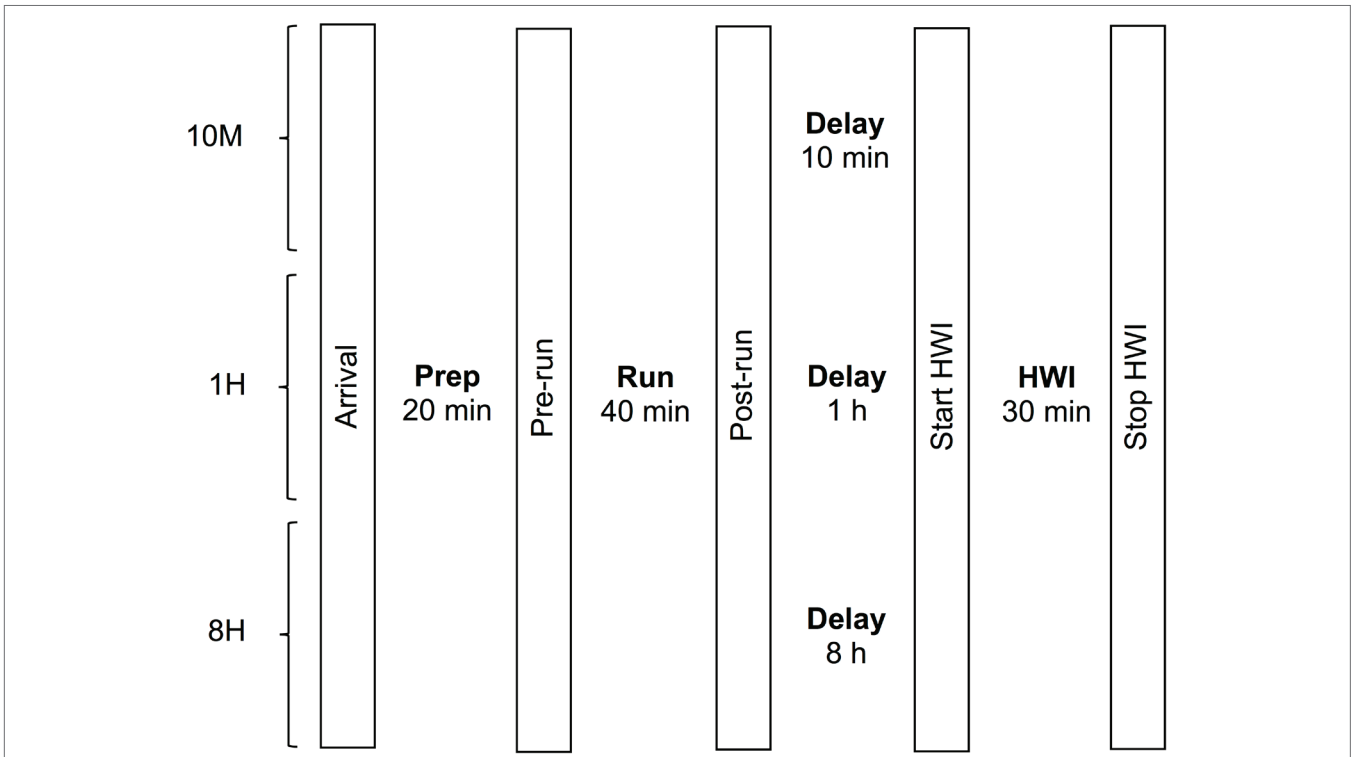

FIGURE 1 | Schematic of the experimental design. 10M, 10 min delay; 1H, 1 h delay; 8H, 8 h delay; HWI, hot-water immersion.

stature (S+M Height Measure 2 m, Rosepark, SA) and skinfold measurement by caliper (Harpenden Calipers, Baty International, West Sussex, United Kingdom) at seven sites including the bicep, tricep, subscapular, supraspinalae, abdominal, mid-thigh, and medial calf, following the International Society for the Advancement of Kinanthropometry recommended protocol (Marfell-Jones et al., 2012).

The Tc was measured continuously using an e-Celsius ingestible telemetric capsule (BodyCap, Caen, France). Participants were instructed to ingest the capsule with water immediately prior to sleep the night before each trial (approximately 8 h prior to each trial). Measurements of skin temperature were taken using a dermal thermal scanner (DermaTemp, Exergen Corporation, MA, USA) on dry skin at four sites (forehead, right calf, right hand, and lower back) before and after exercise/immersion, which allowed for an estimate of mean skin temperature (Tsk) according to the following equation (Nielsen and Nielsen, 1984):

$$\text{Tsk} = 9.429 + (0.137 \times \text{forehead temp}) + (0.102 \times \text{hand temp}) + (0.29 \times \text{back temp}) + (0.173 \times \text{calf temp})$$

Resting body temperature measurements occurred in the climate-controlled laboratory described above (18°C, 65% RH). All participants sat in the climate controlled laboratory wearing exercise clothing for a period of 20 min prior to exercise in all trials. During 10M and 8H, participants spent 10 min in the climate-controlled laboratory wearing shorts only immediately prior to immersion. In 1H, participants spent the entire 60 min in the climate-controlled laboratory prior to immersion; they wore clothing that allowed them to feel comfortable for 50 min, and then shorts only for the final 10 min.

Nude, dry body mass (accurate to 10 g) was recorded prior to the run (equipment same as above) after emptying the bladder. Body mass was also recorded prior to and after immersion following the same procedure. Water bottles were also weighed before and after the exercise to calculate fluid intake during the run so that sweat rate was estimated with the following equation;

$$\text{Sweat rate (SR)} = \left[(\text{change in body mass} + \text{fluid ingested}) / \text{time}\right] / \left[\text{body mass (initial)}\right]$$

Heart rate responses were measured continuously using a Garmin Forerunner 920XT heart rate monitor (Garmin, Neuhausen am Rheinfall, Switzerland).

Measurements of thermal comfort and thermal sensation were recorded at 5 min intervals during exercise and immersion. A four-point scale (1–4) was used to assess thermal comfort (Gagge et al., 1967) and a 17-point scale (0.0–8.0) was used to assess thermal sensation (Young et al., 1987).

Statistical Analyses

The data were analyzed with General Linear Mixed Models and Tukey *post hoc* tests with multiplicity adjusted *p*'s (significance level = 0.05) using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, California USA). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Results are reported as mean ± standard deviation (SD). The magnitudes of any differences between conditions were expressed as standardized differences (effect sizes; ES). The criteria used for interpreting the magnitude of the ES were: ≤0.2 (trivial), >0.2 (small), >0.6 (moderate), >1.2 (large); and > 2.0 (very large) (Hopkins et al., 2009). The ES are reported with uncertainty of the estimates shown as ±90% confidence limits (CL). If the CL crossed both positive and negative trivial ES values, the magnitude was deemed unclear (Hopkins et al., 2009). A sample size calculation was performed (G*Power 3.1.2) with alpha level set at 0.05 and power set at 0.8, which revealed a sample size of nine participants was required to detect a meaningful change in Tc (0.3°C; Zurawlew et al., 2016).

RESULTS

There were no significant differences between conditions for any measured variable during the treadmill runs ($p > 0.05$). The mean treadmill speeds throughout the three running components of the trials were 9.4 ± 0.9, 12.4 ± 1.2, and 9.4 ± 0.9 km·h^{-1}. When all trials were combined, the mean maximum heart rate during the run was 153 ± 12 bpm, and the mean sweat rate during the treadmill run was 0.20 ± 0.03 ml·min·kg^{-1}. There were no significant differences between conditions for temperature of the water during HWI ($p > 0.05$).

The mean and individual Tc during the HWI for each condition is illustrated in **Figure 2**, and the trends in individual core temperature during hot-water immersion across conditions

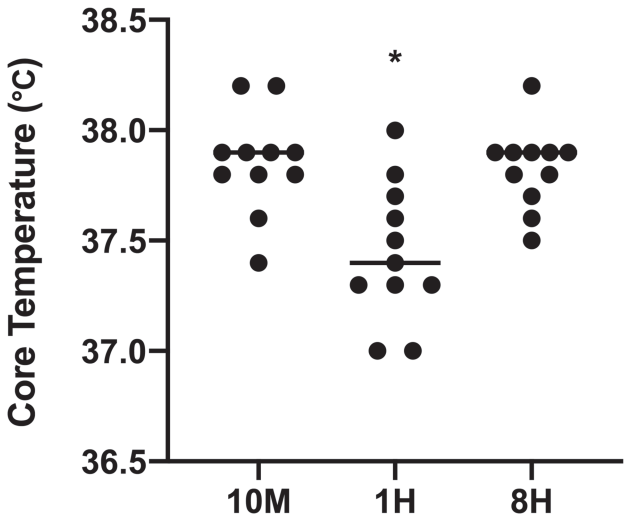

FIGURE 2 | Mean and individual core temperature during hot-water immersion for each condition; 10 min (10M), 1 h (1H), and 8 h (8H) intervals (n = 11). *Significantly different to 10M and 8H. Lines represent the mean and circles represent the individual responses.

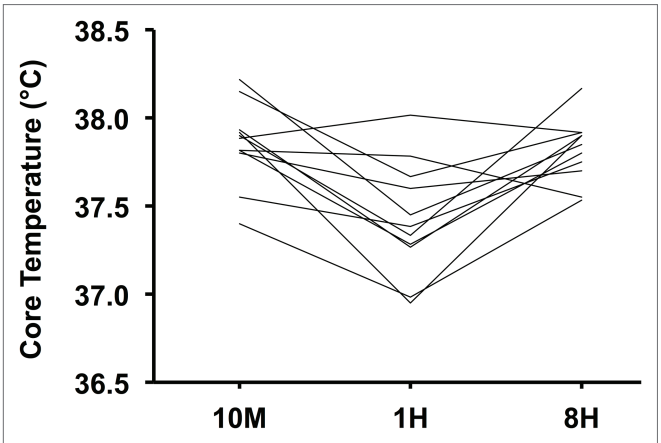

FIGURE 3 | Trends in individual mean core temperature during hot-water immersion across conditions; 10 min (10M), 1 h (1H), and 8 h (8H) intervals (n = 11).

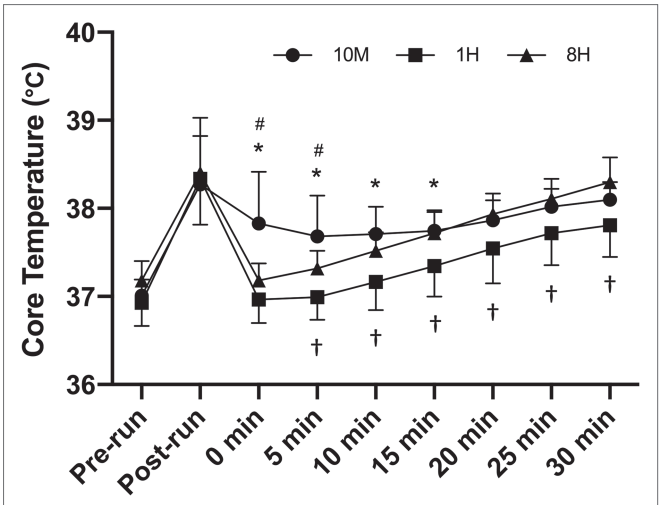

FIGURE 4 | Time course of core temperature changes across the trials for each condition; 10 min (10M), 1 h (1H) and 8 h (8H) intervals (n = 11). *10M sig different to 1H, #10M sig different to 8H, †1H sig different to 8H.

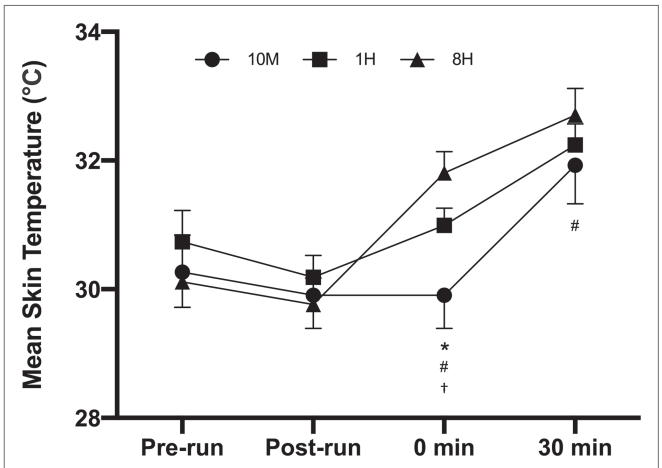

FIGURE 5 | Time course of mean skin temperature changes across the trials for each condition; 10 min (10 M), 1 h (1H), and 8 h (8H) intervals (n = 10). *10M sig different to 1H, #10M sig different to 8H, †1H sig different to 8H.

are illustrated in **Figure 3**. Due to technical problems (the capsule could not connect to the data logger in an 8H trial and we speculate that it had passed), Tc was missing for one participant, and therefore, data were analyzed for 11 participants. The mean HWI Tc was significantly lower in 1H compared to 10M (−0.42 ± 0.33°C; p = 0.0032; ES = 1.70, ±0.72) and 8H (−0.39 ± 0.37°C; p = 0.0140; ES = 1.11, ±0.57). There was no significant difference for mean HWI Tc between 10M and 8H (−0.04 ± 0.20°C; p = 0.8842, ES = −0.14, ±0.43). The peak HWI Tc was significantly higher in 8H compared to 1H (0.49 ± 0.42°C, p = 0.003, ES = 1.25, ±0.74), but there were no significant differences for peak HWI Tc between the other conditions (p > 0.05).

The time course of the Tc responses throughout the trials is illustrated in **Figure 4**. The 10M condition resulted in the highest Tc responses initially, which were significantly higher than 1H from 0 to 15 min (p = 0.0008–0.0163; ES = 1.06–2.95) and 8H from 0 to 5 min (p = 0.0099–0.0449; ES = 1.67–3.03). The Tc in the 8H condition was significantly higher than the 1H condition from 5 to 30 min (p = 0.0079–0.0399; ES = 0.91–1.25).

The time course of the Tsk responses throughout the trials is illustrated in **Figure 5**. Due to technical problems, data were missing for two participants, and therefore, data were analyzed for 10 participants. Upon commencing the HWI, 8H resulted in significantly higher Tsk than both 10M (p < 0.0001; ES = 3.27, ±1.52) and 1H (p = 0.0013; ES = 2.74, ±1.44). The Tsk was also significantly higher for 1H compared to 10M at 0 min (p = 0.0002; ES = 1.90, ±0.92). At the end of the HWI, 8H remained significantly higher than 10M (p = 0.0042; ES = 1.19, ±0.68), but there were no significant differences between the other conditions.

A summary of the heart rate, sweat and perceptual responses during the HWI with effect size comparisons is illustrated in **Table 1**. Due to technical problems, heart rate data were missing for one participant during 8H and therefore data for 11 participants were analyzed. The mean heart rate response was significantly higher in 10M compared to 1H (36 ± 21 bpm; p = 0.0006) and 8H (45 ± 18 bpm; p < 0.0001). The maximum heart rate response was also significantly higher in 10M compared 1H (41 ± 24 bpm; p = 0.0005) and 8H (52 ± 21 bpm; p < 0.0001). There were no other significant differences between conditions for any other variable.

DISCUSSION

The major finding of the current investigation was that a significantly lower Tc response was measured during HWI following a 1 h delay compared to a 10 min delay (−0.42°C),

TABLE 1 | Summary of the heart rate, sweat, and perceptual responses during hot-water immersion with effect size comparisons.

	10M (mean ± SD)	1H (mean ± SD)	8H (mean ± SD)	10M–1H (ES, ±CI)	10M–8H (ES, ±CI)	1H–8H (ES, ± CI)
Mean HR (bpm)	130 ± 19	94 ± 22*	85 ± 11*	1.35, ±0.46	2.81, ±0.59	0.61, ±0.45
Max HR (bpm)	143 ± 21	102 ± 24*	91 ± 13*	1.39, ±0.47	2.77, ±0.60	0.60, ±0.44
SL (ml)	700 ± 376	482 ± 259	665 ± 244	0.51, ±0.68	−0.14, ±0.87	−1.05, ±0.81
SR (ml·min·kg^{-1})	0.29 ± 0.16	0.20 ± 0.10	0.28 ± 0.12	0.53, ±0.70	−0.13, ±0.82	−0.97, ±0.76
Mean TC (AU)	1.8 ± 0.5	1.9 ± 0.5	2.0 ± 0.5	−0.19, ±0.49	−0.43, ±0.63	−0.22, ±0.65
Mean TS (AU)	5.1 ± 0.6	5.2 ± 0.9	5.4 ± 0.7	−0.01, ±0.24	−0.36, ±0.28	−0.35, ±0.39

*10M, 10 min delay; 1H, 1 h delay; 8H, 8 h delay; AU, arbitrary units; bpm, beats per minute; CI, 90% confidence interval; ES, effect size; SD, standard deviation; HR, heart rate; TC, thermal comfort; TS, thermal sensation; SL, sweat loss; SR, sweat rate. *Significantly different to 10M.*

between exercise and immersion (**Figure 2**). Further, mean heart rate was also lower following the 1 h delay, compared to the 10 min delay (−36 bpm). Therefore, within the conditions of the current protocol, we partially accept the hypothesis that delaying HWI by 1 h does reduce acute markers of thermo-physiological strain during a post-exercise HWI session. The second major finding was that, within the conditions of the current protocol, the Tc responses were similar between a 10 min and an 8 h delay between exercise and immersion (0.04°C; **Figure 2**), and peak HWI Tc was greatest in the 8H condition, likely due to a circadian rhythm influence on Tc.

As per **Figure 3**, the individual participants responded similarly to the HWI between trials (i.e., those with the lowest Tc in one trial generally had the lowest Tc in the others), which may be partly explained by individual anthropometrical characteristics. There were differences in the time course of Tc changes throughout the HWI between trials (**Figure 4**). During 10M, the group mean Tc remained stable for the first 15 min of immersion (37.7–37.8°C), before increasing to 38.1°C in the final 10 min. We speculate that the prior exercise and subsequent increased core temperature at immersion onset suppressed the rise in Tc in 10M. For 1H and 8H, pre-immersion Tc was significantly lower prior to immersion compared to 10M due to additional recovery after the exercise (see **Figure 4**). Hence, the Tc profile increased in a linear fashion in these trials, albeit from a higher starting value in 8H, which was the trial with the highest peak Tc at the end of immersion (38.3°C). Considering that the Tc group mean only surpassed 38°C in the final 10 min of immersion in 10M and 8H (and not at all in 1H), the HA potential was likely low for this specific HWI strategy (i.e., 39°C for 30 min to the level of the waist). It should also be noted that athletes may regularly perform exercise at higher intensities than that prescribed in the current study, which may increase the thermo-physiological responses presented.

The HWI strategy presented in the current study (39°C for 30 min to the level of the waist) was deemed a suitable initial exposure based on piloting, but the strategy should become more aggressive (i.e., increased temperature, duration and/or depth) over time to induce a greater rise in Tc and a more sufficient thermal stimulus for adaptation (which also needs to be maintained) to increase the likelihood of inducing meaningful heat adaptations. A previous investigation on post-exercise HWI that successfully induced heat adaptation and performance improvement in runners, implemented a HWI strategy of 40°C for 40 min to the level of the neck (Zurawlew et al., 2016). However, 6/10 participants could not complete this protocol on the first exposure, and therefore, it represents a starting point that is too challenging for many individuals. With this protocol, the researchers demonstrated that core body temperature was increased on average by 1°C throughout the immersion period (following the exercise), across six exposures. In comparison, the current study did not observe such an increase and instead participants completed immersion with a similar core temperature to that observed at the end of the run. Hence, athletes using this technique should aim to quickly increase the demands of the HWI toward 40°C for 40 min to the level of the neck in subsequent exposures as appropriate for the individual. Immersed athletes should be given clear instructions to discontinue HWI when they feel uncomfortably hot or experience any symptoms of pre-syncope or heat illness (i.e., cramping, vomiting, nausea, severe headache, and collapse/fainting). It is also advisable to measure Tc in order to ensure the HWI protocol is both safe and appropriate.

The 8H condition resulted in the highest peak Tc (i.e., at the end of immersion), and a similar mean Tc during HWI compared to 10M. This was somewhat unexpected but may be explained by the higher circadian Tc that occurs in the afternoon (Słomko and Zalewski, 2016). As per **Figures 4, 5**, there were higher pre-immersion Tc and Tsk in 8H compared to 1H, which does suggest a circadian influence. Considering that all trials commenced at a similar time of day (6:00–7:30 am), this meant that the immersion in the 8H trial always commenced between 2:30 and 4:00 pm; a time consistent with the time of day (3:00 and 5:00 pm) that peak circadian rhythm Tc occurs (Słomko and Zalewski, 2016). Hence, the current data suggest that performing HWI during this time is more effective at increasing acute Tc than HWI performed in the morning when there is a delay of at least 1 h between exercise and immersion, and importantly, HA does not appear to be time of day dependent (Zurawlew et al., 2018b). However, performing HWI at this time should be tested within a longer-term heat adaptation study before such recommendations are made explicit for athletes for heat acclimation purposes. Finally, if the HWI is to be conducted in the afternoon, then it may also be beneficial to conduct the training session at this time as well.

Both the mean and maximal heart rates were significantly increased during HWI in 10M compared to both 1H and 8H. It is likely that the 10M condition did not allow for complete

heart rate recovery after the exercise, prior to the immersion, and as such, the participants were subject to increased cardiovascular strain during the HWI in 10M. No significant differences were observed for the sweat responses or the perceptions of thermal comfort and sensation. However, effect size statistics revealed a moderate increase in sweat loss and rate in 8H compared to 1H. It was surprising that there were no differences in thermal perception despite differences in both core temperature and skin temperature, which play a large role in modulating these thermal perceptions. The other interesting finding was that Tsk was significantly higher in 1H compared to 10M before immersion (see **Figure 5**), which may be explained by the convection and evaporation load associated with running, and/or the additional clothing worn by participants during the 1 h delay in 1H (despite the use of a short stabilization period).

The primary objective of the current study design was to maximize ecological validity to provide clear guidelines for athletes on the timing of post-exercise HWI when it is to be implemented outside of the laboratory setting. Indeed, the availability of a hot-bath immediately after training (i.e., within a few minutes) is not practical for most athletes, but this has not been considered previously. Post-exercise HWI that is slightly delayed after a training session (i.e., 10 min or longer) is practical where the athlete has access to a bath at home. The different time delays chosen in the current study reflected likely delays to occur in the field, but investigation into other time delays is also warranted, especially delays of between 20 and 50 min, within which there is likely a threshold where the thermo-physiological response to HWI is reduced, decreasing the potential capacity for HA. Future research could also investigate the effects of post-exercise HWI when exercise is performed in the afternoon, in hotter environments, or after exercise in additional clothing (Stevens et al., 2017). Considering the ecological design, the current study's strengths can also be considered as limitations, for example, the athletes drank to thirst during the exercise, ate their usual diet, and completed their usual activities throughout the day instead of remaining in the laboratory during the delays in the 1H and 8H trials. As such, hydration, the thermic effect of food, and incidental physical activity, which can all contribute to heat storage, were not highly standardized. Fluid ingestion was not measured between exercise and HWI and hydration status was not measured either, but the participants were encouraged to drink during and after the exercise, and there was no difference in measures of body mass between exercise endpoint and starting HWI. We also highlight that this study is only an acute study of the physiological and perceptual responses to the different time delays between exercise and HWI, and more long-term studies are needed to determine any effects on heat adaptation.

It should also be noted that in the 8H trial, the ingestible capsule was in the gastrointestinal tract for an additional 8 h compared to the 10M trial and possibly moved further along the tract. However, this is unlikely to have affected the core temperature observations as previous research determined no differences between measures of core body temperature by rectal probe and ingestible capsule at 1 h (0.15 ± 1 0.11°C) vs. 36 h (0.15 ± 0.14°C), after ingestion (Ducharme et al., 2001). Another study has demonstrated some small gastrointestinal temperature gradients, but the most significant gradient was between the stomach and the small intestine (0.2–0.3°C), and any other gradients were trivial (Kolka et al., 1993). We implemented an 8-h timeframe between ingestion and the first measurement, exceeding the 6-h recommendation between ingestion and measurement to ensure that the capsule clears the stomach (Byrne and Lim, 2007), minimizing the effects of any gastrointestinal temperature gradient.

Overall, the current study provides new recommendations for athletes aiming to maximize the acute thermo-physiological response to post-exercise HWI. Immersion should commence immediately after training (within 10 min) to maximize acute Tc and heart rate responses. If this is not viable, an alternative approach may be to implement HWI in the afternoon when Tc is naturally elevated due to circadian rhythm. In the current design, delays of 1 h between exercise and immersion result in significantly lower Tc responses compared to delays of 10 min and 8 h, and Tc of less than 38°C throughout the whole immersion period (when considering the group mean), and hence, a 1 h delay is not recommended for athletes aiming to maximize the acute thermo-physiological response to post-exercise HWI.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Southern Cross University Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SH collected the data. CS and SH wrote the manuscript. SH, PH, SZ, LT, and CS contributed to the study design and performed critical revisions of the manuscript.

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Performance Changes Following Heat Acclimation and the Factors That Influence These Changes: Meta-Analysis and Meta-Regression

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Heat acclimation (HA) is the process of intentional and consistent exercise in the heat that results in positive physiological adaptations, which can improve exercise performance both in the heat and thermoneutral conditions. Previous research has indicated the many performance benefits of HA, however, a meta-analysis examining the magnitude of different types of performance improvement is absent. Additionally, there are several methodological discrepancies in the literature that could lead to increased variability in performance improvement following HA and no previous study has examined the impact of moderators on performance improvement following HA. Therefore, the aim of this study was two-fold; (1) to perform a meta-analysis to examine the magnitude of changes in performance following HA in maximal oxygen consumption (VO_{2max}), time to exhaustion, time trial, mean power, and peak power tests; (2) to determine the impact of moderators on results of these performance tests. Thirty-five studies met the inclusion/exclusion criteria with 23 studies that assessed VO_{2max} ($n = 204$), 24 studies that assessed time to exhaustion ($n = 232$), 10 studies that performed time trials ($n = 101$), 7 studies that assessed mean power ($n = 67$), and 10 papers that assessed peak power ($n = 88$). Data are reported as Hedge's g effect size (ES), and 95% confidence intervals (95% CI). Statistical significance was set to $p < 0.05$, a priori. The magnitude of change following HA was analyzed, with time to exhaustion demonstrating the largest performance enhancement (ES [95% CI], 0.86 [0.71, 1.01]), followed by time trial (0.49 [0.26, 0.71]), mean power (0.37 [0.05, 0.68]), VO_{2max} (0.30 [0.07, 0.53]), and peak power (0.29 [0.09, 0.48]) ($p < 0.05$). When all of the covariates were analyzed as individual models, induction method, fitness level, heat index in time to exhaustion (coefficient [95% CI]; induction method, −0.69 [−1.01, −0.37], $p < 0.001$; fitness level, 0.04 [0.02, 0.06], $p < 0.001$; heat index, 0.04 [0.02, 0.07], $p < 0.0001$) and induction length in mean power (coefficient [95% CI]; induction length 0.15 [0.05, 0.25], $p = 0.002$) significantly impacted the magnitude of change. Sport scientists and researchers can use the findings from this meta-analysis to customize HA induction. For time to exhaustion improvements, HA implementation should focus on induction method and baseline fitness, while the training and recovery balance could lead to optimal time trial performance.

Keywords: training, adaptation, thermoregulation, athlete, capacity

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INTRODUCTION

Athletes in team and individual sports use a variety of training methods to achieve peak performance. One training modality that has been established is known as heat acclimation (HA) (i.e., training in a hot, artificial environment) or heat acclimatization (i.e., training in a hot, natural environment), repeatedly. HA is the process of intentional and consistent exercise in the heat that results in several positive physiological and perceptual adaptations (Armstrong and Maresh, 1991). A decrease in heart rate, internal body temperature, sweat electrolyte concentration, and perceptual measures and an increase in sweat rate and plasma volume are all positive adaptations that occur throughout HA (Périard et al., 2015; Casadio et al., 2017). While the physiological benefits of HA have been established for many years (Adolph, 1938), a growing body of literature has emerged investigating the many performance benefits of HA in hot and thermoneutral environmental conditions. While the physiological and perceptual benefits of HA are the mechanisms behind enhanced exercise performance, actual result from competition, such as a faster race time, an increased time to exhaustion, or improved aerobic capacity are typically the primary outcomes.

Even still, understanding the mechanisms behind enhanced exercise performance is critical to adopting optimal training programs. Cardiovascular adaptations, including decreases in heart rate and increases in plasma volume, occur within 3–6 days of HA and are known to have a strong influence on exercise performance (Sawka et al., 2011; Périard et al., 2016). Body temperature adaptations, both internal and skin, also occur within 8 days of HA (Armstrong and Maresh, 1991) and are known to improve exercise performance (Nybo and González-Alonso, 2015). Decreases in sweat electrolyte concentration typically occurs within 5–10 days of HA and increase in sweat rate typically occurs within 5–14 days of HA (Armstrong and Maresh, 1991). These adaptations can enhance exercise performance in a hot environment, as these mechanisms improve thermoregulation (Nuccio et al., 2017). These adaptations independently and collectively improve exercise performance by helping the body thermoregulate more efficiently and reduce the overall physiological strain.

In the literature investigating HA, "performance" has been used to describe both physiological and perceptual improvements within a relative bout of exercise. "Performance" has also been used to describe the outcomes from direct measurements, such as time trial and maximal oxygen consumption (VO_{2max}). For this meta-analysis, performance will be defined as the result of any established test that measures exercise ability. Common tests that have been used to assess exercise performance following HA include: VO_{2max}, time to exhaustion, time trial, mean power, and peak power.

While the many physiological and performance benefits of HA have been reported in the literature, there are several methodological discrepancies in the literature that could lead to increased variability in these results, including fitness level, induction length, session duration, exercise intensity, induction method, induction length, environmental conditions of induction, and environmental conditions of testing. Induction method and exercise intensity typically refers to isothermal, controlled work-rate, or self-paced exercise (Daanen et al., 2018). The isothermal induction method involves having individuals exercise to achieve a critical internal body temperature threshold (typically 38.5°C) and maintain that temperature or higher for at least 1 h by adjusting the exercise intensity (Taylor and Cotter, 2006; Taylor, 2014; Périard et al., 2015). The controlled work-rate method involves individuals exercising for a constant intensity for a set duration (Tyler et al., 2016). Both of these methods result in various physiological responses and are thought to influence HA results (Périard et al., 2015). HA induction length refers to the number of days an individual is exposed to exercise in the heat. Previous literature defined various HA protocols as short-term (<7 days), medium-term (8–14 days), and long-term (≥14 days) and concluded that some physiological adaptations (internal body temperature and heart rate) can occur from a short-term HA protocol, however, the extent to which these adaptations translate to specific performance tests are unknown (Tyler et al., 2016). Previous investigations sought to gain a better understanding of induction method and length and reported that isothermal and controlled work-rate protocols yielded similar adaptations and that length did not contribute to additional adaptations (Gibson et al., 2015a,b). Session duration refers to the time per each HA session and typically ranges from 60 to 120 min, with previous research favoring increased duration for physiological benefits (Sawka et al., 2011). In addition to these considerations surrounding HA induction protocols, individuals with high fitness are generally more tolerant to heat and this factor could play a role in the magnitude of performance enhancement from HA (Pandolf et al., 1977; Gardner et al., 1996). Furthermore, environmental conditions during HA induction and testing can modify the response to exercise in the heat and change the magnitude of adaptations to HA, most likely due to the higher physiological strain that ensues in an uncompensable environment (Cheung et al., 2000).

Literature surrounding the practical aspects of HA induction and decay have provided insight into various methods and the many benefits of this method of performance enhancement (Périard et al., 2015). Adaptations in performance tests have been examined in broad sense (i.e., performance vs. exercise capacity), however, no study has examined the effect of HA on specific types of exercise performance tests (Tyler et al., 2016). A recent meta-analysis examined the physiological and perceptual adaptations that occur throughout heat acclimatization induction and decay (Daanen et al., 2018), however, a meta-analysis examining the magnitude of different types of specific performance improvement is absent. Additionally, while several studies have speculated, no previous study has examined the impact of specific moderators on the results observed following HA induction, which could help explain the variability seen in this research. Understanding the magnitude of the performance changes in these tests will be beneficial to athletes, coaches, sports scientists, sport medicine professionals and future researchers who strive to optimize performance and expand the HA literature. Therefore, the aim of this study was two-fold. First, to perform a meta-analysis to examine the magnitude of changes

in performance that results from HA in VO_{2max}, time to exhaustion, time trial, mean power, and peak power. Second, to determine the impact of moderators on HA performance in these performance tests.

MATERIALS AND METHODS

A literature search was conducted using first order search terms ("acclimation," "acclimatization," "adaptation") and second order search terms ("exercise," "endurance," "time trial," "Wingate," "VO_{2max}," "time to exhaustion"). The search was performed in the following databases: PubMed, Scopus, CINAHL, SportDiscus, Academic Search Premier, and Cochrane Library. The search was conducted February 15, 2019.

Selection Criteria

The following search criteria was used to determine the suitability of each paper for this analysis. **Figure 1** demonstrates the selection process for this meta-analysis. This meta-analysis only included a study if it met the following requirements:

1. The full-text was available from a peer-reviewed scientific journal in the English language.
2. The study reported a physical performance test outcome for pre and post HA intervention. Cognitive tests were not included in this analysis because the purpose was to assess the effectiveness of HA on various physical performance tests.
3. Only studies that conducted HA were included (not heat acclimatization). The term "acclimatization" was included in the search terms because "acclimation" and "acclimatization" are sometimes used interchangeably in previous research.
4. The mode of exercise during HA occurred on a cycle ergometer or a treadmill. These methods of exercise have high external validity and will be included to control for variability that may be introduced with other methods (i.e., sauna, stair- stepping).
5. The study reported findings from at least four participants to ensure appropriate power for each of the studies.
6. For studies to be included in the time to exhaustion analysis, the baseline test (prior to the start of HA) should be stopped due to volitional fatigue or the laboratory cut-points (such as internal body temperature >40°C), not because of testing time.

Classification of the Studies

Of the 74 peer-reviewed studies identified, 35 met the inclusion criteria. These studies were categorized by the researchers by type of performance test. Upon review, five types of tests were established: (1) VO_{2max}, (2) time to exhaustion, (3) time trial, (4) mean power, and (5) peak power.

Data Extraction

Studies that involved an additional intervention to HA were included in the analysis only if there was no difference between the control group and the intervention group. In cases that reported differences, only the control group was included.

Study Quality Assessment

The PEDro scale was not used for the inclusion criteria, however, a quality assessment is included in the results section (**Tables 1–5**). On this scale, a "high quality" study will score $\geq$ 7; a "moderate quality" study will score 5 or 6; a "poor quality" study will score $\leq$ 4 (Maher et al., 2003; Yamato et al., 2017). To assess for publication bias, funnel plots of each performance test can be seen in the supplementary material (**Supplementary Figures 1–5**).

Data Analysis

This meta-analysis was performed in Comprehensive Meta-Analysis software (version 2.2.064, Biostat company, Englewood, NJ, USA). Studies included in this analysis reported data to determine the changes within group, between pre and post HA. In the event that correlation values were not available, the lowest available correlation value for that test was utilized to calculate the effect size. Data are reported as mean (M), standard deviation (SD) mean difference (MD), Hedge's g effect size (ES), and 95% confidence intervals (95% CI). Statistical significance was set to $p < 0.05$, a priori.

To determine the effects of moderators on outcomes in performance, a meta-regression was performed. A separate meta-regression was performed for each performance test type and moderators were entered separately as individual models in the analysis. Moderators that were considered for all test types in this analysis included: fitness level, induction length, session duration, exercise intensity, induction method, heat index of induction, and heat index of testing. For the "fitness level" moderator, baseline (prior to HA induction) VO_{2max} levels were utilized. Induction length was entered as a continuous variable as the number of days utilized for HA induction. Session duration was entered as a continuous variable as the total number of minutes for each session throughout HA induction. In cases that involved a progressive duration protocol, the average duration time was entered. Exercise intensity was defined as "low," "moderate," and "high," with "low" being defined as <55% VO_{2max}, "moderate" between 55 and 70% VO_{2max} or isothermal, and "high" being greater that 70% VO_{2max} and "low" was set as the reference group. Induction method was defined as "controlled work rate" or "isothermal," with "controlled work rate" referring to an intensity that was set by the investigator throughout HA induction and "isothermal," referring to HA sessions adjusting the exercise intensity seeking to maintain the internal body temperature to a pre-determined criteria (typically 38.5°C). For this moderator, "controlled work rate" was set as the reference group. Environmental conditions of both induction and testing were measured as heat index and were calculated from reported environmental conditions. Tests were included if they were performed in hot (≥25°C) or thermoneutral (<25°C) environmental conditions. Three of the time to exhaustion tests were performed in thermoneutral environments. Twenty of the time to exhaustion tests were performed in a hot environment. Two studies did not report the environmental conditions of the testing sessions. In terms of mean power, one study conducted the performance test in a thermoneutral environmental condition. Of the VO_{2max} tests, nine were performed in thermoneutral

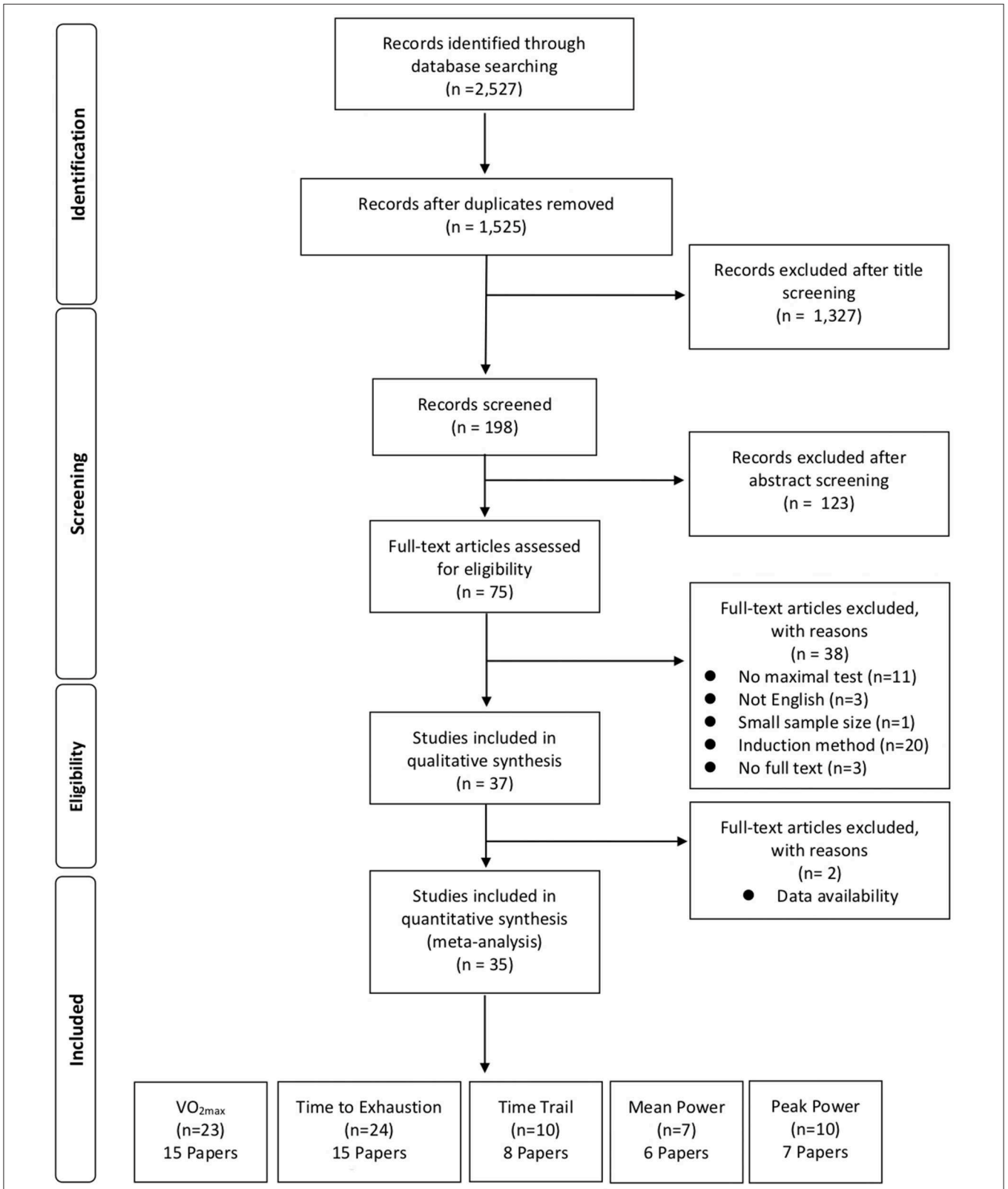

FIGURE 1 | Flow chart summary of the study selection process.

TABLE 1 | VO_{2max} descriptive table.

References	*n*	Induction method~	Induction length (Days)	Session duration (min)	Exercise intensity#	Heat index induction	Heat index testing	Baseline fitness ($ml \cdot kg^{-1} \cdot min^{-1}$)	PEDro
Horstman and Christensen (1982) A & B	6	Controlled work rate	11	68	Low	54	N/A	51.4	5
	4*	Controlled work rate	11	108	Low	54	N/A	47.2	5
King et al. (1985)	10	Controlled work rate	8	90	Low	43	43	46.1	4
Pivarnik et al. (1987)	16	Controlled work rate	6	90	Moderate	43	43	44.2	4
Febbraio et al. (1994)	13	Controlled work rate	7	90	Low	43	39	68.1	7
Aoyagi et al. (1998) A, B, C, & D	6	Controlled work rate	6	60	Low	43	43	47.4	7
	9	Controlled work rate	6	60	Low	43	43	45.1	7
	8	Controlled work rate	6	150	Low	43	43	49.5	7
	8	Controlled work rate	12	150	Low	43	43	48.6	7
Lorenzo et al. (2010)	12	Controlled work rate	10	90	Low	43	39	66.9	6
Chen et al. (2013) A & B	7	Controlled work rate	5	35	Moderate	51	24	53.0	7
	7	Controlled work rate	5	35	Moderate	51	51	53.0	7
Molloy et al. (2013) A & B	9	Controlled work rate	14	30	High	36	22	57.1	6
	7	Controlled work rate	14	30	High	36	22	55.2	6
Keiser et al. (2015)	8	Controlled work rate	10	90	Low	39	39	58.1	7
DiLeo et al. (2016)	10	Controlled work rate	5	90	Low	47	47	50.0	4
Neal et al. (2016a)	10	Isothermal	5	90	Moderate	55	22	63.3	6
Neal et al. (2016b) A & B	8	Isothermal	11	90	Moderate	55	20	56.9	6
	8	Isothermal	11	90	Moderate	55	20	56.9	6
James et al. (2017)	10^	Isothermal	5	90	Moderate	50	37	58.9	6
Rendell et al. (2017)	8	Isothermal	11	90	Moderate	55	22	58.5	6
Willmott et al. (2018) A & B	10	Isothermal	10	60	Moderate	47	21	48.7	6
	10	Isothermal	10	60	Moderate	47	21	48.7	6

~*Induction method was defined as either "controlled work rate," which was defined as a constant intensity for a set duration or "isothermal," which was defined as exercise intensity defined by a pre-determined internal body temperature.*

#*Exercise intensity was defined as either "low," which was <55% VO_{2max}, "moderate," which was isothermal or 55–70% VO_{2max}, and "high," which was >70% VO_{2max}.*

**Included four females.*

^*Included one female.*

conditions and 12 were performed in hot conditions (two not reported). For time trial performance, distance was used as a moderator to see the impact of HA induction on various time trial results.

RESULTS

Search Results

In total, 2,527 articles were found. Of those articles, 35 met the inclusion/exclusion criteria with 23 studies that assessed VO_{2max} ($n = 204$), 24 studies that assessed time to exhaustion ($n = 232$), 10 studies that performed time trials ($n = 101$), 7 studies that assessed mean power ($n = 67$), and 10 studies that assessed peak power ($n = 88$). The fitness level prior to the start of HA was reported (M ± SD; VO_{2max}, 53.7 ± 6.8 $ml \cdot kg^{-1} \cdot min^{-1}$; time to exhaustion 49.2 ± 8.1 $ml \cdot kg^{-1} \cdot min^{-1}$; time trial, 52.3 ± 9.0 $ml \cdot kg^{-1} \cdot min^{-1}$; mean power, 53.8 ± 9.2 $ml \cdot kg^{-1} \cdot min^{-1}$; peak power, 53.1 ± 7.4 $ml \cdot kg^{-1} \cdot min^{-1}$. Most studies investigated only males, however, some of the studies reported female data. The quality of the included manuscripts was assessed by two readers using the PEDro scale (M ± SD; 5.6 ± 1.1). The nature of HA induction does not allow for blinding of the participants. Descriptive information about each of the studies for each performance test type can be seen in **Tables 1–5**.

Impact of HA on Various Performance Tests

HA had a positive impact on performance, regardless of testing type (ES [95% CI]; =0.53 [0.44, 0.63], $p < 0.001$). HA induced positive adaptions for each performance test [MD [95% CI]; time to exhaustion, 144.30 s [128.30, 160.31], $p < 0.001$; time trial, −45.60 s [−22.80, −68.40], $p < 0.001$; mean power 12 W [2.08, 22.37], $p = 0.02$; VO_{2max}, 1.32 $ml \cdot kg^{-1} \cdot min^{-1}$ [0.20, 2.43], $p = 0.02$; peak power 15 W [8.90, 21.09], $p < 0.001$). The magnitude of change following HA induction was analyzed, with time to exhaustion demonstrating the largest performance enhancement, followed by time trial, mean power, VO_{2max}, and peak power (ES [95% CI]; time to exhaustion, 0.86 [0.71, 1.01], $p < 0.001$; time trial, 0.49 [0.26, 0.71], $p < 0.001$; mean power, 0.37 [0.05, 0.68], $p < 0.001$; VO_{2max}, 0.30 [0.07, 0.53], $p = 0.012$; peak power, 0.29 [0.09, 0.48], $p < 0.001$) (**Figure 2**). ES for each of the studies

TABLE 2 | Time to exhaustion descriptive table.

References	*n*	Induction method~	Induction length (Days)	Session duration (min)	Exercise intensity#	Heat index induction	Heat index testing	Baseline fitness ($ml \cdot kg^{-1} \cdot min^{-1}$)	PEDro
Horstman and Christensen (1982)	6	Controlled work rate	11	68	Low	44	44	51.4	5
	4*	Controlled work rate	11	108	Low	44	44	47.2	5
Pandolf et al. (1988)	9	Controlled work rate	10	150	Low	55	55	52.9	6
Nielsen et al. (1993) A & B	13	Controlled work rate	10.5	61	Moderate	37	37	59.0	6
Nielsen et al. (1997)	12	Controlled work rate	10	48.3	Low	61	61	62.0	4
Aoyagi et al. (1998)	6	Controlled work rate	6	60	Low	43	43	47.4	7
	9	Controlled work rate	6	60	Low	43	43	45.1	7
Inoue et al. (1999) A, B, & C	5	Controlled work rate	8	90	Low	49	49	47.0	6
	4	Controlled work rate	8	90	Low	49	49	48.0	6
	5	Controlled work rate	8	90	Low	49	49	30.0	6
Garrett et al. (2009)	10	Isothermal	5	90	Moderate	63	45	57.1	4
Burk et al. (2012)	22	Controlled work rate	11	125.3	Moderate	42	42	53.8	4
Chen et al. (2013) A & B	7	Controlled work rate	5	38	N/A	51	24	53.0	7
	7	Controlled work rate	5	38	N/A	51	51	53.0	7
Kaldur et al. (2014)	21	Controlled work rate	10	100	Low	42	42	53.8	4
Oöpik et al. (2014)	20	Controlled work rate	10	100	Low	42	42	53.2	4
Ashley et al. (2015)	10+	Controlled work rate	10	120	Low	57	57	33.9	7
	8+	Controlled work rate	10	120	Low	57	57	29.2	7
Gibson et al. (2015b) A, B, & C	8	Controlled work rate	10	90	Low	48	42	45.6	6
	8	Isothermal	10	67.4	Moderate	48	42	48.5	6
	8	Isothermal	10	86.1	Moderate	48	42	50.6	6
James et al. (2017)	10^	Isothermal	5	90	Moderate	50	37	58.9	6
Willmott et al. (2018) A & B	10	Isothermal	10	150	Moderate	47	21	48.7	6
	10	Isothermal	10	150	Moderate	47	21	48.7	6

~*Induction method was defined as either "controlled work rate," which was defined as a constant intensity for a set duration or "isothermal," which was defined as exercise intensity defined by a pre-determined internal body temperature.*

#*Exercise intensity was defined as either "low", which was <55% VO_{2max}, "moderate," which was isothermal or 55–70% VO_{2max}, and "high," which was >70% VO_{2max}.*

**Included four females.*

^*Included one female.*

+*Included five females.*

for time to exhaustion (**Figure 3**), time trial (**Figure 4**), mean power (**Figure 5**), VO_{2max} (**Figure 6**), and peak power (**Figure 7**) were demonstrated.

Time to Exhaustion Meta-Regression

When all of the covariates were analyzed as individual models, induction method significantly impacted the magnitude of change seen in time to exhaustion following HA induction (coefficient [95% CI]; −0.69 [−1.01, −0.37], $r^2 = 0.26$, $p < 0.001$) (**Figure 8A**). Fitness level also significantly impacted the change seen in time to exhaustion, however, no variance in the results was explained by this model (coefficient [95% CI]; 0.04 [0.02, 0.06], $r^2 = 0.00$, $p < 0.001$) (**Figure 8B**). The heat index of testing also explained some of the variance seen in this test time (coefficient [95% CI]; 0.04 [0.02, 0.07], $r^2 = 0.18$, $p < 0.001$) (**Figure 8C**). All other covariates did not significantly impact the magnitude of change seen in time to exhaustion following HA (coefficient [95% CI]; induction length, 0.04 [−0.04, 0.13], $p = 0.34$; session duration, 0.01 [−0.00, 0.02], $p = 0.08$; exercise intensity, −0.27 [−0.59, 0.05], $p = 0.10$; heat index of induction, 0.01 [−0.01, 0.04], $p = 0.30$). Of the 19 times to exhaustion tests in a hot environment, 18 saw improved performance, while one saw no changes in performance.

Time Trial Meta-Regression

When all of the covariates were entered as individual models, they did not significantly impact the magnitude of change seen from HA, however, high intensity training was approaching significance and this variable explained 24% of the variance seen in this type of performance test (coefficient [95% CI]; fitness level, 0.00 [−0.02, 0.03], $p = 0.91$; induction length, 0.08 [−0.07, 0.23], $p = 0.28$; session duration, 0.00 [−0.01, 0.01], $p = 0.40$; high intensity, −0.58 [−1.17, 0.02], $p = 0.06$; moderate intensity, −0.23 [−0.65, 0.18], $p = 0.26$; induction method, −0.08 [−0.53, 0.37], $p = 0.74$; heat index of induction, 0.01 [−0.02, 0.05], $p = 0.45$; heat index of testing, 0.03 [−0.01, 0.06], $p = 0.16$). In terms of environmental conditions, time trial performance was improved in all studies, however, only one study investigated a time trial in thermoneutral conditions.

TABLE 3 | Time trial descriptive table.

References	*n*	Induction method~	Induction length (Days)	Session duration (min)	Exercise intensity#	Heat index induction	Heat index testing	Baseline fitness ($ml \cdot kg^{-1} \cdot min^{-1}$)	PEDro
Garrett et al. (2012)	8	Isothermal	5	90	Moderate	61	45	65.0	4
Neal et al. (2016a)	10	Isothermal	5	90	Moderate	55	22	63.3	6
Guy et al. (2016)	8	Controlled work rate	7	N/A	Low	50	50	45.0	7
Lee et al. (2016)	7	Controlled work rate	10	60	Low	41	41	50.7	6
Willmott et al. (2016) A & B	7	Controlled work rate	2	90	Low	45	33	46.1	6
	7	Controlled work rate	4	45	Low	45	33	45.8	6
Wingfield et al. (2016) A & B	10	Controlled work rate	5	90	Low	40	40	44.3	4
	10	Controlled work rate	5	30	High	40	40	41.9	4
James et al. (2017)	10^	Isothermal	5	90	Moderate	50	37	58.9	6
Pethick et al. (2019)	24+	Isothermal	5	90	Moderate	40	38	62.3	6

~*Induction method was defined as either "controlled work rate," which was defined as a constant intensity for a set duration or "isothermal," which was defined as exercise intensity defined by a pre-determined internal body temperature.*

#*Exercise intensity was defined as either "low," which was <55% VO_{2max}, "moderate," which was isothermal or 55–70% VO_{2max}, and "high," which was >70% VO_{2max}.*

^*Included one female.*

+*Included two females.*

TABLE 4 | Mean power descriptive table.

References	*n*	Induction method~	Induction length (Days)	Session duration (min)	Exercise intensity#	Heat index induction	Heat index testing	Baseline fitness ($ml \cdot kg^{-1} \cdot min^{-1}$)	PEDro
Lorenzo et al. (2010)	12	Controlled work rate	10	90	Low	43	39	66.9	6
Brade et al. (2013)	10	Controlled work rate	5	40	High	45	45	55.3	7
Lee et al. (2016)	7	Controlled work rate	10	60	Low	41	41	50.7	6
Neal et al. (2016a)	10	Isothermal	5	90	Moderate	55	22	63.3	6
Wingfield et al. (2016) A & B	10	Controlled work rate	5	90	Low	40	40	44.3	4
	10	Controlled work rate	5	30	High	40	40	41.9	4
Duvnjak-Zaknich et al. (2018)	8	Controlled work rate	8	41	N/A	45	46	54.3	7

~*Induction method was defined as either "controlled work rate," which was defined as a constant intensity for a set duration or "isothermal," which was defined as exercise intensity defined by a pre-determined internal body temperature.*

#*Exercise intensity was defined as either "low," which was <55% VO_{2max}, "moderate," which was isothermal or 55–70% VO_{2max}, and "high," which was >70% VO_{2max}.*

Mean Power Meta-Regression

When all of the covariates were run as individual models, induction length significantly impacted the magnitude of change seen in mean power following HA induction (coefficient [95% CI]; induction length 0.15 [0.05, 0.25], $r^2 = 0.75$ $p = 0.002$) (**Figure 9A**). All other covariates did not significantly impact the magnitude of change seen in mean power from HA, however, fitness level was approaching significance and this variable explained 30% of the variance observed in this performance test (coefficient [95% CI]; fitness level, 0.03 [−0.001, 0.07], $p = 0.06$ (**Figure 9B**); session duration, 0.01 [−0.01, 0.02], $p = 0.36$; high intensity, −0.43 [−1.41, 0.41], $p = 0.28$; moderate intensity, −0.43 [−1.55, 0.69], $p = 0.45$; induction method, −0.23 [−1.33, 0.87], $p = 0.68$; heat index of induction, 0.00 [−0.08, 0.08], $p = 0.94$; heat index of testing, 0.01 [−0.05, 0.06], $p = 0.78$). One study that conducted a performance test in a thermoneutral environmental condition observed improved performance, while five out of the six tests that were performed in the heat saw improvements.

VO_{2max} Meta-Regression

When all of the covariates were run as individual models, they did not significantly impact the magnitude of change seen in VO_{2max} from HA (coefficient [95% CI]; fitness level, −0.01 [−0.02, 0.05], $p = 0.48$; induction method, −0.14 [−0.69, 0.41], $p = 0.62$; session duration, 0.00 [−0.01, 0.01], $p = 0.64$; induction length, 0.03 [−0.06, 0.11], $p = 0.54$; high intensity, −0.44 [−1.26, 0.37], $p = 0.29$; moderate intensity, −0.25 [−0.82, 0.33], $p = 0.41$; heat index of induction, −0.03 [−0.07, 0.02], $p = 0.20$; heat index of testing, −0.01 [−0.02, 0.03], $p = 0.59$). Of the nine thermoneutral VO_{2max} tests, eight observed improvements in performance following HA induction. Of the 12 in hot tests, seven observed performance improvements following HA induction.

Peak Power Meta-Regression

When all of the covariates were run as individual models, they did not significantly impact the magnitude of change seen in peak power from HA (coefficient [95% CI]; fitness level, 0.02 [−0.01, 0.05], $p = 0.22$; induction length, 0.01 [−0.08, 0.10],

TABLE 5 | Peak power descriptive table.

References	*n*	Induction method~	Induction length (Days)	Session duration (min)	Exercise intensity#	Heat Index induction	Heat index testing	Baseline fitness ($ml \cdot kg^{-1} \cdot min^{-1}$)	PEDro
(Castle et al., 2011) A & B	8	Controlled work rate	10	60	Low	37	38	43.3	6
	8	Controlled work rate	10	60	Low	37	38	43.3	6
Brade et al. (2013)	10	Controlled work rate	5	40	High	45	45	55.3	7
Keiser et al. (2015)	8	Controlled work rate	10	90	Low	30	39	61.2	7
Neal et al. (2016a)	10	Isothermal	5	90	Moderate	55	22	63.3	6
(Neal et al., 2016b) A & B	8	Isothermal	11	90	Moderate	55	20	56.9	6
	8	Isothermal	11	90	Moderate	55	20	56.9	6
Rendell et al. (2017)	8	Isothermal	11	90	Moderate	55	20	N/A	6
Willmott et al. (2018) A & B	10	Isothermal	10	60	Moderate	47	43	48.7	6
	10	Isothermal	10	60	Moderate	47	43	48.7	6

~*Induction method was defined as either "controlled work rate," which was defined as a constant intensity for a set duration or "isothermal," which was defined as exercise intensity defined by a pre-determined internal body temperature.*

#*Exercise intensity was defined as either "low," which was <55% VO_{2max}, "moderate," which was isothermal or 55–70% VO_{2max}, and "high," which was >70% VO_{2max}.*

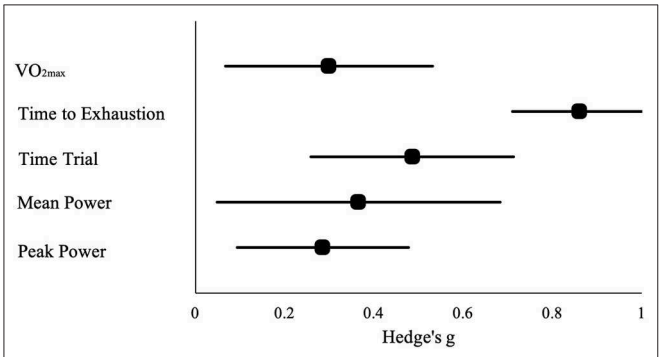

FIGURE 2 | Magnitude of performance changes observed in each performance test following heat acclimation. Data are presented as Hedge's g and 95% confidence intervals.

$p = 0.77$; session duration, 0.00 [−0.01, 0.02], $p = 0.40$; high intensity, −0.24 [−1.02, 0.54], $p = 0.54$; moderate intensity, −0.27 [−0.77, 0.22], $p = 0.28$; induction method, −0.19 [−0.61, 0.22], $p = 0.36$; heat index of induction, −0.02 [−0.04, 0.003], $p = 0.09$; heat index of testing, 0.01 [−0.01, 0.03], $p = 0.56$). Peak power performance improved in all tests, regardless of testing environmental conditions (thermoneutral, $n = 4$; hot, $n = 6$).

DISCUSSION

The largest performance improvement was observed in time to exhaustion with an average improvement of 144.30 s. Tyler et al. demonstrated in a meta-analysis that exercise capacity improved on average 23% (Tyler et al., 2016). Additionally, internal body temperature decreases an average of 0.31°C and heart rate lowers 12 bpm following HA (Tyler et al., 2016). In the present analysis, time to exhaustion tests were terminated when either participants reached their maximal efforts or heart rate/internal body temperature exceeded the lab safety criteria. One possible mechanism that could explain the improvements seen in time to exhaustion include lower internal body temperature (at baseline and during exercise) and heart rate that occur over the course of HA. The second largest magnitude of improvement was observed in time trials (MD, −45.6 s), followed by mean power (MD, 12 W), VO_{2max} (MD, 1.32 $ml \cdot kg^{-1} \cdot min^{-1}$), and peak power (MD, 15 W). Previous research demonstrated a 7% improvement in performance tests following HA (Tyler et al., 2016). These performance improvements following HA were most likely due to increases in maximal cardiac output, lactate threshold and plasma volume, lowered skin temperature and a larger core-to-skin gradient as seen in previous research (Périard et al., 2015). However, VO_{2max} might be impacted through improved fitness induced by exercise training alone compared to HA specifically (Brooks et al., 2015). Finally, peak power is not specifically a measurement of aerobic performance, thus, might not be impacted as substantially as other tests from HA.

One potential moderator that could explain some of the variance between studies that could not be accounted for in this analysis is the number of days of rest following HA before testing. There were a wide variety of reporting methods for the metric that does not allow for certainty in this analysis. For example, many manuscripts reported completing the performance test "within x number of days," meaning some participants may have completed the test the day after HA induction and other participants may have completed the test on day x after the end of HA induction. In general studies reported completing the tests anywhere from one to seven days following HA induction. A recent paper by Daanen et al. demonstrated that internal body temperature was lowered three and seven days following HA induction compared to the day immediately following HA induction. Thus, leading one to believe that performance adaptations may also be improved with a few days of recovery following HA induction, however, future research is needed (Daanen et al., 2011). Another meta-analysis has extensively examined the timeline of HA decay and concluded that internal body temperature and heart rate responses typically

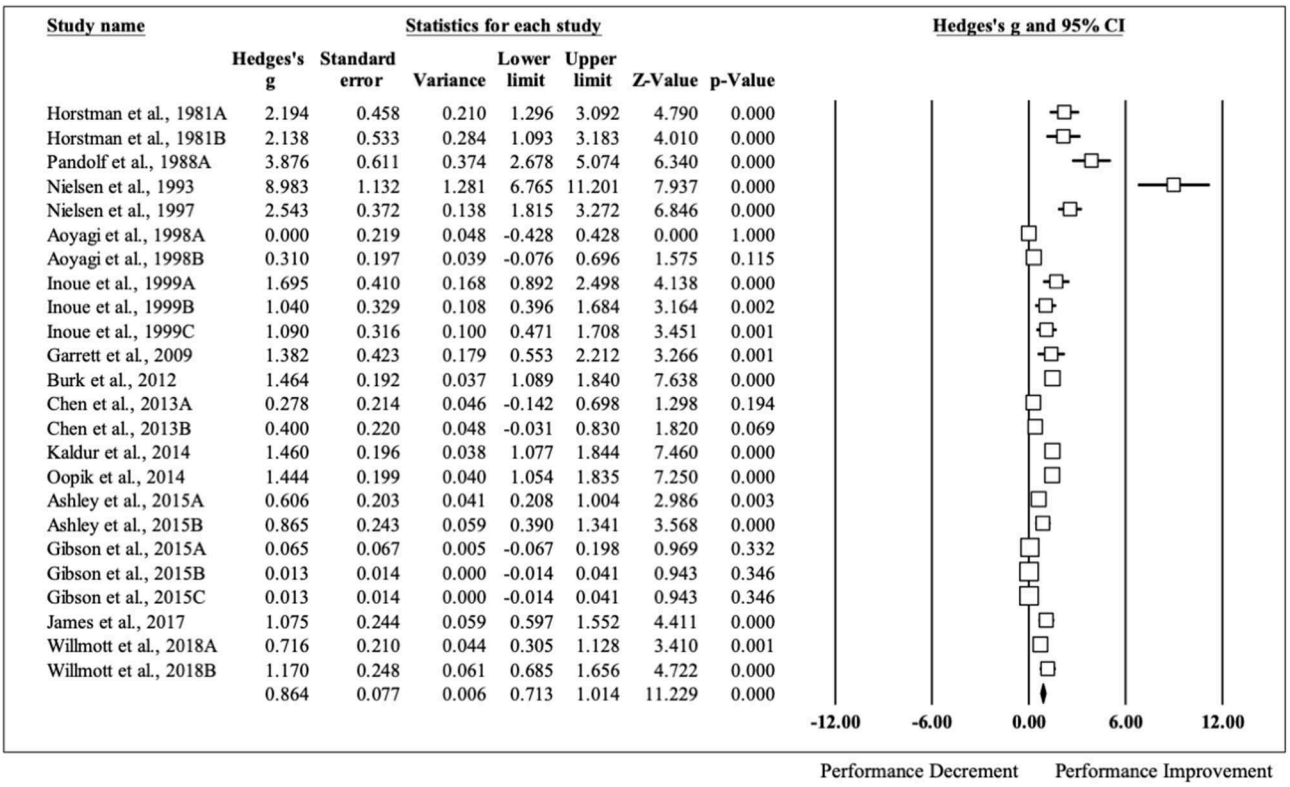

FIGURE 3 | Time to exhaustion forest plot. Data are presented as Hedge's g and 95% confidence intervals.

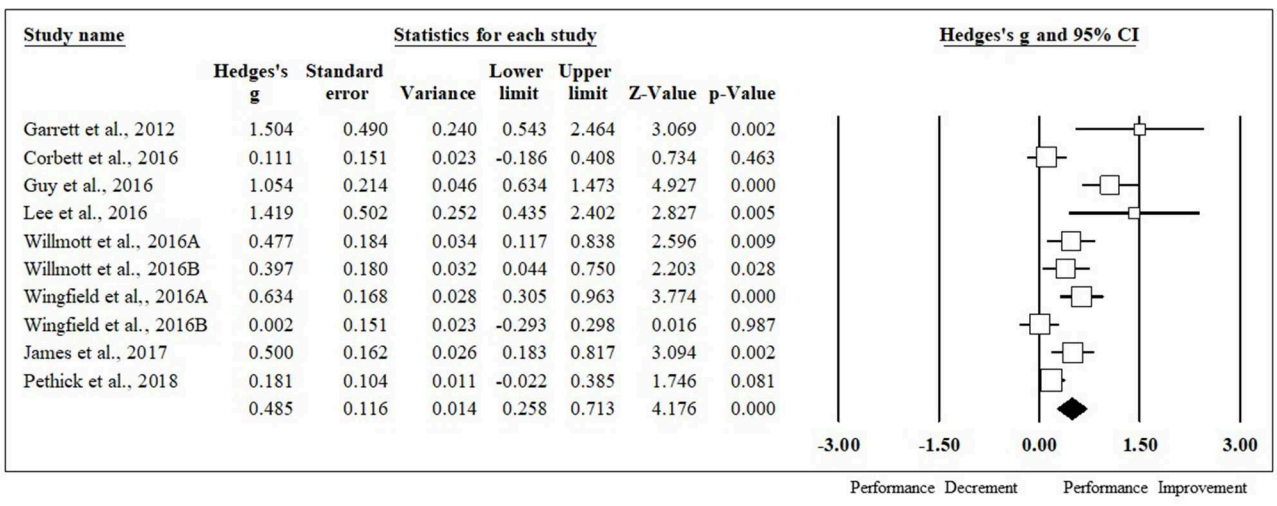

FIGURE 4 | Time trial forest plot. Data are presented as Hedge's g and 95% confidence intervals.

decay at a rate of 2.5% per day (Daanen et al., 2018). The importance of recovery has even been examined and reported when seeking optimal training improvements in a thermoneutral environment and 96 h of rest following training was suggested (Waldron et al., 2019). The results from both of these manuscripts point to the importance of finding the appropriate balance between recovery and acclimation decay for optimal performance results.

While differentiating the performance outcomes between HA and training alone is of importance, the current analysis did not examine these differences. Of the manuscripts included in this analysis, only 11 included a control group (time trial, $n = 5$; time

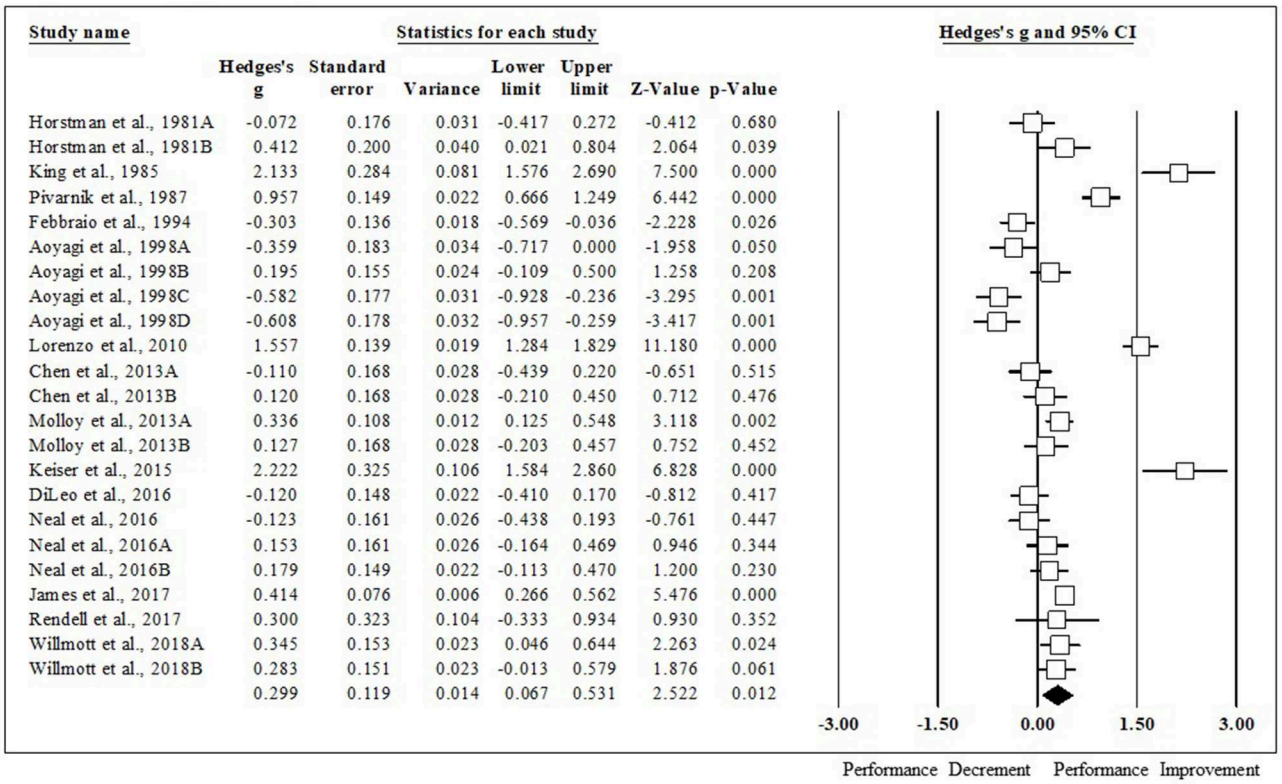

FIGURE 5 | VO_{2max} forest plot. Data are presented as Hedge's g and 95% confidence intervals.

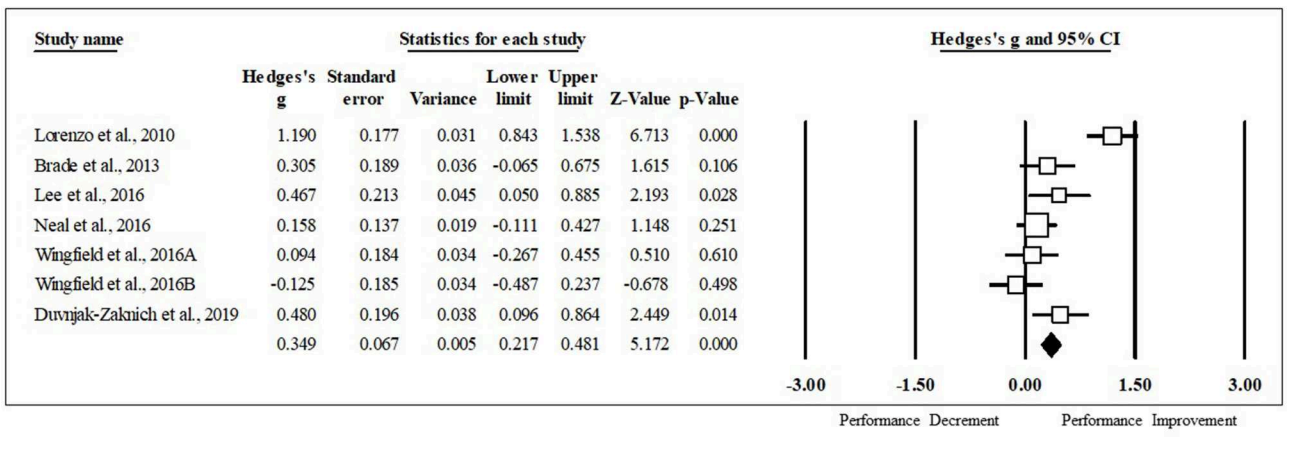

FIGURE 6 | Mean power forest plot. Data are presented as Hedge's g and 95% confidence intervals.

to exhaustion, $n = 4$; VO_{2max}, $n = 6$; peak power, $n = 3$; mean power, $n = 2$). HA appears to improve time trial performance compared to controls (Guy et al., 2016; Lee et al., 2016; Willmott et al., 2016; James et al., 2017). One short term HA protocol (4–6 days) did not elicit statistically significant improvements in time trial performance compared to a control group, however, moderate to large effect sizes were reported (Willmott et al., 2016). Time to exhaustion improved with HA in all studies, but not with control groups (Nielsen et al., 1993; Chen et al., 2013; James et al., 2017; Willmott et al., 2018). In terms of VO_{2max}, the performance differences between HA and training are unclear, as some studies reported differences between the groups and other did not (Lorenzo et al., 2010; Chen et al., 2013; Keiser et al., 2015; James et al., 2017; Rendell et al., 2017; Willmott et al., 2018). Peak power may improve with HA compared to training alone, however, the results are unclear and future research is

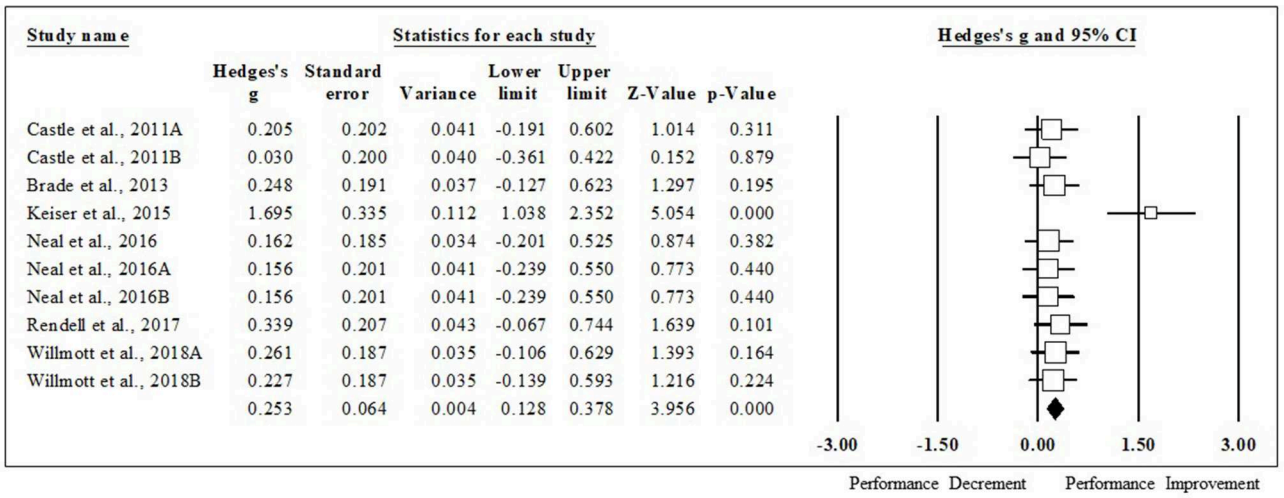

FIGURE 7 | Peak power forest plot. Data are presented as Hedge's g and 95% confidence intervals.

needed (Keiser et al., 2015; Rendell et al., 2017; Willmott et al., 2018). Both studies that assessed mean power demonstrated improved performance benefits from HA compared to a control group (Lorenzo et al., 2010; Lee et al., 2016). To determine the true performance changes of HA compared to training alone, future studies should aim to include a control group within their study design.

Time to Exhaustion

As previous research has clearly established, HA is an effective strategy to improve time to exhaustion and this was evident in the current meta-analysis, as no study reported decrements. The study that observed the largest performance improvement (ES = 8.98) took place with the participants who held the highest VO_{2max} (62.0 $ml \cdot kg^{-1} \cdot min^{-1}$), hypothetically giving them a higher training ceiling (Nielsen et al., 1993; Chen et al., 2013; James et al., 2017; Willmott et al., 2018). The HA induction took place over the course of 10 days for ∼48 min per session at a low exercise intensity (120 beats per minute; ∼45% VO_{2max}) and in the most extreme environmental conditions (ambient temperature, 35.4 ± 0.05°C; relative humidity, 87.2 ± 0.04%) of any study included in this analysis (Nielsen et al., 1997). Pandolf et al. also observed large improvements in time to exhaustion following HA (ES = 3.88) with a controlled work rate exercise intensity for 150 min over 10 days in relatively fit, middle-age individuals (VO_{2max} = 52.9 $ml \cdot kg^{-1} \cdot min^{-1}$) (Pandolf et al., 1988). The purpose of this particular research was to examine differences in young and middle age males over the course of HA who were matched for several morphological factors and the magnitude of performance time change was much larger for the younger group than the middle age group, due to the younger group reaching exhaustion much sooner than the middle age group at the beginning of HA, however, the middle-age group was not included in this analysis since their baseline test did not meet the inclusion criteria (Pandolf et al., 1988). The authors of this study hypothesized that the higher training volume of the middle aged men explained their thermoregulatory advantage at the beginning of HA, as they reported running on average 20 more miles per week than the younger men, pointing to the importance of previous training for improved thermoregulation capabilities (Pandolf et al., 1988). Despite this difference, HA induction successfully allowed the younger men to reach the same thermoregulatory capacity as middle aged men (Pandolf et al., 1988).

Two studies included in this meta-analysis did not observe any time to exhaustion performance improvements following HA induction (Pandolf et al., 1988; Aoyagi et al., 1998). One potential explanation of these findings in one of these studies is the low exercise intensity of the test (walk at 1.34 $m \cdot s^{-1}$ to exhaustion), allowing participants to complete the test to completion before HA induction ensued (Aoyagi et al., 1998). Similarly, the other group in the Pandolf et al. study was able to tolerate the test well on the first day of HA, most likely due to their training history (Pandolf et al., 1988).

Of the moderators entered into the meta-regression, induction method and fitness level appear to explain some of the variance seen in this type of performance test following HA. Controlled work rate exercise intensity during HA appears to hold a slight advantage over isothermal (controlled work rate ES = 1.00; isothermal ES = 0.31). One possible mechanism to explain this finding is the potential increase in area under the heating curve with controlled work rate exercise intensity during HA, as the isothermal method might actually lead to a lower overall thermal load since the exercise is adjusted to maintain a temperature of 38.5°C (Bardis et al., 2013).

While recent evidence suggests that peak internal body temperatures of 39°C are not more advantageous than the traditional isothermal temperature of 38.5°C (Gibson et al., 2015b, 2019), there are perhaps greater improvements with increased levels of hyperthermia (>39.0°C), especially in elite level athletes. Data from the Union Cycliste Internationale Road

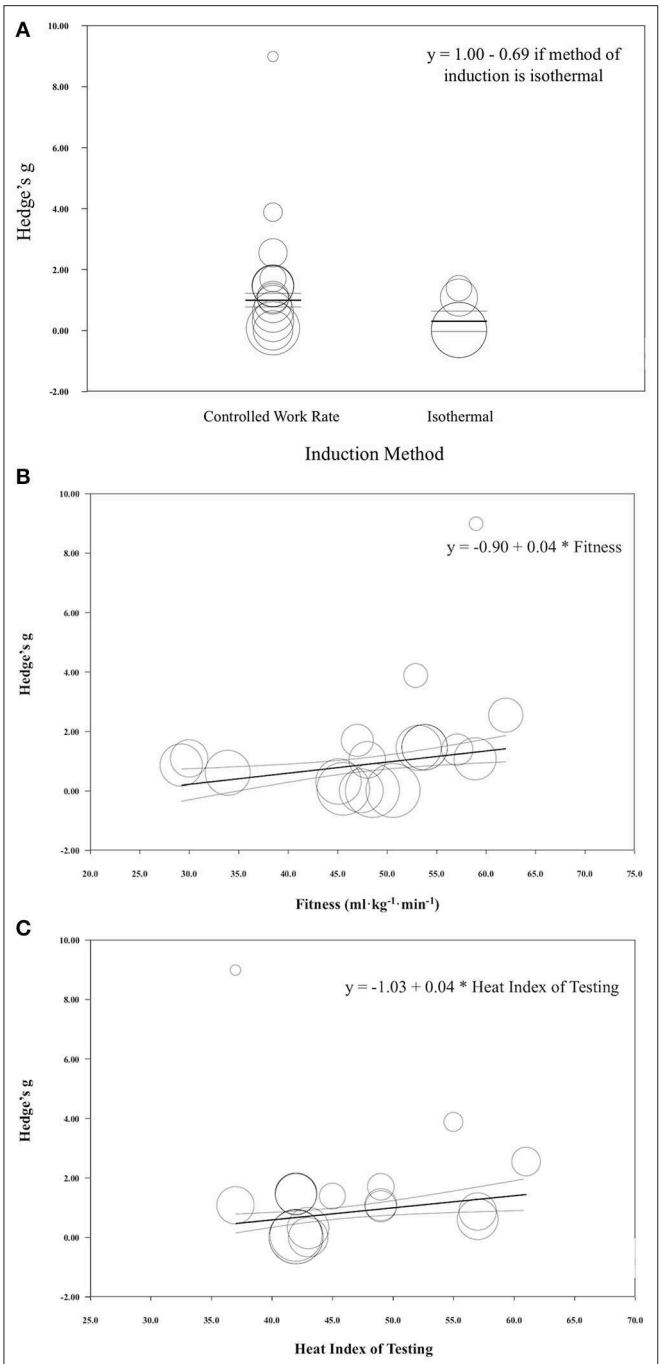

FIGURE 8 | (A) Regression of Hedge's g on induction method for time to exhaustion exercise performance following heat acclimation. Solid black bars represent the mean Hedge's g. Each circle represents individual studies. The size of the circle represents the weight of that study that was applied in the analysis. Smaller circles indicate lower weight and larger circles indicate higher weight. **(B)** Regression of Hedge's g on fitness level for time to exhaustion exercise performance following heat acclimation. Solid black bars represent the mean Hedge's g. Each circle represents individual studies. The size of the circle represents the weight of that study that was applied in the analysis. Smaller circles indicate lower weight and larger circles indicate higher weight. **(C)** Regression of Hedge's g on heat index for time to exhaustion exercise performance following heat acclimation. Solid black bars represent the mean *(Continued)*

FIGURE 8 | Hedge's g. Each circle represents individual studies. The size of the circle represents the weight of that study that was applied in the analysis. Smaller circles indicate lower weight and larger circles indicate higher weight.

Cycling World Championship demonstrated the capability of elite level athletes to tolerate internal body temperatures well above what is often reported in the HA literature (as high as 41.5°C), however, future research is needed in this area. An increased thermal load has the potential to drive HA through several mechanisms, including, an increased cardiac response, skin temperature, and sweat rate (Shibasaki et al., 2006; Périard et al., 2016). While increased internal body temperature has the potential to elicit greater HA adaptations, a valid measure of internal body temperature (ingestible thermistor or rectal temperature) and professionals trained in recognizing and treating exertional heat illness is needed when intentionally inducing HA in this way to ensure athlete safety.

Fitness level also appeared to impact the results seen in this performance test, as studies with higher starting VO_{2max} values appeared to have greater improvement in this type of performance test. For example, an individual with a VO_{2max} of 60 $ml \cdot kg^{-1} \cdot min^{-1}$ (predicted ES = 1.50) is likely to achieve a larger magnitude of performance changes following HA compared to an individual with 40 $ml \cdot kg^{-1} \cdot min^{-1}$ (predicted ES = 0.7). Because of the stimulation of sweating and skin blow flow (Piwonka et al., 1965), improved evaporative cooling (Gisolfi and Robinson, 1969), greater cardiac stability (Strydom and Williams, 1969), changes in fluid dynamics (Senay, 1979), earlier onset of sweating (Baum et al., 1976; Nadel, 1979), and greater sweat sensitivity (Wells et al., 1980), aerobically trained individuals appear to show partial HA benefits (Armstrong et al., 1987).

Time Trial

Time trial performance is arguably the most applicable in the sport setting and every study included in this meta-analysis demonstrated faster times following HA induction. Time trial was improved by −0.76 min on average. Garrett et al. saw the largest improvement in time trial performance (ES =1.50) following HA using the isothermal method for 90 min over 5 days in participants holding the highest VO_{2max} (65.0 $ml \cdot kg^{-1} \cdot min^{-1}$) in the most extreme environmental conditions (ambient temperature, 39.5°C; relative humidity, 60%). A previous review by Periard et al. demonstrated aerobically fit individuals can develop adaptations to HA rapidly (Périard et al., 2015). Lee et al. also demonstrated large improvements following HA induction (ES = 1.42) (Lee et al., 2016). The HA induction took place with a controlled work rate for 60 min over the course of 10 days with relatively fit individuals (VO_{2max} = 50.7 $ml \cdot kg^{-1} \cdot min^{-1}$). Willmott et al. showed the smallest improvements following HA with 30 min of exercise at a high intensity controlled work rate for 5 days in 32°C and 60% relative humidity (ES = 0.002). Previous research suggested 60–120 min of exercise duration to induce optimal adaptations following HA, therefore, 30 min of exercise for each session in this study might not be enough to elicit optimal adaptations (Sawka et al., 2011).

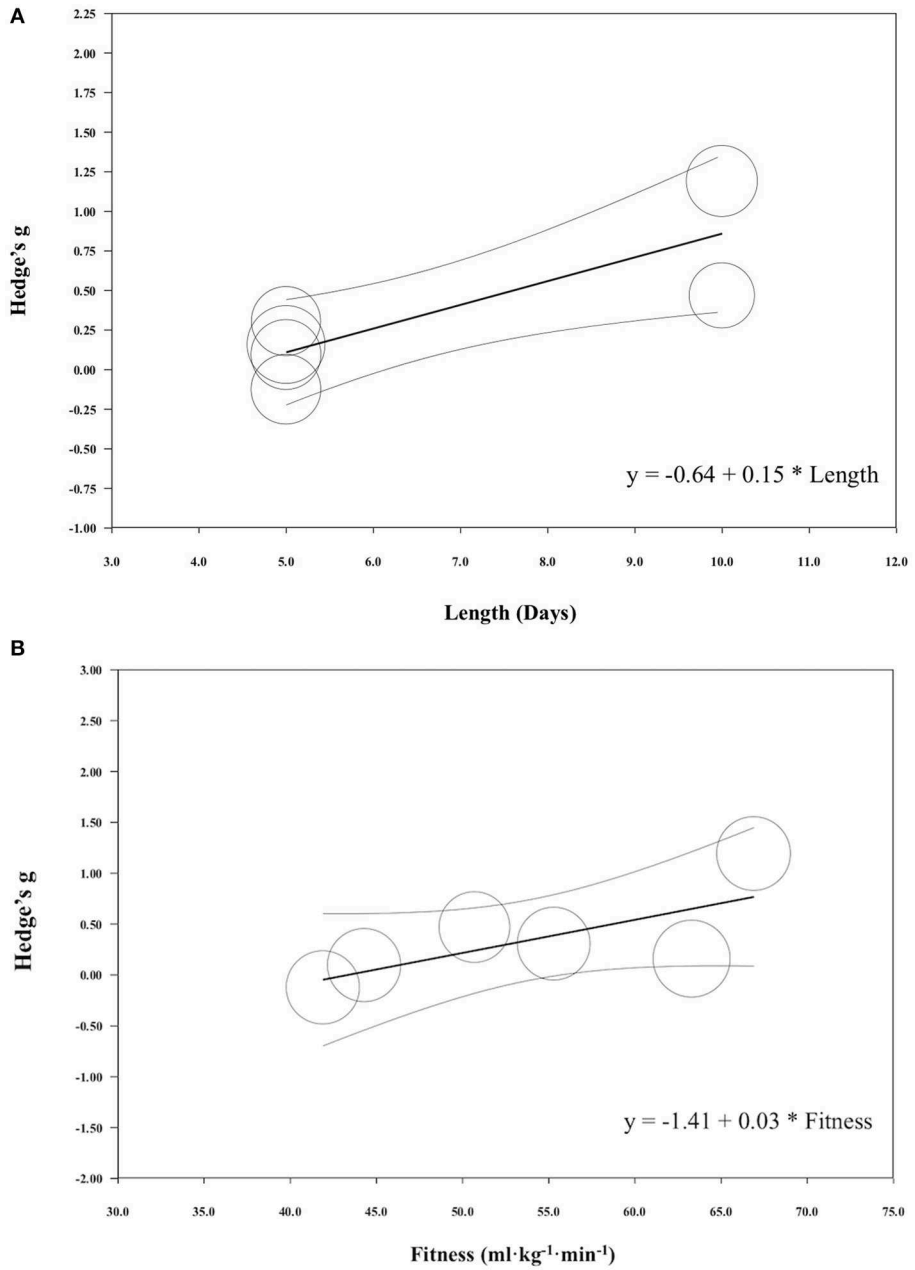

FIGURE 9 | (A) Regression of Hedge's g on induction length for mean power exercise performance following heat acclimation. Solid black bars represent the mean Hedge's g. Each circle represents individual studies. The size of the circle represents the weight of that study that was applied in the analysis. Smaller circles indicate lower weight and larger circles indicate higher weight. **(B)** Regression of Hedge's g on fitness level for mean power exercise performance following heat acclimation. Solid black bars represent the mean Hedge's g. Each circle represents individual studies. The size of the circle represents the weight of that study that was applied in the analysis. Smaller circles indicate lower weight and larger circles indicate higher weight.

Of the moderators entered into the meta-regression, exercise intensity might explain some of the variance (24%) seen in this type of performance test following HA even though it was not significant. Low intensity exercise induced large adaptations in time trial performance (High intensity, ES = 0.00; Moderate intensity; ES = 0.35; Low intensity, ES = 0.58), which could be due to lower levels of fatigue from HA that might be seen with high or moderate exercise intensity.

VO_{2max}

It has been well-established that fitness level contributes substantially to someone's ability to thermoregulate and that individuals with higher fitness levels already demonstrate some

physiological parameters of HA (Pandolf et al., 1977). One interesting phenomenon that is evident from this meta-analysis is that there are also improvements of VO_{2max} following HA induction. Keiser et al. observed a 9.6% improvement in VO_{2max} following 10 days of 90 min low intensity HA sessions (Keiser et al., 2015). Lorenzo et al. also demonstrated large improvements in VO_{2max} following 10 days of 90 min, low intensity HA sessions (MD ± SD, -4.5 ± -0.5 ml·kg^{-1}·min^{-1}) (Lorenzo et al., 2010). VO_{2max} was lower following HA in eight studies, unlike time to exhaustion and time trial performance tests that did not demonstrate any negative outcomes following HA.

There are several factors that could help explain these negative findings, including the fatigue, training impulse, and the participant's starting fitness levels. Similar to any novel training, HA introduces new stress to the body and can lead to fatigue. Daanen et al. recently demonstrated further performance improvements following HA when a break was initiated at the cessation of induction prior to the performance test (Daanen et al., 2011), allowing the participants time to recover and reap the full benefits of HA. Aoyagi et al. demonstrated the largest decrement in VO_{2max} following HA (MD ± SD, -1.4 ± -0.4 ml·kg^{-1}·min^{-1}), that involved 150 min (longest exercise duration) of 12 days of HA with only one rest day (Aoyagi et al., 1998). Similarly, Febbraio et al. saw decrements in VO_{2max} following HA induction (MD ± SD, -1.5 ± -0.6 ml·kg^{-1}·min^{-1}) in highly fit participants, however, the test took place within 24 h of the final HA session, which might not have allowed the full adaptations to take place (Febbraio et al., 1994). There were no moderators that largely impacted the magnitude of changes seen in VO_{2max}.

Power

Power is another critical performance measurement that can be applicable in sport settings. In this meta-analysis, mean power and peak power were analyzed. Mean power was improved by 12 W, on average. Lorenzo et al., reported the largest improvements following HA (Lorenzo and Minson, 2010). HA induction took place with 90 min of low intensity, controlled work rate for 10 days at 40°C and 30% relative humidity. Duvnjak-Zaknich et al. also showed large improvements following HA in mean power (ES = 0.480), in which the HA induction took place with 41 min of controlled work rate exercise for 8 days at 35°C and 60% relative humidity (Duvnjak-Zaknich et al., 2018). Lee et al. also showed large improvements in mean power (ES = 0.467). However, Wingfield et al. demonstrated negative mean power result following 30 min of high intensity controlled work rate HA for 5 days and the smallest improvement following 90 min of low intensity control work rate for 5 days in 33°C and 60% relative humidity (Wingfield et al., 2016). The studies showing larger improvements achieved longer length of HA induction. In addition to this point, Wingfield et al. measured mean power during five times of 6 s sprints, while other studies performed mean power during aerobic exercise test, such as 60 min exercise. HA induction could be more beneficial to improve mean power during aerobic exercise following longer duration of induction length.

Induction length explained 75% of the variance seen in power output following HA. For example, when HA induction length was 10 days, the predicted magnitude of change was ES = 0.86 and when it was 5 days, the predicted magnitude of change was only ES = 0.11. This finding is in line with original research which pointed to the full adaptations of HA taking 10 days (Armstrong and Maresh, 1991). However, these findings should be interpreted with caution, as there were only seven studies included in this analysis and other variables, such as the participant's previous training history, were not accounted for and could contribute to this variability. In fact, fitness level was approaching statistical significance in the regression model and may contribute to the variability with increased statistical power.

Peak power was improved 15 W, on average. Keiser et al. showed the largest improvements (ES = 1.695) which took place with 90 min of low intensity exercise for 10 days in 33°C and 39% relative humidity, while other studies showed smaller improvements following HA (ES = 0.030–0.339). Peak power was measured during a graded exercise test, repeated short sprint test, and longer duration exercise. There were no moderators that significantly impacted the results seen in peak power, most likely due to this type of test not directly measuring aerobic capacity, but more likely anaerobic capacity.

Limitations

While the goal of this meta-analysis was to provide an overview of various performance tests, this meta-analysis was not without limitations. One limitation of this meta-analysis was that some papers did not report correlations which was necessary to calculate ES in the statistical software. In this case, the lowest correlation value was used to achieve the most conservative outcomes. Even though females were included in this analysis, it is unclear if the current findings can be extrapolated to this population due to the variety of or lack of control over menstrual cycle status. For example, one study did not control for menstrual cycle status and simply reported that the findings were not different when females were excluded from the analysis (Pethick et al., 2019). Another study reported completing pre-tests and HA during the follicular phase of the menstrual cycle and post-tests during the luteal phase (James et al., 2017). Still, some did not report information about menstrual cycle status (Horstman and Christensen, 1982; Ashley et al., 2015). A further limitation of this meta-analysis was that each type of performance test had slightly different testing methods. For example, the distance of the time trial was not the same among the studies but were still categorized as a time trial. The moderator analysis may help with the interpretation of these results. While the physiological mechanisms behind power, endurance, and sprint tests cannot be understated, there were not enough peak power and mean power studies to utilize these categories as moderators in the current analysis. Another limitation was when data needed to calculate effect size was not reported in text or tables and it was demonstrated in figures, the data was estimated using a ruler. Additionally, while all studies reported the use of internal body temperature assessment, very few reported the actual internal body temperature data during HA induction and this meta-analysis could not include this information as a moderator.

However, a previous review indicated an increased internal body temperature during HA is a critical factor to induce adaptations (Périard et al., 2015). Future research should ensure that the internal body temperature data during the HA sessions are reported.

CONCLUSIONS AND PRACTICAL APPLICATION

A wide range of HA induction protocols have been investigated in this meta-analysis. The largest performance improvement was observed in time to exhaustion followed by time trial, mean power, VO_{2max}, and peak power following HA. The results observed in these performance tests were each impacted differently by specific moderators. Performance enhancements were greater in time to exhaustion tests when a controlled work rate method was utilized for HA and when the participants of these studies began the HA with higher baseline fitness levels, as indicated by VO_{2max}. Time trial results were improved if the HA induction involved low exercise intensity, which could be related to the participants in these studies not experiencing fatigue from high intensity HA. Longer HA induction (i.e., 10 days) appeared to elicit greater adaptations in mean power than short HA induction (i.e., 5 days). Sport scientists and researchers can use the findings from this meta-analysis to customize the design of HA induction protocols to maximize the adaptations of specific performance tests.

AUTHOR CONTRIBUTIONS

CB and YS developed the idea of the meta-analysis, completed the analysis, and created the figures. CB, YS, and LF worked to review and code all of the manuscripts. LF created the tables. DC verified the manuscript review and analytical methods. All authors reviewed and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01448/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effectiveness of Short-Term Heat Acclimation on Intermittent Sprint Performance With Moderately Trained Females Controlling for Menstrual Cycle Phase

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Introduction: Investigate the effectiveness of short-term heat acclimation (STHA), over 5-days (permissive dehydration), on an intermittent sprint exercise protocol (HST) with females. Controlling for menstrual cycle phase.

Materials and Methods: Ten, moderately trained, females (Mean [SD]; age 22.6 [2.7] y; stature 165.3 [6.2] cm; body mass 61.5 [8.7] kg; $\dot{V}O_{2\ peak}$ 43.9 [8.6] mL·kg^{-1}·min^{-1}) participated. The HST (31.0°C; 50%RH) was 9 × 5 min (45-min) of intermittent exercise, based on exercise intensities of female soccer players, using a motorized treadmill and Wattbike. Participants completed HST1 vs. HST2 as a control (C) trial. Followed by 90 min, STHA (no fluid intake), for five consecutive days in 39.5°C; 60%RH, using controlled-hyperthermia (∼rectal temperature [T_{re}] 38.5°C). The HST3 occurred within 1 week after STHA. The HST2 vs HST3 trials were in the luteal phase, using self-reported menstrual questionnaire and plasma 17β-estradiol.

Results: Pre (HST2) vs post (HST3) STHA there was a reduction at 45-min in T_{re} by 0.20°C (95%CI −0.30 to −0.10°C; d = 0.77); $\overline{T}_{sk}$ (−0.50; −0.90 to −0.10°C; d = 0.80); and $\overline{T}_{b}$ (−0.25; −0.35 to −0.15°C; d = 0.92). Cardiac frequency reduced at 45-min (−8; −16 to −1 b·min^{-1}; d = 1.11) and %PV increased (7.0; −0.4 to 14.5%: d = 1.27). Mean power output increased across all nine maximal sprints by 56W (−26 to 139W; d = 0.69; n = 9). There was limited difference (P > 0.05) for these measures in HST1 vs HST2 C trial.

Discussion: Short-term heat acclimation (5-days) using controlled-hyperthermia, leads to physiological adaptation during intermittent exercise in the heat, in moderately trained females when controlling for menstrual cycle phase.

Keywords: female, menstrual cycle, dehydration, fluid-regulation, plasma volume

INTRODUCTION

The worldwide popularity of football results in competitive matches being held in a whole host of environmental conditions, some of which can be in excess of 30°C with high levels of RH (Ozgunen et al., 2010). The 2016 Olympic Games in Brazil and the future 2020 Olympics in Tokyo, Japan (Gerrett et al., 2019) are examples of this.

It has been reported that the menstrual cycle plays a significant role in athletic performance in the heat (Avellini et al., 1979; Tenaglia et al., 1999; Janse de Jonge et al., 2012). However, research is limited and recent evidence has been contradictory. It has demonstrated little effect of the menstrual cycle (Sunderland and Nevill, 2003), oral contraceptive pill (OCP) usage (Lei et al., 2017) and in trained females who have smaller fluctuations in hormonal response (Lei and Mundel, 2018). However, the possible attenuation of endurance performance during heat stress has been reported (Avellini et al., 1979; Tenaglia et al., 1999; Constantini et al., 2005; Janse de Jonge et al., 2012) but this is not a universal finding (Kolka and Stephenson, 1997; Sunderland and Nevill, 2003; Lei et al., 2017). Secondly, there is limited research on fluid handling across the menstrual cycle with OCP usage (Stachenfeld et al., 1999; Stachenfeld, 2008). This supports the need for a robust research design in the present study. It is recognized that resting core temperature and the temperature threshold for the activation of the thermoregulatory responses, cutaneous vasodilation and sweating in females is elevated during the luteal phase of the menstrual cycle (Stachenfeld et al., 2000; Sunderland and Nevill, 2003). Especially, in those taking the OCP (Charkoudian and Johnson, 1997; Rogers and Baker, 1997) which can rise by ∼0.2–0.6°C in the early follicular phase (Stephenson and Kolka, 1985; Kolka and Stephenson, 1989; Grucza et al., 1993; Rogers and Baker, 1997). Many women feel that the menstrual cycle status negatively affects athletic performance (Wilson et al., 1991; Bruinvels et al., 2016) but in reality it has been reported that most physical aspects of athletic performance are not hindered by the menstrual cycle (Lebrun et al., 1995; Constantini et al., 2005; Wiecek et al., 2016; Tounsi et al., 2017). In summary, research on how the menstrual cycle phase affects exercise performance in unacclimated females in the heat is limited and contradictory. Furthermore, the limited information on female sex hormones, OCP and fluid regulation by Stachenfeld and colleagues (Stachenfeld et al., 1999; Stachenfeld, 2008) supports the research design of the present study.

There are fundamentally three models in which active heat acclimation can be achieved; (i) constant work-rate, (ii) self-regulated exercise and (iii) the controlled hyperthermia or isothermic technique (Taylor and Cotter, 2006). The controlled hyperthermia technique has been postulated to provide greater heat adaptation than the constant work-rate and self-regulated work-rate methodologies. On the basis that core temperature elevation is a key consideration for successful heat acclimation associated with high skin temperature and sweating response, as exercise alone is not a sufficient stimulus for adaptation (Hessemer et al., 1986). In contrast, there has been evidence to demonstrate isothermic and fixed intensity heat acclimation methods induce similar heat adaptation in the short and long-term. However, it is suggested controlled-hyperthermia is a more efficient and practical method for heat adaptation, especially for athletes tapering before competition (Gibson et al., 2015). The addition of a permissive dehydration stimulus, that is restricting fluid intake during acclimation has received recent attention in the literature (Akerman et al., 2016) and with a female cohort (Kirby et al., 2019). However, the benefits of permissive dehydration has not been reported universally (Neal et al., 2016). In our previous work using a male cohort (Garrett et al., 2014), permissive dehydration during acclimation has been shown to improve the fluid regulatory mechanisms by improving the reabsorption of water and Na^+ resulting in PV expansion. This indicated that the adaptive response may be enhanced rather than impaired by dehydration acclimation and this work had a euhydration control. Furthermore, we have demonstrated that the use of permissive dehydration with STHA (5-days) has been shown to provide heat adaptation for moderately (Garrett et al., 2009) and highly trained (Garrett et al., 2012) male athletes. From a practical perspective it provides a very light exercise load that minimizes both additional exercise strain and the disruption of quality training during the tapering period for competition (Garrett et al., 2011).

Most heat acclimation protocols have been carried out using male cohorts. Furthermore, the limited research that has been conducted using female participants, to the author's knowledge only three have used the controlled hyperthermia technique. Mee et al. (2015) reported that in response to STHA of 5-days with permissive dehydration, there was limited physiological change. Improvements in cardiovascular stability, lower core temperature and sudomotor adaptation required a longer, medium-term heat acclimation (MTHA) of 10-days (Mee et al., 2015). Similarly, Kirby et al. (2019) determined nine-, but not 4-days heat acclimation with permissive dehydration, improves self-paced endurance performance in females (Kirby et al., 2019). Daanen and Herweijer (2015) demonstrated limited responses to STHA of 3-days in a younger versus an older female population (Daanen and Herweijer, 2015). However, these studies may not give us all the information we need given their research designs have mixed OCP and non-users. Furthermore, they have not controlled for menstrual cycle phase.

The aims of the present study are to evaluate the physiological and performance effects of a STHA (5-day) protocol, using the controlled hyperthermia technique with no fluid intake, on an intermittent HST with a female cohort and controlling for menstrual cycle phase. It is hypothesized that STHA will improve thermoregulation in a cohort of young, moderately trained female game players when controlling for menstrual cycle phase.

Abbreviations: f_c, cardiac frequency ($b \cdot min^{-1}$); $\dot{Q}$, cardiac output ($L \cdot min^{-1}$); HST, heat stress test; $\dot{V}O_{2\,max}$, maximum oxygen uptake ($L \cdot min^{-1}$or $mL \cdot kg^{-1} \cdot min^{-1}$); MPO, mean power output (W); $\overline{T}_b$, mean body temperature (°C); $\overline{T}_{sk}$, mean skin temperature (°C); $\dot{V}O_2$, oxygen consumption ($L \cdot min^{-1}$ or $mL \cdot kg^{-1} \cdot min^{-1}$);$\dot{V}O_2$ peak, peak oxygen uptake ($L \cdot min^{-1}$or $mL \cdot kg^{-1} \cdot min^{-1}$); PPO, peak power output (W); %PV, percentage plasma volume (%); $[alb]_p$, plasma albumin ($mg \cdot mL^{-1}$); $[aldo]_p$, plasma aldosterone ($pg \cdot mL^{-1}$); $[cortisol]_p$, plasma cortisol ($ug \cdot dl^{-1}$); $[Na^+]_p$, plasma sodium ($mmol \cdot L^{-1}$); TP_p, plasma total protein ($mg \cdot mL^{-1}$); T_{re}, rectal temperature (°C); RH, relative humidity (%); STHA, short-term heat acclimation; $color_u$, urine color (units); osm_u, urine osmolality (mOsm/kg); SG_u, urine specific gravity (units).

MATERIALS AND METHODS

Experimental Design and Overview

Ten moderately trained female participants undertook a 5-day STHA regime, with no fluid replenishment during each daily acclimation session. Participants' thermoregulatory, cardiovascular and fluid-regulatory status were measured at rest and in response to an intermittent, exercising HST, administered the week before and after the 2nd day after the STHA regime to ensure 1 day of rest. Participants were asked to refrain from strenuous exercise immediately before and 24 h prior to each HST, as it has been demonstrated that lower resting core temperature contributes to reduced physiological strain during acclimation (Kampmann et al., 2008). A general overview of the STHA protocol for the moderately trained females is shown in **Figure 1**.

Participants

Ten, moderately trained, females (Mean [SD]; age 22.6 [2.7] years; stature 165.3 [6.2] cm; body mass 61.5 [8.7] kg; cardiac output 5.5 [1.3] L; and $\dot{V}O_{2\ peak}$ 43.9 [8.6] $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) participated. They were games players and oral contraceptive pill users (combined). Participants completed pre-exercise medical questionnaires and informed consent to participate in the study. All participants were in good health. The study had ethical approval (No. 1516177) from the University of Hull ethics committee following the World Health Organization declaration of Helsinki guidelines.

Protocol

Experimental Standardization

All participants were fully informed of all experimental procedures (orally and written). Prior to experimental testing participants completed pre-exercise medical questionnaires and informed consents. Each female participant used a monophasic, oral contraceptive pill (OCP) and dose of hormone concentration differed between individuals depending on their specific medication. All participants were previously unacclimated to the heat and this study was completed outside the British summertime to minimize seasonal acclimatization effects. To minimize circadian rhythm affects HSTs and acclimations occurred at the same time of day. Participants were asked to refrain from strenuous exercise for 24 h prior to HSTs and using a food diary follow a consistent food intake. They were asked to refrain from caffeine and alcohol consumption 12 h before all testing procedures.

As a methodological control participants completed HST2 and HST3 in the same phase of their menstrual cycle (luteal phase), in the active pill portion of the OCP. This was reported by menstrual cycle questionnaire. This detailed the start of the menstrual cycle, premenstrual symptoms and contraceptive medication. This was confirmed by baseline measurement of plasma 17β-estradiol (**Table 1**).

Short-Term Heat Acclimation (STHA)

The STHA protocol consisted of five consecutive day's heat exposure (39.5°C; 60% RH) for 90 min a day, using the controlled hyperthermia technique (Garrett et al., 2009), with permissive dehydration (Garrett et al., 2014). Participants cycled (Monark 824E, Monark Exercise AB, Varberg, Sweden) against a self-selected resistance at 60 rpm attaining a T_{re} 38.5°C, as quickly as possible and maintained for the 90 min of exposure by regular adjustment of workload. However, an initial workload of 60 watts for the first 5 min duration was the same for all participants at the start of each day's trial. Elevation of T_{re} to the same point during heat exposures was to increase workload progressively during the week. The fluid-regulatory hormone aldosterone $[aldosterone]_p$, electrolytes (Na^+, K^+, and Cl^-), proteins (total protein $[TP]_p$; Albumin $[alb]_p$), cortisol $[cortisol]_p$

TABLE 1 | Mean ± SD plasma 17β-estradiol in HST2 (pre-) and HST3 (post-) STHA in the luteal phases of the menstrual cycle.

Menstrual cycle week (n = 8)	3	3
Heat stress test	HST2	HST3
Plasma 17β-estradiol ($pg{\cdot}mL^{-1}$)	29.7 ± 16.4	28.7 ± 8.0

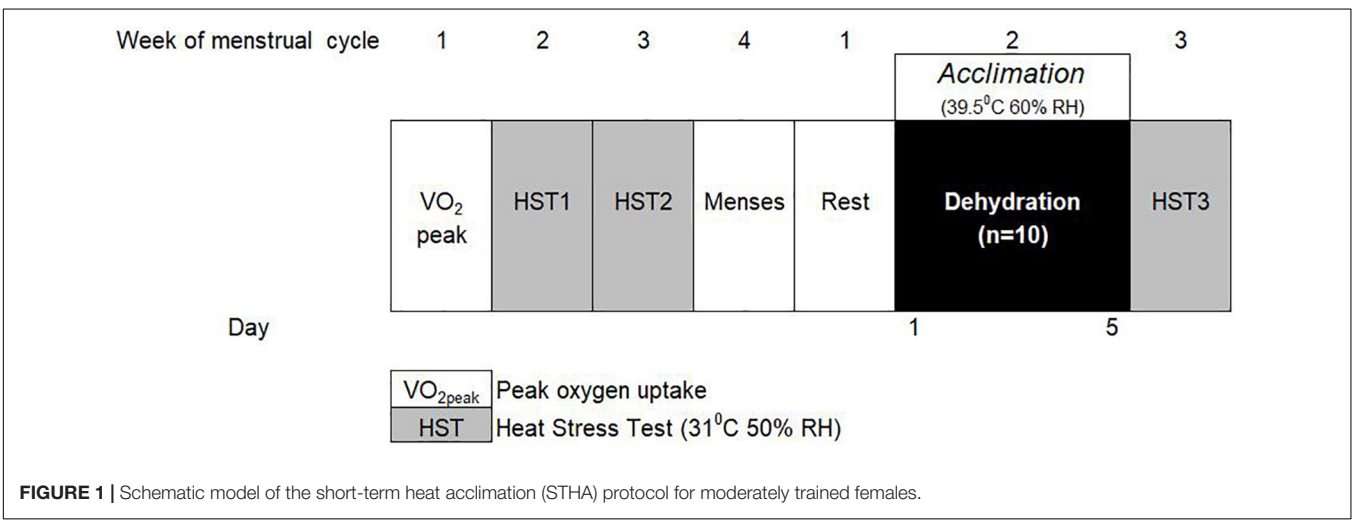

FIGURE 1 | Schematic model of the short-term heat acclimation (STHA) protocol for moderately trained females.

and percentage change in plasma volume (%PV) were measured at rest and end of acclimation bouts on day 1 and day 5. Time (minutes) time to a T_{re} of 38.5°C and work (J) was recorded on each day of acclimation.

Urinary measures

Urine samples were obtained before and after day 1 and day 5 acclimation. Using fresh urine samples, urine specific gravity and urine color were measured using a calibrated refractometer (Uricon-N, Urine specific gravity refractometer, Atago Co., Tokyo, Japan) and urine color chart (Armstrong et al., 1994, 1998), respectively. Urine volume was recorded and urine osmolality was analyzed after the experiment.

Blood measures

Plasma for the measurement of the fluid regulatory hormone aldosterone (200 μl) was stored using chilled K-EDTA tubes (1.6 mg·ml^{-1}). Measurement of aldosterone and cortisol used the Coat-A Count aldosterone procedure. The intra-assay coefficient of variation for aldosterone and cortisol was 8.8 and 12.1%, respectively, for duplicate measures. All samples for a given individual were analyzed within the same assay. Plasma Na^+, K^+, and Cl^- was analyzed using duplicate colorometric analysis (Cobas Mira Plus, New Jersey, United States).

Changes in the concentration of hemoglobin [Hb] and haematocrit [Hct] were used to determine the relative change in plasma volume described by Dill and Costill (1974). Venous blood samples (5 mL) were taken from an antecubital vein (Vacutainer Precision Glide 21-gauge needle, Becton Dickinson Vacutainer Systems) by phlebotomy without stasis and immediately analyzed – in sexplicate – for [Hb] (Willoughby et al., 2002), (Model OSM3, Radiometer, Copenhagen, Denmark), and [Hct] (using a Hawksley Microhematocrit centrifuge [Sussex, United Kingdom] and a Micro-capillary reader [Damon/IEC Division, Mass, United States]). The percentage change in plasma volume was analyzed from day 1 to 5 of the acclimation regime, during HSTs and calculated using a mathematical equation (Dill and Costill, 1974).

Aerobic Fitness Testing and Cardiac Output

Participants performed an incremental ramp exercise test to volitional exhaustion on a treadmill (h/p/Cosmos, Model Pulsar 3p, Traunstein, Germany), for determination of peak oxygen uptake ($\dot{V}O_{2\ peak}$) and velocities for individualisation of the HSTs. This procedure involved a starting velocity of 5 km/hr with workload increments of 0.1 km/hr every 6 s (1 km/hr/min), until volitional exhaustion. Breath by breath expired air was collected via a metabolic cart system (Cortex Metalyzer 3B, Cortex Biophysic, Leipzig, Germany). Participants RPE (Borg, 1982) and f_c (Polar FS1, Polar Electro, OY, Finland) were recorded every minute. All participants were given verbal encouragement in the latter stages of the incremental test.

Baseline cardiac output was measured using a breath-by-breath cardiac output analyser (Innocor, Innovision, Odense, Denmark). Prior to measurement, calibration of the cardiac output analyser was completed. Each participant had a fresh mouthpiece connected to a bacterial filter (Innovision, Odense, Denmark). A nose clip (Innovision, Odense, Denmark) was then placed over the participant's nose to prevent any expired air escaping. Participants were instructed to breathe in synchronization (∼5 breaths, ∼15 s) with the on-screen demonstration until measurement was complete.

Heat Stress Test (HST)

The HST took place in an environmental chamber (Type SSR 60-20H, Design Environment, Gwent, United Kingdom) set to ambient temperature of 31°C; 50% RH. Pre-exercise urine and blood measure (%PV) were taken prior to entering the chamber. The HST consisted of 9 × 5 min blocks of intermittent exercise on a treadmill (Pulsar 3p, h/p/Cosmos, Traunstein, Germany) and cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom). Each 5-min block consisted of intermittent treadmill running; standing (recovery), walking (50% HRmax), jogging (60% HRmax), low- (70% HRmax), moderate- (85% HRmax) and high-intensity (95% HRmax), ending with a 6-s maximal cycle ergometer sprint. Treadmill velocity changed every 5–11 s and percentage time spent at each velocity was adapted from collegiate level football match play characteristics (Vescovi and Favero, 2014). Sprint characteristics; peak power output (PPO) and mean power output (MPO) were used as performance measures.

Prior to acclimation, as a control trial, two HST's (HST1 vs. HST2) were performed and separated by 1 week. The post acclimation HST3 was performed within a week (5 ± 2 days) of the final acclimation day to prevent the decay of acclimation (Garrett et al., 2009) and we acknowledge there may be some variability in the magnitude of response (Waldron et al., 2019). To control for menstrual cycle phase the HST2 (Pre-) and HST3 (Post-) STHA trials were performed in week three of the menstrual cycle (luteal phase), in the active pill portion of the OCP for all participants. Baseline measures of plasma 17β-estradiol were taken prior to HST2 and HST3 trials in the luteal phase of the menstrual cycle and there was no difference ($P = 0.87$) observed (**Table 1**).

Body temperature

Core body temperature was measured using a rectal thermistor (U thermistor, Grant Instruments Ltd., Cambridge, United Kingdom) was self-inserted to a depth of 10 cm beyond the anal sphincter. Skin temperature was measured using skin thermistors (Type EUS-U-V5-V2, Grant Instruments Ltd., Cambridge, United Kingdom) placed on four, left sided, sites: chest, bicep, thigh, and calf, secured using micropore tape. Mean skin temperature ($\overline{T}_{sk}$) (Ramanathan, 1964) and mean body temperature ($\overline{T}_b$) (Sawka et al., 1996) were measured. Temperature data was recorded at 1-min intervals on a portable data logger (2020 series data logger, Grant Instruments Ltd., Cambridge, United Kingdom).

Data analysis

Sample size was based upon results from the previous limited research on females (Mee et al., 2015; Kirby et al., 2019), using our permissive dehydration protocol during STHA (Garrett et al., 2014). Where a statistical difference was observed in primary outcomes. The stress response of dependent measures in STHA and HSTs were analyzed for normal distribution by

using the Shapiro–Wilk and the Brown–Forsythe test determined equal variance. All data were normally distributed and two-way repeated measures ANOVA was used to determine main effects between day 1 and 5 acclimation, pre vs post HSTs and interaction over time for all dependent measures. Pairwise multiple comparison procedures were analyzed using post-hoc Bonferroni correction *t*-tests when appropriate. The change in thermal markers on day 1 and day 5 of acclimation were analyzed using paired *t*-test analysis. Work output from day 1 to day 5 of acclimation was analyzed using one-way analysis of variance ANOVA, with repeated measures and Bonferroni correction *t*-tests to isolate differences between days. Data is reported for ten moderately trained females unless otherwise stated. Where appropriate data is reported as mean differences ± SD with 95% confidence intervals (95% CI) and the magnitude of effect using Cohen's *d* effect sizes (where 0.2–0.59 small; 0.6–1.19 moderate; 1.2–1.99 large; 2.0–4.0 very large).

RESULTS

All ten participants completed the 5-day STHA protocol and three HSTs (HST1; HST2; HST3). The HST1 versus HST2 was a control trial taken 1 week apart with no intervention. The HST2 versus HST3 with the STHA intervention took place in week 3 of the menstrual cycle (luteal phase) for all ten participants. Due to issues with venepuncture measures blood parameters were analyzed for eight participants only. Similarly, eight participants had baseline plasma 17β-estradiol measured before HST2 and HST3 trials in the luteal phase.

Acclimation

Thermal Stress and Strain

Thermal stress and strain from days 1 to 5 of heat acclimation are presented in **Table 2** and work completed in **Figure 2**.

Measures of ambient temperature (T_a) and RH indicated that the thermal stress was similar on day 1 and 5 of acclimation. Similarly, the thermal strain was consistent between days illustrated by mean cardiac frequency (f_c) and rectal temperature (T_{re}) responses. Time to 38.5°C was longer on day 5 cf. day 1 (**Table 2**; $P = 0.04$). Therefore, less work was performed on day 1 cf. day 5 (**Table 2** and **Figure 2**; $P = 0.02$).

TABLE 2 | Thermal stress and strain on the first (Day 1) and last day (Day 5) of short-term heat acclimation (STHA) for ten moderately trained females.

	Day 1	Day 5	*P*-value
T_a (°C)	39.6 ± 0.1	39.7 ± 0.2	0.14
RH (%)	60.0 ± 0.2	60.1 ± 0.1	0.72
Mean f_c (b·min^{-1})	144 ± 22	141 ± 19	0.11
Mean T_{re} (°C)	38.29 ± 0.46	38.24 ± 0.47	0.24
Time to T_{re} 38.5°C (min)	36.70 ± 6.36	44.62 ± 11.04	**0.04**
Work (KJ)	18.98 ± 5.94	23.03 ± 5.14	**0.02**
Body mass change (%)	−1.7 ± 0.6	−1.8 ± 0.7	0.80
%PV change	0.9 ± 13.1	0.7 ± 12.0	0.98

Ambient temperature (T_a), RH cardiac frequency (f_c), rectal temperature (T_{re}), time to T_{re} 38.5°C and work on day 1 and 5 of acclimation undertaken with no fluid intake. Data presented as mean ±SD for ten female participants. Significant differences by paired t-test are shown in bold.

Urinary Measures

To determine hydration status urine color ($color_u$), urine osmolality (osm_u), urine specific gravity (SG_u) and body mass were measured at rest and 90 min, on day 1 and 5 of acclimation (**Table 3**). There was no main effect ($P > 0.05$) and interaction across time ($P > 0.05$) for $color_u$, osm_u, SG_u and body mass on day 1 and 5 of STHA.

Blood Measurements

Blood measures and percentage change on the first day (Day 1) to the last day (Day 5) of acclimation after 90-min heat exposure are presented in **Table 4**.

There was no main effect for $[aldo]_p$ between day 1 and 5 ($F = 1.583$; $P = 0.25$) or interaction across time ($F = 0.755$; $P = 0.41$). Similarly, there was no main effect between day 1 and 5 for $[Na^+]_p$ ($F = 1.106$; $P = 0.32$) or interaction across time ($F = 0.983$; $P = 0.36$). The measure $[TP]_p$ demonstrated no main effect between day 1 and 5 ($F = 0.93$; $P = 0.37$) or interaction across time ($F = 0.198$; $P = 0.67$). There was no main effect between day 1 and 5 ($F = 0.0618$; $P = 0.81$) for $[alb]_p$ or interaction across time ($F = 0.50$; $P = 0.50$). In contrast, there was a significant main effect between day 1 and 5 for $[cortisol]_p$ ($F = 15.303$; $P = 0.01$), and interaction across time ($F = 10.775$; $P = 0.02$). Bonferroni-corrected post-hoc comparisons showed a significant difference between pre and post measures within day 1 ($P = 0.002$) but not on day 5 ($P = 0.57$).

Heat Stress Test

Measurements were taken at rest and across the 45 min in HSTs. Data is presented for ten female participants unless otherwise stated.

Control Study

The HST1 versus HST2 was a control trial taken 1 week apart with no intervention. There was a limited change for T_{re}, $\overline{T}_{sk}$, $\overline{T}_b$, f_c, and %PV ($P > 0.05$). Similarly, in the sprint performance test the PPO and MPO demonstrated limited change ($P > 0.05$).

Intervention Study

The HST2 trial took place 1 week before the STHA (5-days), with no fluid intake intervention. The post HST3 occurred within 7-days of the last acclimation.

Body temperatures

Figure 3 presents mean ± SD rectal temperature (T_{re}), mean skin temperature ($\overline{T}_{sk}$) and mean body temperature ($\overline{T}_b$), pre- to post acclimation in hot conditions (31°C; 50% RH; $n = 10$).

There was no main effect for T_{re}($F = 1.411$; $P = 0.27$) after STHA but there was a significant interaction across time ($F = 2.991$; $P = 0.004$). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 40 ($P = 0.01$) and 45 min ($P = 0.007$). At 45 min T_{re} reduced by 0.20°C (95%CI −0.30 to −0.10°C; $d = 0.77$: Moderate). There was a significant main effect for $\overline{T}_{sk}$ ($F = 5.252$; $P = 0.05$) after

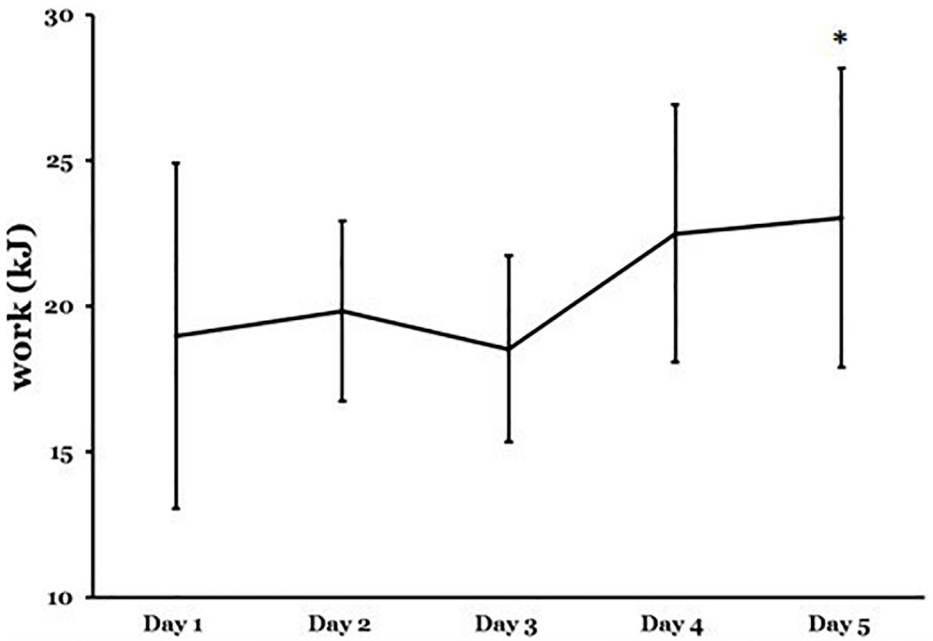

FIGURE 2 | Work output on the first day (Day 1) to the last day (Day 5) of acclimation after 90-min heat exposure. Data are mean ±SD are for ten moderately trained females. Significant difference $*p < 0.05$; Day 1 to the last day of acclimation analyzed using one-way analysis of variance (ANOVA) with repeated measures and Bonferroni correction *t*-tests to isolate differences between days.

TABLE 3 | Urinary measures of hydration ($color_u$, osm_u, SG_u) and nude body mass, at rest and end-exercise, on day 1 and 5 of short-term heat acclimation.

	Day 1:rest	Day 1:end	Day 5:rest	Day 5:end
$Color_u$ (units)	2 ± 1	4 ± 2	3 ± 1	4 ± 2
osm_u (mOsm/kg)	379 ± 292	447 ± 181	379 ± 267	396 ± 271
SG_u (units)	1.008 ± 0.007	1.012 ± 0.007	1.008 ± 0.006	1.010 ± 0.008
Body mass (kg)	62.3 ± 9.9	61.2 ± 9.8	62.5 ± 9.8	61.4 ± 9.7

Data presented as mean ±SD for ten female participants. A two-way repeated measures ANOVA and post-hoc Bonferroni correction t-tests when appropriate was used to determine the differences from rest to end-heat exposure, on day 1 and 5 of short-term heat acclimation.

TABLE 4 | Blood measures and percentage change from rest to end-exposure on the first day (Day 1) versus the last day (Day 5) of acclimation after 90-min heat exposure.

	$[aldo]_p$ ($pg{\cdot}mL^{-1}$)	$[Na^+]_p$ ($mmol{\cdot}L^{-1}$)	$[TP]_p$ ($mg{\cdot}mL^{-1}$)	$[alb]_p$ ($mg{\cdot}mL^{-1}$)	$[cortisol]_p$ ($ug{\cdot}dl^{-1}$)
Day 1					
Acclimation					
Rest	216 ± 131	140 ± 2	72.8 ± 3.2	670 ± 36	172 ± 63
End	417 ± 99	141 ± 1	78.3 ± 3.0	716 ± 33	307 ± 47*
%Change	48%	1%	7%	6%	44%
Day 5					
Acclimation					
Rest	187 ± 64	139 ± 1	71.6 ± 4.8	666 ± 41	190 ± 47
End	332 ± 143	142 ± 2	77.6 ± 5.7	717 ± 52	200 ± 67
%Change	44%	2%	8%	7%	5%

*Data are mean ±SD for eight moderately trained females. A two-way repeated measures ANOVA and post-hoc Bonferroni correction t-tests when appropriate was used to determine the differences from rest to end-heat exposure, on day 1 and 5 of short-term heat acclimation. *P < 0.05 at rest versus end-heat exposure (90 min) on day 1 and 5.*

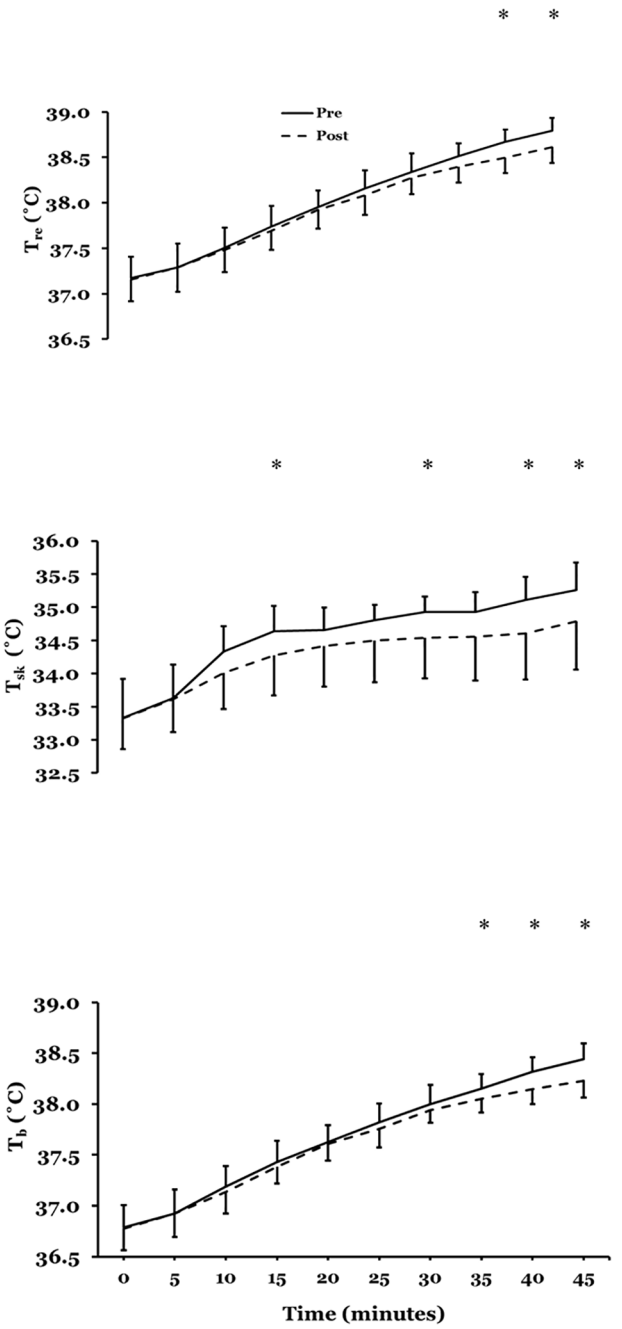

FIGURE 3 | Mean ± SD rectal temperature (T_{re}) (upper plate), mean skin temperature ($\overline{T}_{sk}$) (mid plate) and mean body temperature ($\overline{T}_b$) (lower plate), pre- to post acclimation in hot conditions (31°C; 50% RH; n = 10). *$P < 0.05$ post-hoc Bonferroni correction t-tests.

STHA and interaction across time (F = 4.689; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 15 (P = 0.043), 30 (P = 0.03); 35 (P = 0.004); 40 (P = 0.009) and 45 min (P = 0.009). The reduction in $\overline{T}_{sk}$ at 45 min was −0.50 (−0.90 to −0.10°C; d = 0.80: Moderate). There was no main effect for $\overline{T}_b$ (F = 4.419; P = 0.07) after STHA but there was a significant interaction across time (F = 3.942; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 35 (P = 0.01), 40 (P = 0.002) and 45 min (P = 0.001). At 45 min $\overline{T}_b$ reduced by −0.25 (−0.35 to −0.15°C; d = 0.92: Moderate).

Cardiac frequency and percentage change in plasma volume (%PV)

There was a significant main effect for cardiac frequency (F = 7.702; P = 0.02) after STHA and interaction across time (F = 2.485; P = 0.02). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at rest (P = 0.001), 5 (P = 0.04) and 45 min (P = 0.003). Cardiac frequency reduced at rest (−13; −18 to −7 b·min^{-1}; d = 1.04: Moderate) and at 45-min (−8; −16 to −1 b·min^{-1}; d = 1.11: Moderate). There was an increase in %PV from baseline post STHA by 7.0% (−0.4 to 14.5%, d = 1.27: Large).

Psychophysiological

There was a significant main effect for thermal comfort (F = 27.156; P = 0.001) after STHA and interaction across time (F = 3.378; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference from 10 to 45 min ($P < 0.05$). Thermal comfort reduced at 45 min by −1 (−1.5 to −0.5 units; d = 0.89: Moderate). There was a significant main effect for thermal sensation (F = 19.462; P = 0.002) after STHA and interaction across time (F = 4.533; P = 0.001). Bonferroni-corrected post-hoc comparisons showed a significant mean difference at 0 to 10 and 20 to 45 min ($P < 0.05$). Thermal sensation reduced at 45 min by −1 (−1.5 to −0.0 units; d = 0.67: Moderate). There was a significant main effect for RPE (F = 5.831; P = 0.04) after STHA and interaction effect across time (F = 2.853; P = 0.006). Bonferroni-corrected post-hoc comparisons showed a significant mean difference 20 (P = 0.04), 25 (P = 0.04) 30 (P = 0.02), 40 (P = 0.01) and 45 min (P = 0.01). RPE decreased at 45 min by −2 (−4 to 0 units; d = 0.70: Moderate).

Repeated sprint performance

The PPO and MPO were measured across all nine, 6-s maximal sprints in the 45-min protocol (**Figure 4**).

There was no main effect for PPO (F = 1.458; P = 0.26) after STHA or significant interaction effect across all nine sprints (F = 0.397; P = 0.91). There was not a main effect for MPO (F = 3.064; P = 0.12) after STHA but the interaction across all nine sprints was close to significance (F = 0.296; P = 0.06). The MPO increased across all nine maximal sprints by 56W (−26 to 139W; d = 0.69: Moderate; n = 9).

DISCUSSION

Effectiveness of Short-Term Heat Acclimation

The adaptations from short-term (5-d) heat acclimation with no fluid intake during acclimation, using the controlled hyperthermia technique, reduced exercising cardiovascular strain in females controlling for menstrual cycle phase. The cardiovascular stability was due to increased heat loss rather

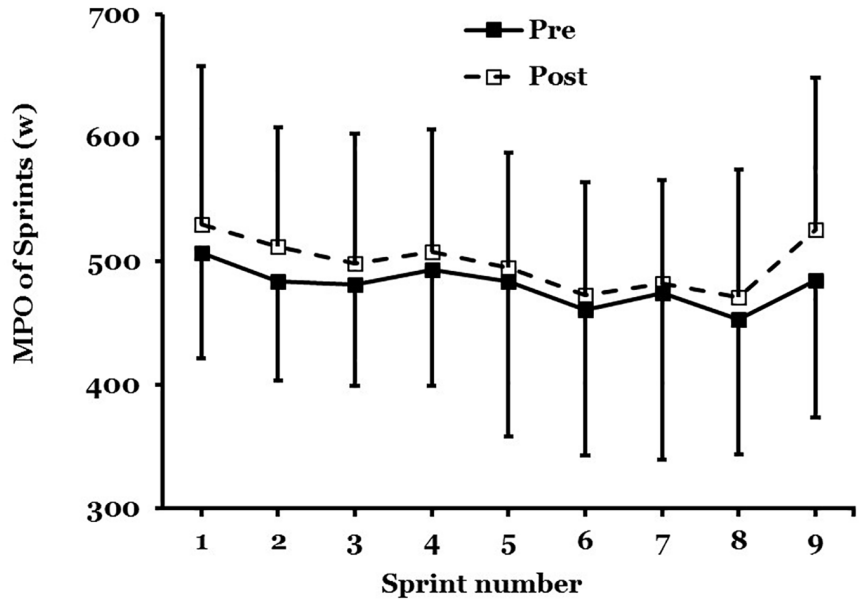

FIGURE 4 | Mean ± SD of mean power output (MPO) in maximal sprint performance, pre- to post acclimation in hot conditions (31°C; 50% RH; n = 9).

than lower heat content (∼resting core temperature), at the time of day of testing HSTs. This concurs with our previous work that has used no fluid intake during acclimation but with moderately (Garrett et al., 2009) and highly trained males (Garrett et al., 2012). However, it is in contrast with the limited work on STHA with females, using controlled-hyperthermia with permissive dehydration (Mee et al., 2015; Kirby et al., 2019).

Adaptation to Exercise in the Heat and Menstrual Cycle Phase

The post acclimation HST3 was performed within a week of the final acclimation day to prevent the decay of acclimation (Garrett et al., 2009). It has long been recognized that the menstrual cycle plays a significant role in athletic performance (Avellini et al., 1979; Tenaglia et al., 1999; Janse de Jonge et al., 2012). Therefore, to control for menstrual cycle phase the HST2 (Pre-) and HST3 (Post-) STHA trials were performed in week 3 of menstrual cycle (luteal phase), with all participants using oral contraceptive pills (combined). This was determined by menstrual cycle questionnaire and baseline measures of plasma 17β-estradiol. This was measured prior to HST2 and HST3 intervention trials and there was no statistical difference observed (**Table 1**). It has previously been reported that heat adaptation in females is not affected by menstrual cycle phase (Lei et al., 2017; Lei and Mundel, 2018) or the use of oral contraceptive pill (Armstrong et al., 2005). However, menstrual cycle phase and the associated changes in female sex hormones can influence core temperature (Inoue et al., 2005), the overall thermoregulatory set point range (Charkoudian and Stachenfeld, 2016) but a limited effect on whole body heat loss has been reported (Notley et al., 2018).

The present results using a female cohort and controlling for menstrual cycle phase, undergoing STHA of daily controlled hyperthermia with no fluid intake, demonstrated that the participants experienced adaptation to the heat. This was indicated by the characteristic features of acclimation. A decrease in T_{re} by −0.2°C (**Figure 3**; top panel), $\overline{T}_{sk}$ by −0.5°C (**Figure 3**; mid panel) and $\overline{T}_{b}$ by −0.25 (**Figure 3**; lower panel) was observed. Similar body temperature measures have previously been reported by the author, using the hyperthermia-control technique but with male participants (Garrett et al., 2009, 2012, 2014). In contrast, the research group of Mee et al. (2015) reported that the employment of the controlled hyperthermia model with permissive dehydration successfully attenuated T_{re} during a 30-min run in the heat after 5 days in males (−0.39 ± 0.36°C) but not in females (−0.07 ± 0.18°C). Yet after a further 5 days of acclimation the females T_{re} response was similar by 0.48 ± 0.27°C (Mee et al., 2015). This indicates that a female population requires a longer-term intervention than the 5-days STHA we employed (Mee et al., 2015). Similarly, Kirby et al. (2019) determined nine-, but not 4-days heat acclimation improves self-paced endurance performance in females, using hyperthermia control with permissive dehydration (Kirby et al., 2019). However, this was over a shorter 4-day STHA. Furthermore, in these two studies there were method differences with the present work. The exercise protocols were short in duration (∼30 min), self-paced/fixed speed trials and importantly menstrual OCP phase was not controlled.

End-exercise f_c decreased by 8 b·min^{-1}. Increased cardiovascular stability is recognized as one of the most rapidly occurring adaptations to the heat (Garrett et al., 2009, 2011). Furthermore, a 7.0% PV expansion from baseline was observed in this study. Previous research suggests intravascular

fluid expansion resulting from such increases in colloid-osmotic pressure (Senay et al., 1976; Senay, 1979) explains a majority of PV expansion (Goto et al., 2010). Therefore, this suggests that the PV expansion may have contributed to the greater cardiovascular stability that was observed. This is similar to the findings of Pethick et al. (2018) who demonstrated induced plasma volume expansion in a female cohort following 5-days high-intensity heat acclimation (Pethick et al., 2018).

Repeated Sprint Performance

This study demonstrated that an increase in MPO was close to significance across all nine maximal sprints after STHA of 5-days (**Figure 4**). This improvement in intermittent performance is supported by Sunderland et al. (2008) who developed a heat acclimation protocol (4-days) for female team sports (Sunderland et al., 2008). They reported a reduced rate of rise in rectal temperature and a 33% improvement in distance run during a repeated shuttle run performance test after STHA in a female cohort. From a practical perspective, an improvement in sprint performance is a valuable asset in team sport situations. Work-rate during team sports matches are largely determined by the oppositions playing style of the opposing team and individuals (Ozgunen et al., 2010), the ability to maintain repeated sprint performance can determine when a games player gets to the ball first and outrun the opposition.

Fluid Regulation Response to Repeated Heat Stress

In the present study, participants experienced the same thermal load and this is the basis of using the controlled hyperthermia technique for heat acclimation. Individual's experienced a mild hypohydration of ∼2% body mass (**Table 2**). This is similar to the imposed hypohydration administered by Judelson and colleagues, who reported a modification in the hormonal and metabolic response to resistance exercise, influencing the post-exercise circulatory milieu (Judelson et al., 2008). The research design of this study is supported by recommendations from earlier work with females on sex hormones and fluid regulation by the Stachenfeld research group (Stachenfeld et al., 1999; Stachenfeld, 2008).

Fluid Regulatory Hormones, Electrolytes and Plasma Volume Expansion

In the present study, after 90-min exercise $[aldo]_p$ did not significantly increase across acclimation bouts (**Table 4**) and this is in contrast to what has previously been reported (Judelson et al., 2008). The principal effects of aldosterone are the retention of Na^+ and therefore water from the urine output to maintain extracellular fluid volume and thus blood volume. However, in the present study, an exercise-induced response of increased $[Na^+]_p$ was not clearly evident after the no fluid intake acclimation regime (**Table 4**). Therefore, this is in contrast with previous findings (Brandenberger et al., 1989; Francesconi et al., 1993; Allsopp et al., 1998) who reported a strong relationship between increased Na^+ with $[aldo]_p$ response. In the present study, using the ΔPV (Dill and Costill, 1974) technique, there was an acclimation induced increase in resting %PV across HSTs by 7.0 ± 6% in the present study. This is similar to Pethick et al. (2018) who successfully induced plasma volume expansion in a female cohort, following 5-days high-intensity heat acclimation (Pethick et al., 2018).

Stress Hormone Response

In the present study the time to reach 38.5°C significantly increased (21.6%) from day 1 to 5 resulting in an associated increase in work (21.3%) (**Table 2**). Mean time to reach 38.5°C has been shown to be longer during STHA, using the controlled hyperthermia technique for females (51 ± 7 min), in comparison with males (48 ± 9 min) (Mee et al., 2015). Similarly, in the present study, a much larger % difference in time to 38.5°C was observed in comparison with previous studies using male cohorts with the same protocol (Garrett et al., 2009, 2012, 2014; Neal et al., 2015). The stress hormone, cortisol, significantly increased during acclimation on day 1 but this response was not observed on day 5. Despite a greater time to 38.5°C and more work being completed, hence, indicating a heat adaptive response (**Table 4**). This agrees with previous observations on male cohorts suggesting heat acclimation reduces cortisol levels during exercise in the heat (Francesconi et al., 1983; Armstrong et al., 1989) but such findings are not universal (Finberg and Berlyne, 1977; Sunderland et al., 2008).

LIMITATIONS AND FUTURE DIRECTIONS

In order to standardize menstrual cycle phase each female participant used a monophasic, oral contraceptive pill (OCP) but a potential limitation was that the dose of hormone concentration differed between individuals depending on their specific medication.

For future directions, information is limited on the physiological mechanisms of fluid regulation in females, following STHA. Therefore, a comparison of euhydration versus dehydration STHA, may provide a greater understanding of this area. To the authors knowledge, our earlier work (Garrett et al., 2014) is the only study to have done this but with male participants.

CONCLUSION

In summary, this work has established the effectiveness of STHA for 5 days, using the controlled-hyperthermia technique with no fluid intake (Garrett et al., 2009, 2012, 2014), on intermittent activity in hot environments with a female cohort, controlling for menstrual cycle phase. The current research suggests these methods of heat acclimation in a female cohort enhances thermoregulation and cardiovascular stability

during intermittent exercise in the heat. These improvements may provide protection from exertional heat related illnesses associated with exercise performance. This work adds to the limited body of literature available and this is particularly important given the 2020 Olympics will be held in the hot and humid conditions of Tokyo in Japan.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Hull Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AG conceived and designed the research. JG, ED, VB, and JS conducted the experiments. JB, HJ, DG-S, and RB contributed to the blood handling and analysis. AG and GA analyzed the data. AG and JG wrote the manuscript. All authors read and approved the manuscript.

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OPINION
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Integrating Heat Training in the Rehabilitation Toolbox for the Injured Athlete

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INTRODUCTION

Musculoskeletal injuries are arguably one of the most severe impediments athletes may encounter in their career. Depending on the severity of the sustained injury, time-loss could be substantial, leading to profound de-conditioning effects within the cardiovascular, metabolic and muscular systems. For instance, following ACL reconstruction, athletes' return to sport may range between 16 and 52 weeks (Anderson et al., 2019). Case studies in elite soccer players have reported profound losses in whole body fat-free mass (5.8 kg), as well as in lean leg mass (0.9–1.5 kg) following immobilization and inactivity during this period (Milsom et al., 2014; Anderson et al., 2019). Moreover, a 4–20% decline in maximal aerobic capacity (VO_{2max}) has been reported following 2–8 weeks of physical de-conditioning, owing largely to the decline in blood volume, and consequently stroke volume and cardiac output (Mujika and Padilla, 2001). In this context, there is a crucial need to optimize rehabilitation programs, such that the physical demands associated with return to sport and beyond are well-tolerated.

Here we propose that an athlete's rehabilitation program may be optimized by incorporating repeated heat exposures or heat acclimation, given the evidence in support of this modality to minimize skeletal muscle and cardiovascular de-conditioning during periods of disuse and reduced physical activity. For example, there is emerging evidence demonstrating remarkable benefits of heat stress on the regulation of muscle mass (Selsby and Dodd, 2005; Selsby et al., 2007; Ihsan et al., 2014; Hafen et al., 2019). Moreover, training in the heat allows for maintaining a lower absolute work load for a given relative exercise intensity, reducing the mechanical demands of the rehabilitating athlete. While it is important to note that some (Shvartz et al., 1977; Sawka et al., 1985; Lorenzo et al., 2010), but not all (Karlsen et al., 2015; Keiser et al., 2015; Neal et al., 2016) studies have reported improved exercise performance in cooler environments following heat acclimation, harnessing the benefits through passive or active heat acclimation remains a promising strategy to minimize cardiovascular de-conditioning in a rehabilitating athlete, whose training load is considerably reduced. This opinion piece presents how repeated heat exposures may be incorporated in the rehabilitation toolbox at various stages of the return to sport journey (**Figure 1**).

MUSCLE ADAPTATIONS TO HEAT THERAPY TO MINIMIZE MUSCLE ATROPHY WHEN IMMOBILIZED

During the first phase of the rehabilitation program, including pre- and post-surgery, heat exposure may be used to minimize the loss in muscle mass (**Figure 1**). Indeed, the effects of heat stress on the regulation of muscle mass has been relatively well-studied in cell cultures and rodents, demonstrating remarkable benefits. For instance, heat stress has been shown to enhance the

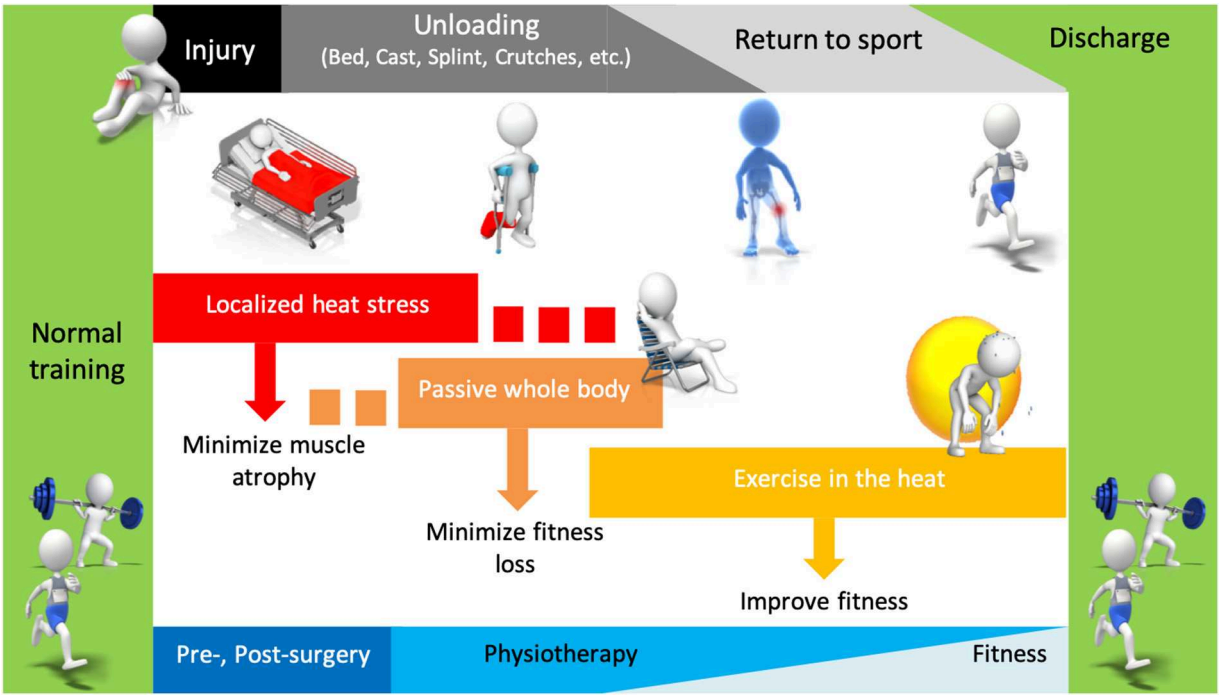

FIGURE 1 | Depiction of how the therapeutic use of passive or active heat stress may be utilized at different stages of rehabilitation to limit muscle atrophy and decline in fitness, optimizing the return to sport.

recovery of muscle mass within atrophied muscles (Goto et al., 2004; Selsby et al., 2007), as well as attenuate the loss in muscle mass in aging (Ohno et al., 2012), disuse (Selsby and Dodd, 2005), and pharmacological (Tsuchida et al., 2017) models of muscle atrophy. Moreover, heat exposure has been shown to preserve muscle mass and minimize protein degradation in a variety of muscle trauma models including ischemic injury (Garramone et al., 1994), crush injury (Takeuchi et al., 2014), exercise-induced muscle damage (Touchberry et al., 2012), and pharmacologically-induced muscle toxicity (Kojima et al., 2007; Shibaguchi et al., 2016).

The potential for heat-based interventions to be an integrative part of a rehabilitation strategy has, therefore, gathered considerable enthusiasm lately. This surge in interest is in-part due to recent studies in humans demonstrating improved muscle contractile function (Racinais et al., 2017b) or attenuated loss of muscle mass during immobilization (Hafen et al., 2019) following short-term heat exposure. Specifically, Racinais et al. (2017b) showed that 11 days of passive whole body heat exposure (60 min @ 48–50°C, 50% RH) resulted in an increased peak twitch amplitude, improved maximal voluntary torque, as well as the relative torque/electromyographic relationship. Additionally, more recent data by Hafen et al. (2019) showed that the decline in muscle mass following 10 days of lower limb immobilization was in-part attenuated when 120 min of localized heating of the vastus lateralis was administered daily. The preserved muscle mass following heat treatment coincided with the protein abundance of the transcriptional co-activator PGC-1α, HSP 70, and HSP 90, as well as minimized loss of mitochondrial respiratory chain protein content and mitochondrial respiratory function (Hafen et al., 2019). These findings are in general agreement with evidence gathered from cultured cells and rodents, where heat stress had been shown to mitigate muscle atrophic pathways through HSPs, PGC-1α, and mitochondrial signaling (Sandri et al., 2006; Romanello et al., 2010; Cannavino et al., 2015).

In addition to a mitochondrial and HSP centered mechanism, data gathered from cell cultures and rodent models implicate the Akt-mTOR cascade as a potential pathway by which heat stress might preserve muscle mass during immobilization. For instance, acute exposures (30–60 min) to environmental heat (39–41°C) has been shown to increase anabolic signaling (i.e., Akt-mTOR pathway), in line with increased muscle protein content (Goto et al., 2003; Uehara et al., 2004; Ohno et al., 2012; Yoshihara et al., 2013). Apart from initiating protein synthesis *per se*, activation of Akt has also been shown to suppress protein degradation through inactivating FOXO3, a key transcription factor regulating the expression of atrophic genes (Sandri et al., 2004). Nevertheless, while there is considerable evidence indicating that Akt-mTOR signaling may be up-regulated following heat stress, further evidence involving humans is further needed to verify this pathway within immobilization and injury models.

PASSIVE HEAT ACCLIMATION TO MINIMIZE DE-CONDITIONING EFFECTS WHEN NOT TRAINING

In addition to protecting muscle mass, passive heat exposures may also help to maintain cardiovascular fitness from the

onset of injury (**Figure 1**). Indeed, the cardiovascular and thermoregulatory adaptations conferred by repeated heat exposures are somewhat similar to the adaptations acquired through exercise *per se*, but with some specificities to improve thermoregulation. Importantly, a large part of the cardiovascular and thermoregulatory adaptations conferred by exercise heat stress may also be obtained through passive exposures in the condition that they sufficiently trigger an increase in body temperature, circulation, and sweating (Racinais et al., 2015; Périard et al., 2016). With recent studies demonstrating the accrue of such adaptations following passive heat exposures (Brazaitis and Skurvydas, 2010; Racinais et al., 2017a,b), there is intuitively an interest in developing heat-related therapeutic modalities for injured athletes restricted from training. The purpose of such modalities would be to induce cardiovascular adaptations (e.g., blood volume expansion) that aid in attenuating the loss of fitness, given the deterioration in cardiovascular adaptation substantially accounts for the decrease in VO_{2max} during de-conditioning (Mujika and Padilla, 2001). Practical passive heat acclimation strategies include hot water immersion or sauna bathing. Saunas are typically 80–90°C, whereas water temperature when immersing into a bath should be 40–42°C to induce adaptation, while remaining tolerable. These modalities are typically undertaken for 30–60 min, depending on whether heating was preceded by exercise. Guidelines regarding passive heating strategies are comparable to exercise heat acclimation and include consecutive days of exposure with a minimum of 5–7 exposures for initiating adaptations (Heathcote et al., 2018).

HEAT TRAINING TO OPTIMIZE THE RELATIVE/ABSOLUTE WORKLOAD IN INJURED ATHLETES

Once the athlete can progressively load the injured limb but is still unable to undertake heavy training, heat exposure during mild exercise may enhance the re-conditioning process (**Figure 1**). Indeed, exercise heat exposure provides a unique challenge to the cardiovascular system, which must not only supply blood to exercising muscles (i.e., oxygen delivery), but also to the peripheral vasculature (i.e., heat loss). When prolonged exercise is undertaken under heat stress and whole-body temperature increases, the cardiovascular response is progressively exacerbated compared to that of temperate conditions (Périard et al., 2013). This exacerbation is characterized by a compromise in the maintenance of mean arterial blood pressure and cardiac output, as heart rate drifts upward and stroke volume decreases (Rowell, 1974; Coyle and Gonzalez-Alonso, 2001). This forces the cardiovascular system toward a functional limit (i.e., VO_{2max}) (Nybo et al., 2001; Arngrimsson et al., 2003) with a progressive hyperthermia-induced reduction in VO_{2max} increasing the relative exercise intensity (i.e., $\%VO_{2max}$) for a given absolute workload (Périard et al., 2011; Périard and Racinais, 2015). Thus, the hyperthermia-induced dissociation between relative and absolute exercise intensity (Périard and Racinais, 2015) may allow injured athletes to train at a given heart rate or percentage of VO_{2max} for a lower absolute mechanical load in hot than temperate conditions.

Although the exacerbated cardiovascular response inherent with exercising under heat stress impairs the ability to perform optimally in hot environments, it may provide a pathway for injured athletes to train at high relative intensities without having to produce high levels of mechanical work with an injured limb. For example, injured athletes could exercise at a workload equivalent to a particular $\%VO_{2max}$, which as the session progressed, would result in increasing cardiovascular (Wingo, 2015) and metabolic (Febbraio et al., 1994) responses. To expedite these responses and avoid having to produce high levels of mechanical work in the early part of exercise sessions, prior to the development of whole-body hyperthermia, exercise may be preceded by passive heating.

Ultimately, we suggest that injured athletes may benefit from training in the heat as the cardio-metabolic load associated with a given mechanical workload increases progressively with the development of hyperthermia. This may help maintain fitness or attenuate the loss of aerobic capacity associated with a prolonged rehabilitation process following an injury, particularly in those unable to fully load the injured limb.

CONCLUSION

In summary, the therapeutic use of either exercise or passive heat exposure is a novel and emerging modality to assist with post-injury rehabilitation or re-conditioning. The use of such modalities can be targeted toward minimizing cardiovascular de-conditioning in athletes who are unable to undertake their usual mechanical loading. Moreover, drawing from the extensive research in rodent and cell cultures, as well as emerging evidence in humans, the use of passive heat application may minimize the decline in muscle mass during immobilization or periods of severely restricted exercise. There is a need though, to undertake further research toward optimizing the delivery of passive and active heat treatment modalities (i.e., whole-body vs. limb only, optimal muscle temperature and duration, dose-response, etc.). Such information would aid in the development of evidence-based heat therapy protocols for both sporting and clinical situations.

AUTHOR CONTRIBUTIONS

MI, JP and SR conceived and contributed to writing the manuscript. All authors provided critical feedback approved the final version of the manuscript.

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frontiers in Physiology

BRIEF RESEARCH REPORT
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Heat Acclimation Does Not Modify Q_{10} and Thermal Cardiac Reactivity

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Heat acclimation (HA) is an essential modifier of physiological strain when working or exercising in the heat. It is unknown whether HA influences the increase of energy expenditure (Q_{10} effect) or heart rate (thermal cardiac reactivity TCR) due to increased body temperature. Therefore, we studied these effects using a heat strain database of climatic chamber experiments performed by five semi-nude young males in either non-acclimated or acclimated state. Measured oxygen consumption rate (VO_2), heart rate (HR), and rectal temperature (T_{re}) averaged over the third hour of exposure were obtained from 273 trials in total. While workload (walking 4 km/h on level) was constant, heat stress conditions varied widely with air temperature 25–55°C, vapor pressure 0.5–5.3 kPa, and air velocity 0.3–2 m/s. HA was induced by repeated heat exposures over a minimum of 3 weeks. Non-acclimated experiments took place in wintertime with a maximum of two exposures per week. The influence of T_{re} and HA on VO_2 and HR was analyzed separately with mixed model ANCOVA. Rising T_{re} significantly ($p < 0.01$) increased both VO_2 (by about 7% per degree increase of T_{re}) and HR (by 39–41 bpm per degree T_{re}); neither slope nor intercept depended significantly on HA ($p > 0.4$). The effects of T_{re} in this study agree with former outcomes for VO_2 (7%/°C increase corresponding to $Q_{10} = 2$) and for HR (TCR of 33 bpm/°C in ISO 9886). Our results indicate that both relations are independent of HA with implications for heat stress assessment at workplaces and for modeling heat balance.

Keywords: heat acclimation, metabolic rate, heart rate, body temperature, rectal temperature, Q_{10} coefficient, heat strain, heat stress

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INTRODUCTION

Heat acclimation (HA) refers to adaptations of physiological functions to repeated exposures to heat stress enhancing the tolerance to that stressor and, thus, reducing physiological strain (Taylor, 2014). This manifests, among others, in increased sweat rates accompanied by reduced rates of energy expenditure, heart rates, and body temperatures when exercising in the heat with relevance in military (Sawka et al., 2011), occupational (Strydom et al., 1966; Kampmann, 2000), or sports context (Garrett et al., 2011; Périard et al., 2015).

The above-mentioned heat strain indicators are interlinked, e.g., by the well-known temperature dependency of the rates of chemical and physiological processes (van't Hoff, 1884), which is conveniently described as Q_{10} coefficient, defined as "*the ratio of the rate of a physiological process at a particular temperature to the rate at a temperature 10°C lower*"

(IUPS Thermal Commission, 2003). Using oxygen uptake rate (VO_2) as indicator of metabolic rate and rectal temperature (T_{re}) characterizing body temperature, this is mathematically expressed as (Chaui-Berlinck et al., 2002):

$$Q_{10} = \left(VO_2 / VO_{2,ref}\right)^{10/(T_{re}-T_{re,ref})} \quad (1)$$

$VO_{2,ref}$ refers to the oxygen uptake rate at a reference rectal temperature, e.g., $T_{re,ref}$ = 36.8°C. Re-arranging Eq. 1, it expresses percentage change in oxygen uptake rate ($\%VO_2$) due to a change in T_{re} (ΔT_{re}) as follows (Bröde and Kampmann, 2019):

$$\%VO_2 = \left(Q_{10}^{\Delta T_{re}/10} - 1\right) \times 100 \quad (2)$$

Q_{10} coefficients for biological systems typically vary between 2 and 3 (Chaui-Berlinck et al., 2002; IUPS Thermal Commission, 2003; Seebacher et al., 2015), with relevance not only during hyperthermia (Nadel et al., 1971; Howells et al., 2013), but also during body cooling (Erecinska et al., 2003). Furthermore, the setting Q_{10} = 2 is applied in human thermoregulation models (Werner and Buse, 1988; Fiala et al., 2012). A recent study on the influence of core temperature on oxygen uptake with 11 young acclimated males (Kampmann and Bröde, 2015) confirmed this with Q_{10} = 2.1 on average, corresponding to a 7% increase in VO_2 per degree rise in T_{re} according to Eq. 2. However, there was large inter-individual variation from Q_{10} = 1, i.e., no increase in VO_2 due to T_{re}, to Q_{10} = 8, corresponding to 23% VO_2 increase per degree rise in T_{re}.

There are reports on decreased Q_{10} after acclimation to heat indicating a reduced sensitivity of metabolic rates to increasing environmental temperature in ectotherms (Sandblom et al., 2014; Seebacher et al., 2015). Aiming at a comparative human study related to body temperature, we would like to extend the preceding works and analyze "whole organism" Q_{10} effects with acclimated compared to non-acclimated participants.

Body temperature also influences heart rate (HR) with a typical increase of 30–40 bpm per degree rise in T_{re} (Vogt et al., 1973; Kuhlemeier and Miller, 1978; Kampmann, 2000; ISO 9886, 2004; Bröde and Kampmann, 2019). This increase is termed "thermal cardiac reactivity" (TCR), and also "thermal pulses" (Kampmann et al., 2001) or "thermal heart rate component" (ISO 8996, 2004; Dubé et al., 2019), and shows considerable inter-individual variation between 16 and 60 bpm/°C (Kampmann, 2000; Bröde and Kampmann, 2019). An earlier study (Kuhlemeier and Miller, 1978) estimated TCR from pooled intra- and inter-individual data under different workloads of workers classified in "hot" and "cold-neutral" professions during summer and winter months, thus considering "natural" acclimation effects. The authors reported 6–7 bpm lower HR in summer compared to winter, and a 5–6 bpm reduction in HR in "hot" professions compared to the reference group, but did not allow for changes in the slope, i.e., TCR, depending on acclimation in their analyses, which were performed using the estimated overall value of 29 bpm/°C. Thus, it is unclear, whether acclimation changes TCR.

Q_{10} and TCR are relevant for the assessment of thermal stress and strain in different fields of application, e.g., as a potential source of error when estimating metabolic rate from heart rate measurements (ISO 8996, 2004; Malchaire et al., 2017). Here, TCR may induce an overestimation bias (Bröde and Kampmann, 2019) requiring dedicated correction procedures (Vogt et al., 1973; Kampmann et al., 2001; Dubé et al., 2019). Q_{10} also helps to explain the reduced cycling gross efficiency observed with increasing body temperature (Daanen et al., 2006).

Recently, Q_{10} and TCR were explicitly and implicitly applied for the non-invasive determination of core temperature from peripheral signals including heart rate, sometimes also involving the estimation of metabolic rate. Algorithms have been developed typically for work in protective clothing in industry (Richmond et al., 2015), firefighting (Kim, 2018), and military scenarios (Buller et al., 2013; Welles et al., 2018; Hunt et al., 2019).

For those applications, it is important to know whether the underlying algorithms will require adjustments considering the heat acclimation state of the individual. A recent pooled analysis (Bröde et al., 2009) of the changes in T_{re} (ΔT_{re}) and HR (ΔHR) after 5 days of short-term HA observed in 23 females and 34 males showed a significant positive correlation with ΔHR increasing with ΔT_{re} by 32.6 bpm/°C, close to the 33 bpm/°C reported for TCR in international standards (ISO 9886, 2004). Thus, TCR may have a role in explaining the effects of HA. However, it is unknown whether Q_{10} or TCR will depend on HA.

Therefore, the aim of this research was to study the influence of HA on Q_{10} and TCR using an extensive heat strain database compiled from controlled climatic chamber experiments.

MATERIALS AND METHODS

Heat Strain Database

We used a heat strain database of climate chamber experiments conducted previously at IfADo (Wenzel et al., 1989; Kampmann, 2000) according to the ethical principles of the Declaration of Helsinki with approval by IfADo's local Ethics Committee. **Figure 1** illustrates the recordings of rectal temperature (T_{re}) and heart rate (HR) for a typical heat stress exposure.

We searched our database for individuals having performed series of experiments in both non-acclimated (HA0) and acclimated (HA1) states. Inclusion criteria were a minimum number of 15 experiments per series with comparable workload and clothing in order to determine Q_{10} and TCR on an individual level. We retrieved 273 trials organized in 10 series, which originated from five semi-nude young fit males in either HA0 or HA1 state. The number of experiments in each series varied depending on acclimation state and individual between 15 and 47 experiments, with total figures of 118 trials for HA0 and 155 for HA1. The personal characteristics (mean ± SD) of the participants were 20.2 ± 0.8 years of age, 1.84 ± 0.02 m of body height, 71.4 ± 7.5 kg of body weight, 1.9 ± 0.1 m^2 of body surface area, and 47.1 ± 9.8 mL/min/kg of peak rate of oxygen uptake.

As the procedures have been described in detail elsewhere (Kampmann, 2000), they are only briefly summarized here. Each trial consisted of treadmill work with constant workload

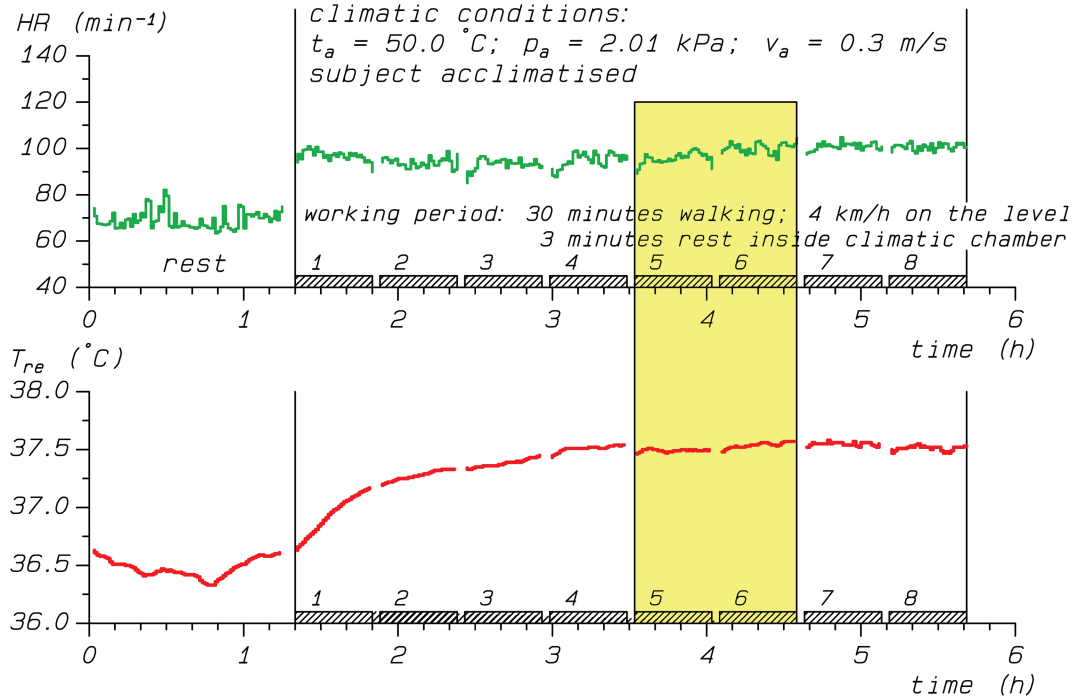

FIGURE 1 | Time course of heart rate (HR, upper panel) and rectal temperature (T_{re}, lower panel) during a heat stress experiment. The yellow shaded area marks the time interval with averaged values of HR = 98 bpm and T_{re} = 37.5°C used for analyses, while VO_2 (not shown) was 714 mL/min.

of walking 4 km/h on the level for at least 3 h organized in 30 min work periods interrupted by 3 min breaks for determining body weight loss (**Figure 1**). The participants were exposed to varying levels of heat stress with conditions characterized by different combinations of air temperature (range 25–55°C), water vapor pressure (0.5–5.3 kPa), and air velocity (0.3–2.0 m/s). Mean radiant temperature was equal to air temperature.

Rectal temperatures were recorded continuously using a thermistor probe (YSI 401, Yellow Springs) inserted 10 cm past the anal sphincter, as well as heart rates, which were obtained using ECG electrodes. T_{re} and HR were stored as 1-min averages, and means calculated over the third hour of exposure were used for further analyses (**Figure 1**). They were matched to oxygen uptake rates (VO_2) obtained toward the end of the third hour of exposure by collecting the expired air with Douglas bags (Douglas, 1911). We determined the oxygen and carbon dioxide concentrations with a paramagnetic gas analyzer (Servomex) and infrared analyzer (UNOR Mark 2), respectively. The VO_2 calculations based on the Haldane transformation (Poole and Whipp, 1988) are detailed in Rutenfranz and Wenzel (1980), while the methods were historically reviewed recently (Shephard, 2017).

Heat Acclimation Protocol

HA was induced by repeated experiments in warm-humid climates (air temperature 38–40°C with 65–70% relative humidity) over 3–4 weeks in a way that the subjects could sustain 3 h of heat exposure reaching a T_{re} of 38.5°C. To counteract a decay in acclimation over the weekend (Daanen et al., 2018), HA was re-established on Mondays and measurements for the series started the day after.

Non-acclimated exposures took place in wintertime in order to avoid seasonal adaptation, and with a maximum of two exposures per week on non-consecutive days to prevent short-term HA effects.

Data Analysis and Statistics

Statistical analysis was performed using R version 3.6.1 (R Core Team, 2019). The influence of T_{re}, which was centered to a reference value of 36.8°C, and HA on VO_2 and HR was analyzed separately with linear mixed model ANCOVA (Bates et al., 2015). The models included random intercepts and T_{re} slopes for individuals nested within acclimation status with tests for statistical significance carried out applying Kenward-Roger approximations for denominator degrees of freedom (Kuznetsova et al., 2017).

RESULTS

Figure 2 illustrates the influence of T_{re} on HR (**Figure 2A**) and VO_2 (**Figure 2B**), respectively. While the information for T_{re} and HR was almost complete (only 5 missing HR values), 34 VO_2 observations were missing, the majority (24) for ID5 in HA1 due to a defect in the O_2 analyzer requiring repairing

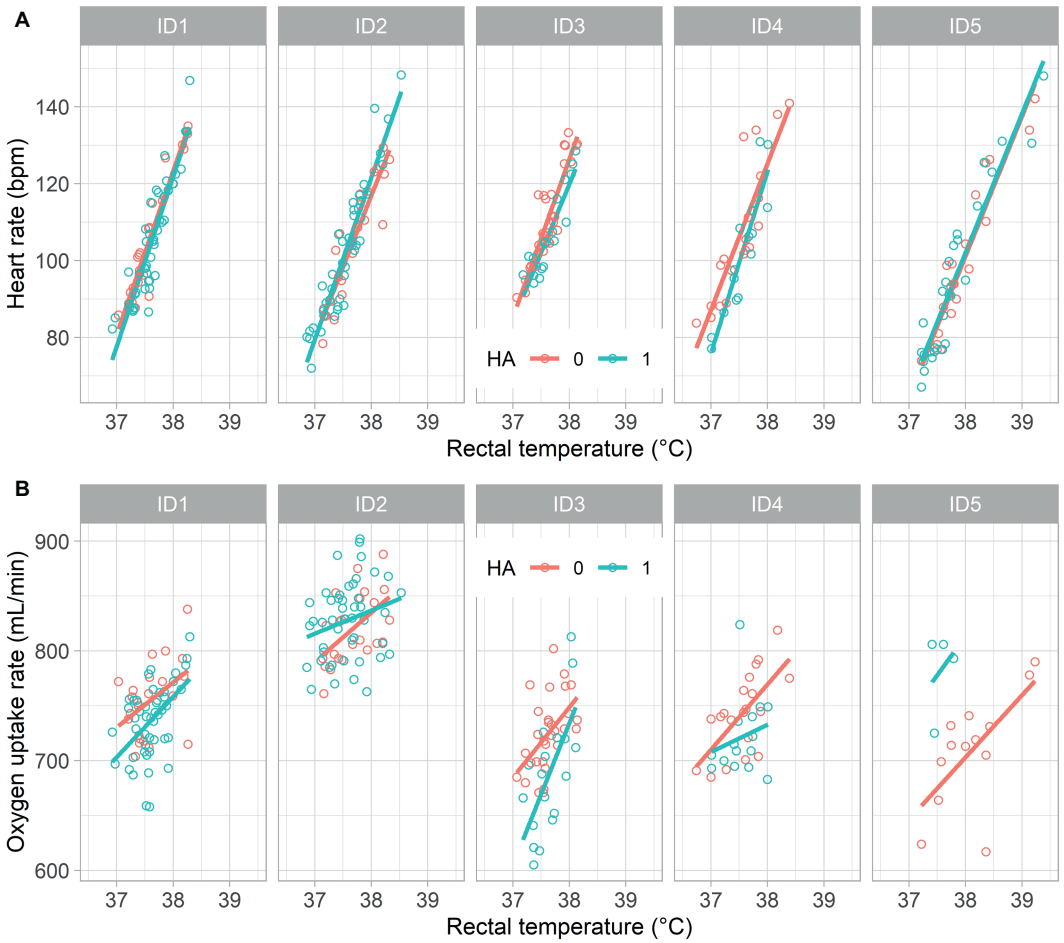

FIGURE 2 | Measured values and linear regression lines illustrating the influence of rectal temperature on **(A)** heart rate (thermal cardiac reactivity) and on **(B)** oxygen uptake rate (Q_{10} effect) for five participants (ID1–ID5) in non-acclimated (HA0) and acclimated (HA1) states, respectively.

TABLE 1 | Mixed effects ANCOVA results for the influence of T_{re}, centered to a reference value of 36.8°C, and heat acclimation (HA) on HR (thermal cardiac reactivity) and on VO_2 (Q_{10} effect).

Parameter	HR (bpm)	VO_2 (mL/min)
Intercept @T_{re} = 36.8°C for non-acclimated	71.9 ± 3.3 (**p < 0.0001**)	702.8 ± 30.5 (**p < 0.0001**)
T_{re} slope for non-acclimated	39.0 ± 1.9 (**p < 0.0001**)	50.7 ± 10.9 (**p = 0.0024**)
HA1: intercept adjustment for acclimated	−3.6 ± 4.6 (p = 0.4560)	8.7 ± 43.4 (p = 0.8456)
T_{re}*HA1: slope adjustment for acclimated	2.0 ± 2.7 (p = 0.4716)	−1.0 ± 16.5 (p = 0.9516)

T_{re}, rectal temperature; HR, heart rate; VO_2, oxygen uptake rate; HA1, acclimated. Data are parameter estimates ± SE with values of p for the null hypotheses of zero estimates in brackets resulting from linear mixed model analyses including random intercepts and slopes for participants nested within acclimation status. Bold p-values indicate statistically significant results ($p < 0.05$).

while the series of exposures had to be continued. Nevertheless, linear regression lines showed positive correlations with T_{re} for both dependent variables in each series.

The parameter estimates from the statistical analysis (**Table 1**) indicate that on average HR rose from 72 bpm at reference T_{re} = 36.8°C by 39 bpm per degree increase in T_{re}, i.e. TCR was 39 bpm/°C for non-acclimated individuals. When acclimated, the intercept was reduced by 4 bpm, while TCR slightly increased to 41 bpm/°C. However, while the TCR effect was highly statistically significant ($p < 0.0001$), adjustments due to HA to both intercept and slope were non-significant ($p > 0.4$).

Similar to TCR, rising T_{re} also significantly ($p < 0.01$) increased VO_2 by about 7% per degree increase of T_{re} compared to the reference VO_2 at T_{re} = 36.8°C for both HA0 and HA1; neither slope (i.e., Q_{10}) nor intercept depended significantly on HA ($p > 0.8$).

DISCUSSION

Our results regarding the impact of T_{re} on HR conform with reports of TCR between 30 and 40 bpm/°C in previous studies (Vogt et al., 1973; Kuhlemeier and Miller, 1978; Bröde and Kampmann, 2019) and in ISO 9886 (2004). They also agree

with former effect sizes for VO_2, as the observed increase of 7%/°C corresponds to a Q_{10} coefficient around 2, which were reported as mean value in human trials (Kampmann and Bröde, 2015) and used in advanced models of human thermoregulation (Werner and Buse, 1988; Fiala et al., 2012).

A novel finding of our study was that heat acclimation did neither modify thermal cardiac reactivity nor influence Q_{10}.

In contrast, a Q_{10} decrease after acclimation to warm conditions was reported for ectotherms and interpreted as lowered sensitivity to increasing environmental temperatures under climate change scenarios (Sandblom et al., 2014). However, those lowered Q_{10} were calculated across states of acclimation presuming that acclimation will shift the otherwise unchanged temperature-response function to the right (Seebacher et al., 2015). The latter would conform to the invariance regarding heat acclimation of the intra-individually determined Q_{10} in our study. On the other hand, the shift of the intercept observed in **Table 1** was minimal and non-significant.

There are limitations within this study that only used observations retrieved from an existing database of semi-nude fit young males performing light to moderate work. It would be worthwhile to verify our results involving other populations, e.g., females or elderly, under higher activity levels or working in protective clothing. Future studies might further include heart rate variability (HRV) measurements quantifying the sympathetic and vagal impacts on HR. Earlier studies had indicated vagal dominance following HA (Flouris et al., 2014), and negative correlations of vagal tone with T_{re} and HR (Brenner et al., 1997). However, as HRV calculations require beat-to-beat (RR) intervals, we could not perform these analyses with our aggregated HR data.

Nevertheless, our analyses of 273 experiments indicate that intra-individually determined Q_{10} and TCR remain unaltered following heat acclimation. This stability could have implications for the development and application of methods using the Q_{10} and TCR relationships for the heat stress assessment at workplaces (Malchaire et al., 2017; Bröde and Kampmann, 2019), and for the modeling of heat balance, e.g., for predicting core temperature from non-invasive signals when working with protective clothing in industrial, military, or firefighting operations (Richmond et al., 2015; Kim, 2018; Welles et al., 2018).

DATA AVAILABILITY STATEMENT

The raw data including the R scripts used for the analyses supporting the conclusions of this article are provided as **Supplementary Material** to this article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IfADo's Local Ethics Commission. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BK and PB designed and conceived the analyses. BK collected the data. PB organized the database and performed the statistical analysis. Both authors interpreted the data, and wrote the manuscript, and, after critically reviewing and providing significant editing of its content, approved the final article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01524/full#supplementary-material

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REVIEW
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Heat Adaptation in Military Personnel: Mitigating Risk, Maximizing Performance

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The study of heat adaptation in military personnel offers generalizable insights into a variety of sporting, recreational and occupational populations. Conversely, certain characteristics of military employment have few parallels in civilian life, such as the imperative to achieve mission objectives during deployed operations, the opportunity to undergo training and selection for elite units or the requirement to fulfill essential duties under prolonged thermal stress. In such settings, achieving peak individual performance can be critical to organizational success. Short-notice deployment to a hot operational or training environment, exposure to high intensity exercise and undertaking ceremonial duties during extreme weather may challenge the ability to protect personnel from excessive thermal strain, especially where heat adaptation is incomplete. Graded and progressive acclimatization can reduce morbidity substantially and impact on mortality rates, yet individual variation in adaptation has the potential to undermine empirical approaches. Incapacity under heat stress can present the military with medical, occupational and logistic challenges requiring dynamic risk stratification during initial and subsequent heat stress. Using data from large studies of military personnel observing traditional and more contemporary acclimatization practices, this review article (1) characterizes the physical challenges that military training and deployed operations present (2) considers how heat adaptation has been used to augment military performance under thermal stress and (3) identifies potential solutions to optimize the risk-performance paradigm, including those with broader relevance to other populations exposed to heat stress.

Keywords: heat acclimation, heat acclimatization, heat adaptation, heat stroke, heat syncope, heat illness, heat stress

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INTRODUCTION

Heat stress, through occupational exposure to strenuous physical exercise and/or environmental extremes of heat and humidity, presents a perennial challenge (Hancock et al., 2007) to military personnel. The consequences of incomplete or inadequate heat adaptation may be fatal; either directly, as with heat stroke, or through impaired physiological functioning and increased

susceptibility to military hazards, including combat. Augmenting physical and mental performance through heat adaptation may be pivotal to military operational success (Nindl et al., 2013).

Over the last two decades, many Western militaries have conducted land-based campaigns in climatically-severe regions (World and Booth, 2008; Cox et al., 2016a). These operations frequently evolved into mature missions allowing for targeted physical training prior to deployment and large-scale graded acclimatization practices upon arrival to operational theaters. In more recent history, several militaries have contributed to internationally-sponsored security projects including the protection of internally displaced civilian populations (Bailey et al., 2019) and the containment of infectious diseases outbreaks, such as Ebola (Dickson et al., 2018). Achieving peak occupational performance and developing and maintaining resilience in the face of thermal threat has been a critical endpoint in all such circumstances.

Routinely, the 'special population' fulfilling these roles is drawn from the general population of its parent nation and allied entities. Historically, the study of military personnel has provided insights into acclimatization mechanisms and strategies that have been generalizable to other occupational groups and the wider civilian population (Conn, 1963). The concept of relative climatic adaptation has been attributed to the renowned British Naval officer and clinician-scientist, Sir James Lind (Lind, 1771; Périard et al., 2015). As research findings on heat adaptation in this special population may be applied in other groups, so those learned elsewhere may have potential to prove militarily advantageous.

Illustrated by research data from uniformed personnel observing traditional and more contemporary acclimatization practices – and with particular reference to recent United Kingdom (UK) military experience – this article (1) characterizes the physical challenges that modern training and operations present (2) considers how heat adaptation has been used or may be applied to protect and augment the health and performance of military personnel and (3) identifies potential solutions to optimize the risk-performance paradigm that operates both during and after heat acclimatization.

METHODS

No articles were dismissed in this review but, where appropriate, comment has been made on the limitations of the study presented, i.e., limited subject numbers and confounding methodological issues. Historically, based journals have been included where appropriate to indicate the limitations in current research. Use of the terms 'military,' 'armed forces,' 'heat acclimation,' 'heat acclimatis(z)ation,' heat adaptation and 'heat illness' were employed. Search engines included PubMed, Web of Knowledge and directly from the appropriate academic journals. Studies previously reported from our group's work with the United Kingdom military are used to illustrate certain concepts, including novel unpublished data.

THE THERMAL CHALLENGE OF MODERN MILITARY OPERATIONS, TRAINING, AND CEREMONIAL DUTIES

In a volatile and increasingly complex world, governments of globally-facing nations foster contingency for a wide variety of overseas commitments (Ministry of Defence, 2018). These may be conducted through changing weather and elevation, over a range of timescales and set across challenging geographies: from short notice deployments to more enduring overseas operations (Edholm, 1969; Shapiro et al., 1981). Performance requirements are equally diverse arising from the scope of potential missions confronting the military and associated bodies, such as host nation civilian agencies and non-governmental aid organizations. Dedicated military taskings may include training and mentoring to allied forces; counter-terrorism/insurgency work; and full-scale war, including 'peer-to-peer' conflict conducted at large-scale, high tempo and over substantial physical areas. Other activities in which the military may contribute, or play a complementary role, include medical and engineering support to humanitarian relief efforts; law enforcement; firefighting responses; and management of other natural or man-made disasters and hazards (Ministry of Defence, 2018).

Operational capability may be impaired when unacclimatized personnel are required to travel at short-notice to a severe climate or to over-ride intrinsic or extrinsic thermal safeguards in the face of physical or tactical threats. For example, operational pressures may on occasion limit time or opportunity for full acclimatization, in favor of getting 'boots on the ground' with a view to achieving immediate military effects. From a physiological standpoint, however, inadequate or sub-optimal heat adaptation may have an array of consequences, the most feared of which is heat stroke and its associated morbidity and mortality (Abriat et al., 2014). Heat stroke has been defined as central nervous system (CNS) dysfunction, multiorgan failure and extreme hyperthermia, with core body temperature (Tc) usually $> 40.5°C$ (Epstein and Yanovich, 2019). It arises from failure to dissipate excessive body heat and is presently considered more preventable than treatable, being associated with a high mortality rate where treatment is delayed (Leon and Bouchama, 2015). Heat stroke and lesser forms of incapacity may be defined occupationally as 'heat illness': an 'all embracing term including those individuals who become incapacitated through exhaustion or syncope as a result of a rise in core body temperature'(Ministry of Defence, 2017). The occurrence of heat illness in military personnel falls with heat acclimation/acclimatization (Bean and Eichna, 1943; Edholm, 1969). This is particularly-well evidenced for minor episodes of heat illness characterized by heat syncope, or syncope upon cessation of exercise (post-exertional syncope), with prompt recovery of consciousness on prostration (Bean and Eichna, 1943). It is less clear whether risk of more severe illness, such as heat stroke, is substantially ameliorated in the heat adapted phenotype (Lim and Mackinnon, 2006). An increased hospitalization risk for heat illness (as a surrogate for severity) has been reported in unselected military personnel

considered acclimatized by attending physicians (Stacey et al., 2015). Hospitalization (Carter et al., 2005), and heat stroke deaths (Malamud et al., 1946) are less prevalent in military recruit populations originating from hotter regions, as opposed to cooler. However, it is unclear whether this is secondary to long-term physiological adaptation and/or modification of exertional behaviors and practices. In military recruit populations training in hot regions 77% of cases of heat illness (all forms) and 75% of heat stroke deaths have been observed to occur in the first 8 weeks of training, though the remainder of cases after this point are not insignificant proportions, considering the expected time course for acclimatization (Borden et al., 1945).

When an episode of heat illness occurs during, or soon after, strenuous physical exertion, it may be termed Exertional Heat Illness (EHI). This has been the predominant form of heat illness to affect British Army personnel in recent history, with EHI representing 96% of military cases reported by military doctors between 2009 and 2013 (Stacey et al., 2015) and continuing to make up the majority of referrals for specialist assessment. Most United Kingdom cases are incapacitated during training in temperate climates (Stacey et al., 2016). However, during the first 6 months of operational deployment to the extreme-dry heat of Iraq in 2003, 849 heat-implicated casualties were reported among United Kingdom military personnel, requiring 766 hospital admissions and 161 aeromedical evacuations to the United Kingdom (Bricknell, 2003). Three hundred of these cases presented to a dedicated Heat Illness Unit in July alone (incidence 50 per 1,000 deployed personnel) (Bolton et al., 2006) 90% had been affected within the first 10 days of arrival into theater and only a minority (14%) had been undertaking heavy work at the time of incapacity. This experience was reflected in Schickele's historic 1947 analysis of fatal heat stroke among military recruits in the continental United States, in which over 80% of 157 cases confirmed at post-mortem suffered heat stroke in relation to 'average activity' (drill, guard duty, relatively short marches) versus only 14% affected during heavy exercise (Schickele, 1947). Nearly all deaths occurred above a critical threshold of elevated heat and/or humidity, though some cases occurred with heavy physical exercise below the 'heat death line.' At the multinational field hospital in Camp Bastion, Afghanistan, heat illness was also a leading cause of admission, ranking second only to infectious diseases among all diagnoses made in internal medicine (2011 to 2013, summer and non-summer presentations) (Cox et al., 2016a). In the experience of the authors, the case load in Afghanistan represented a mixture of exertional, non-exertional, and intermediate heat illness (i.e., incapacitation where only light levels of physical activity were implicated in pathogenesis). Thus incapacity sustained with exposure to heat stress may manifest with excessive external (climatic, both macro and microclimate) and internal (metabolic) load, with each type of stressor capable of causing incapacity at lower 'doses' when operating synergistically. Heat stress also impacts on other duties, particular to the military, such as precipitating syncope or heat exhaustion during drill (Smalley et al., 2003; Wallace et al., 2006; Budd, 2008) often during large state ceremonial parades (Parsons et al., 2015). The effect of heat stress on reducing orthostatic tolerance has been well-demonstrated (Crandall et al., 2010a; Crandall and Wilson, 2015; Schlader et al., 2016a,b).

HEAT ADAPTATION: HEALTH PROTECTION AND AUGMENTED PERFORMANCE IN MILITARY PERSONNEL

The thermal challenge of military service raises questions over the optimal ways for parent organizations to exercise their duty of care and facilitate mission objectives. Heat adaptation is a key component of managing the overarching risk presented and, in the military as elsewhere, serves two key purposes: (1) the prevention of heat-related illnesses and (2) the improvement of physical performance and mental functioning. Crucially for military tasks, this may include improved decision-making under heat stress (Cheung et al., 2016).

Acquisition of the heat-adapted state occurs through repeated or continuous exposure to heat stress and accompanying elevation in Tc and may be achieved with artificial exposure to heat, by residence in a natural hot climate, or with physical training sufficient to raise Tc in less severe conditions (Leon and Bouchama, 2015). Phenotypic changes that are associated with repeated heat stress include altered sweating and skin blood flow responses, decreased metabolic rate, plasma volume (PV) expansion and improved cardiovascular stability (Nielsen, 1998; Shapiro et al., 1998; Patterson et al., 2004). In the hot environment, acquisition of these adaptations associates with improved thermal comfort (Sato et al., 1990), lower physiological strain (Taylor, 2014) and restored performance capacity for equivalent bouts of exercise conducted in less severe conditions. In some studies, performance is also enhanced on return to the thermoneutral environment, by way of increased maximal aerobic capacity (Sawka et al., 1985; Lorenzo et al., 2010). A core physiological change in heat adaptation, as described by Conn and Johnston (1944), is increased sweat rate and decreased concentration of sweat sodium and chloride (Leon and Bouchama, 2015) due to changes in the eccrine glands (Sato et al., 1990). Sweating, through changes in the CNS, occurs earlier and at a lower core temperature (Taylor, 2014).

Bass and Henschel defined acclimatization as 'the dramatic improvement in the ability to work in the heat which occurs within 4 to 7 days of first exposure' (Bass and Henschel, 1956). Over this period, rapid, demonstrably effective (Garrett et al., 2009, 2012) but ultimately incomplete (Pandolf, 1998; Gibson et al., 2015) adaptation ensues with improved cardiovascular stability and heart rate decrease, in association with PV expansion (Armstrong and Maresh, 1991). Horvath and Shelley described attaining a stable state of improved physical and mental functioning with fuller acclimatization (Horvath and Shelley, 1946) encompassing the features of longer term heat adaptation such as behavior changes resulting in reduced heat stress, e.g., reduced work and greater use of shelter (Périard et al., 2015), and improved thermal sensation (Chalmers et al., 2014). Acclimation status has been categorized by the number of exposures to heat stress (Chalmers et al., 2014; Tyler et al., 2016) as short-term (up to seven exposures), medium- term (eight to 14 exposures), and long-term (15 or more exposures).

Heat adaptation from exercise-heat stress is commonly induced through three mechanisms (Daanen et al., 2018):

(1) constant work-rate exercise (Nadel et al., 1974; Nielsen et al., 1993, 1997) (2) self-paced exercise (Nelms and Turk, 1972) and (3) isothermic acclimation (Regan et al., 1996; Garrett et al., 2009). Additional novel methods that have been proposed include matching exercise intensity to the observed decrease in heart rate (Périard et al., 2015; Tyler et al., 2016) or constant-rate exercise to a fixed rating of perceived exertion (RPE) (Omassoli et al., 2019). For the purposes of simplicity and economy, military organizations have favored standardized exposures (Gibson et al., 2015), commonly at a constant work-rate (Daanen et al., 2018), with some approaches being more self-paced. In recent decades, Western military forces have regained significant experience in deploying large numbers of personnel to thermally-stressful environments for demanding combat and peace support operations. This has seen the introduction of empirical guidance to achieve safe and effective group-based acclimatization, drawing on earlier lessons learned in the wake of hot weather training and campaigns during World War 2 (Bean and Eichna, 1943; Schickele, 1947; Conn, 1963), and the propagation of general principles in support of this aim (**Table 1**). In this context, it has been stated that approximately 2 weeks of progressive heat exposure and physical work should expedite near-complete acclimatization, with the average service person expected to have achieved ∼50 and ∼80% of ultimate physiological adaptation, respectively, by the completion of the first and second weeks of appropriate training (STO/NATO, 2013) (**Figure 1**). An example of a validated acclimatization schedule for physically fit personnel to follow on arrival to a hot region is provided at **Table 2**. This is reproduced from United Kingdom Defense policy on prevention of climatic illness and injury (Ministry of Defence, 2017). The specific impact and potential implications of following this protocol are discussed below.

TABLE 1 | Heat acclimatization strategies that can be considered before and after military deployment to a hot region (STO/NATO, 2013).

(1) Mimic the deployment climate.

(2) Ensure adequate heat stress by:
- Invoking profuse sweating.
- Using exercise and rest to modify the heat strain.
- Having 4 to 14 days of heat exposures.
- Maintaining the daily duration of at least 120 min.

(3) Start early (1 month before deployment).
- Performance benefits may take longer than physiological benefits.
- Be flexible with training.
- Build confidence.
- Pursue optimum physical fitness in the current climate.

(4) Methods.
- Pre-deployment: climate controlled room or hot weather.
- Integrate with training by adding additional acclimatization sessions; inserting.
- Acclimatization with training; alternating acclimatization days with training days, and no detraining.
- Mimic the deployment environment by working out in a warm room wearing sweats (*sic*) if you are in a cool/temperate environment.

(5) On arrival.
- Start slowly at reduced training intensity and duration and limit heat exposure.
- Increase heat and training volume (intensity and duration) as tolerance permits.
- Acclimatize in heat of day.
- Physical training should be conducted in coolest part of day.
- Use work/rest cycles or interval training.
- Be especially observant of salt needs for the first week of acclimatization.
- Sleeping in cool or air-conditioned rooms will not affect heat acclimatization status and will aid recovery from heat stress.

Over the short term moderate intensity exercise appears to induce a more efficient physiological adaptation (Houmard et al., 1990) in terms of time spent exercising in the heat. But, for simple approaches utilizing constant work rate, it has been argued that the 'forcing function' of fixed environmental heat exposure and endogenous heat production is attenuated by the effects of progressive heat adaptation, in a process of habituation (Taylor, 2000, 2014). Such protocols may be convenient to apply and effective at restoring the ability to perform a standard bout of work in the new (hot) conditions, but may limit or slow progress toward maximum acclimatization. Furthermore, excessive thermal stress in the absence of close monitoring – as may be seen with large numbers of military participants working to a constant level or group pace, especially early in the course of adaptation – has potential to induce significant heat-related morbidity, with group-paced activities associating with hospitalization among United Kingdom heat illness casualties (Abriat et al., 2014). Yet whilst a self-paced heat acclimatization program would be seemingly protective in this regard, such practices are not an absolute safeguard, with soldiers exceeding pre-defined heart rate and temperature limits, particularly in competitive groups (Armstrong et al., 1986).

Isothermic or controlled hyperthermic acclimation commonly manipulates the endogenous thermal load under constant exogenous heat stress (Regan et al., 1996), but can also vary environmental conditions to reach and sustain a target Tc. These regimens associate with greater physiological adaptations in a shorter space of time, with incorporation of physical exercise (Hellon et al., 1956; Bain et al., 2013; Charlot et al., 2017) being more efficient than a passive strategy (Harrison, 1985; Périard et al., 2015; Racinais et al., 2015; Tyler et al., 2016). Often isothermic regimens utilize both forms of heat exposure (Daanen et al., 2018). Approaches of this kind could hold considerable appeal for military planners and commanders intent on taking personnel through a rapid transition to a more severe environment, as with deployment of high readiness troops into an emerging or escalating operational theater. This methodology is resource intensive however and may be unsuited to large scale implementation, i.e., in groups of military personnel above sub-unit size (20–30 pax). Whether it would always be operationally effective to strictly observe sub-maximal physiological thresholds is a matter of military debate, at least as it applies to routine training in the heat (Hunt et al., 2016). Attaining measures of heart rate, Tc or derived/composite parameters such as the Physiological Strain Index (Buller et al., 2018) could ensure

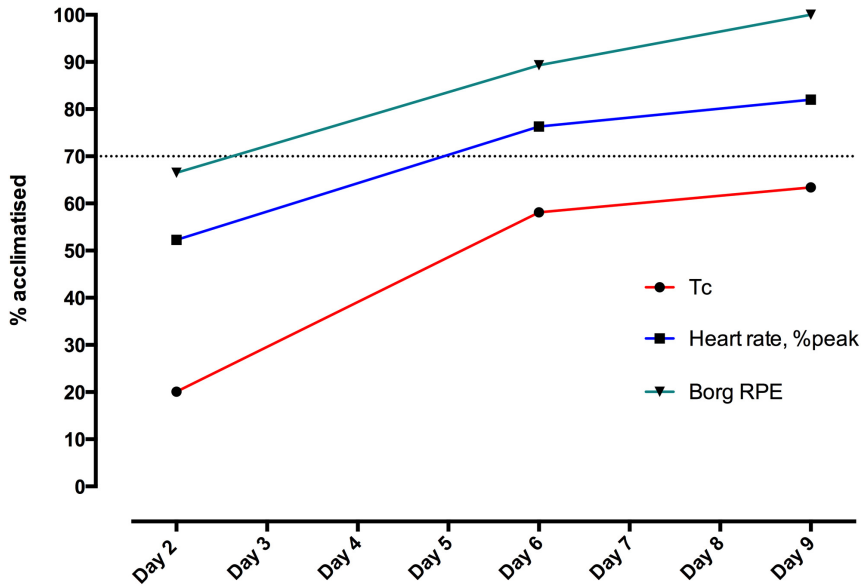

FIGURE 1 | Heat adaptation in 21 volunteers, displayed as progress toward full (100%) acclimatization status on Day 23 in Cyprus, relative to pre-deployment baseline in United Kingdom (0% acclimatized). Volunteers performed the acclimatization protocol described in **Table 1**, with 60 min stepping at 50% VO_2max in a climatic chamber controlled to WBGT 34°C, at United Kingdom baseline and on Days 2, 6, 9, and 23 following arrival in Cyprus (substituted for usual acclimatization exercise on these days). Acclimatization status (%) was calculated by parameter, in terms of the end-exercise value relative to that on Day-23. Tc, core body temperature; Borg RPE (Borg, 1973), Relative Perceived Exertion according to Borg's scale (may need to reference: as below). Dotted line indicates 70% adaption.

safe initial induction of heat adaptation, whatever the method selected to achieve it.

PRACTICES AND PITFALLS IN GENERATING AND SUSTAINING HEAT ADAPTATION IN MILITARY POPULATIONS

Drawing parallels with civilian athletic groups, whom may also be rapidly required to perform in hot climates with minimal preparation (Périard et al., 2015), military personnel should maintain a high state of physical fitness. Military guidance developed within the North Atlantic Treaty Organization (NATO) (**Figure 1**) identifies an increased need for monitoring 'the least fit soldier, who will have the most difficult time (to acclimatize) (STO/NATO, 2013). This assertion is supported by findings that: (1) physical fitness improves physiological responses to exercise in the heat (Armstrong and Maresh, 1991) (2) heat-related illnesses are reported at lower rates in trained personnel than (putatively less well-conditioned) recruits undergoing induction to the Armed Forces (Carter et al., 2005; Stacey et al., 2015) (3) aerobically well-trained participants may be 'primed' to heat adaptation with an indirect relationship

TABLE 2 | A validated acclimatization schedule for physically fit personnel to follow on arrival to a hot region reproduced from United Kingdom Defense policy on prevention of climatic illness and injury (Ministry of Defence, 2017).

Day	Dress	Target WBGT Index (°C WGBT)	Duration (min)	Activity
1	No activity. rest, eat, drink, and sleep (for 24 h)			
2	T-shirt and shorts	26–30	1 × 50	Walk at 6 km/h (3.7 miles/h)
3	T-shirt and shorts	26–30	2 × 50	Walk at 6 km/h; rest for 15 min; resume walking.
4	T-shirt and shorts	26–30	100	Walk at 6 km/h
5	T-shirt, combat jacket, lightweight trousers, and body armor	26–30	2 × 50	Walk at 6 km/h for 50 min then remove body armor and jacket and rest for 15 min; resume walking.
6	T-shirt, combat jacket, lightweight trousers, and body armor	26–30	100	Walk at 6 km/h
7	T-shirt, combat jacket, lightweight trousers and body armor and webbing (10 kg)	26–30	2 × 50	Walk at 6 km/; remove webbing, rest for 15 min; resume walking.
8	T-shirt, combat jacket, lightweight trousers and body armor and webbing (10 kg)	26–30	100	Walk at 6 km/h

Personnel undertaking acclimatization should be allowed fluids as required

existing between VO_2max and the number of days required to acclimatize (Pandolf et al., 1977; Garrett et al., 2011). The NATO guidance also identifies a second high-risk group: 'the most motivated soldiers, who may overdo their physical activity and be susceptible to becoming heat casualties' (STO/NATO, 2013). Highly motivated personnel can be found throughout any military organization, but are often concentrated among the fittest elements, including the infantry, marines, and other 'frontline' occupations. These units are trained to engage in dismounted ground close combat (DGCC) and may be required to close with and kill enemy forces, among other physically-arduous functions. Reported heat stroke rates have been 7- to 11- times higher in the US Army and Marine Corps than in the Navy or Air Force and all forms of heat illness are significantly commoner in combat versus other occupational roles (Armed Forces Health Surveillance Bureau, 2019). Individuals in these higher risk groups may be required to deploy more quickly, risking inadequate time for acclimatization before action, plus exposure to a multitude of other factors that may delay or interrupt full heat adaptation. Whilst in most cases one single predisposing factor (Epstein, 1990; Epstein et al., 1999) is sufficient to reduce the ability of the individual to tolerate heat during physical activity – or even with passive exposure – aggregating factors can further increase risk for heat illness (Gardner et al., 1996). Motivation to accomplish a mission, whilst disregarding early signs and symptoms for heat-related disability, is a contributing factor that can significantly exacerbate this situation (Shibolet et al., 1976; Epstein et al., 1999).

In advance of deployment to a hot environment, United Kingdom military guidance directs commanders, physical training staff and individual service personnel to build or improve aerobic fitness over 3 to 4 weeks, aiming to 'increase and maintain heart rate above 65% of maximum heart rate for periods of 40 min initially, then extending to an hour.' This should be followed by 10–14 days of individual or group exercise to 'raise and maintain an elevated body temperature for at least 1 h each day'… 'checked by visual assessment of sweating,' such that a total of 6 weeks dedicated readiness-training has been completed pre-deployment (Ministry of Defence, 2017). In prescribing sub-maximal exercise in time-limited bouts, with allocated rest days (at least 2 per week) and progress toward more thermally-stressful exposures immediately prior to deploying, this approach has the potential to protect, both less and more fit, individuals during preparation for, and on arrival to, a hot environment. In what may be a busy run-in to an overseas exercise or operational mission, unit schedules and individual availability can conceivably limit full compliance with this strategy.

Following arrival to a hot environment, the performance of military duties may usefully be used to induce heat acclimatization, so long as personnel are not critically stressed and/or affected by heat illness in the process. It is common practice (e.g., French and United Kingdom guidance) to promote an initial rest day, with no exercise permitted, in order to allow for transient factors specifically associated with preceding travel that may otherwise undermine heat tolerance (**Table 2**). The impact of significant troop numbers being incapacitated during early acclimatization can degrade operational capability, challenge the capacity of deployed healthcare systems and, as described above in relation to United Kingdom deployment to Iraq, dictate that many affected personnel are re-patriated (Bricknell, 2003; Bolton et al., 2006). This is particularly the case for severe cases of heat illness or if local healthcare assets lack expertise in the risk stratification and discrimination of casualties whom remain at increased risk of heat stroke, and subsequently require protection from further exposure, or where those with minor heat related debility are removed unnecessarily. The military guidance (**Table 2**) therefore advocates a slow start to acclimatization, with reduced and time-limited exercise bouts, gradually increasing in intensity and length toward pre-acclimatization norms.

In assessing the impact of progressive aerobic activity added into a program of 'usual outdoor military activities,' Charlot et al. (2017) showed an accelerated adaptation versus individuals not exposed to dedicated training, with greater improvements in post-exertional heart rate, thermal discomfort, and RPE following the first week of acclimatization (Charlot et al., 2017). These bouts were of relatively short duration (32 rising to 56 min sub-maximal interval running bouts) by the standards often observed in military protocols, but had the advantage of being complemented by and accommodated within the operationally-focused work schedule mandated from arrival to the hot environment. Performed within military logistic-time constraints the investigators failed to demonstrate a greater reduction in Tc – or blunting of its rise with exertion – in the training group, but cited their improved cardiovascular stability as theoretically conferring better protection against non-heat stroke illnesses, such as heat exhaustion and syncope. Coupled with the significant and substantial reductions in thermal discomfort and RPE observed in both groups following early acclimatization, the authors pointed to the potential for exercise performance to be sustained – and less severe forms of heat illness potentially bypassed – despite limited improvements in heat dissipation and thus Tc responses. The risk of 'high intensity tasks' causing adverse effects, such as heat stroke, after 7 days of heat acclimatization, with or without training' was highlighted. This warning has been echoed in United Kingdom guidance that full acclimatization may take longer than 15 days; that this process may be extended further when a substantial period of travel or crossing of multiple time-zones precedes arrival to the hot environment; and that living or working in air-conditioned accommodation may slow its development (Ministry of Defence, 2017). Charlot et al. (2017) also identified a dissociation between objective physiological variables and subjective psychological variables, with sweat volumes and Tc responses failing to keep pace with large improvements in thermal discomfort and RPE. In a formal evaluation of the United Kingdom regimen provided in **Table 1** – undertaken in British paratroopers, marines and specialized infantry, deployed from the United Kingdom to Cyprus, and adapted to incorporate serial testing of heat tolerance (Stacey et al., 2018b) – the most substantial early reductions in exercising heat strain parameters were seen for RPE, followed by heart rate and then Tc (**Figure 1**). Progress toward final

acclimatization status (deployment Day 21) followed the same pattern, with Tc lagging behind RPE and heart rate even after the first full week of the protocol. The need for caution as heat adaptation progresses, highlighted by this relative discrepancy, has been explained as: participants 'may feel larger improvements in thermal and exercise discomfort, likely increasing intensity during training sessions or operational tasks based on these positive feelings/sensations. . . (but) the maximum decrease in core temperature was not yet achieved (Charlot et al., 2017). Work conducted in US Navy personnel has shown adaptation discordance between mean skin temperature (>90% progress toward Day 21 acclimatization status after first week) and both heart rate and rectal temperature (only 37 and 38% complete at 7 days, respectively) (Naval Medical Command, 1988) as improved perceptions of the severity of exertion and thermal stress experienced with acclimatization are thought to derive through greater reductions in facial temperature (Malgoyre et al., 2018). The mean lowering of skin temperature may be an important factor in improved physical capacity and the associated potential for sustaining heat stroke – e.g., with unsafe levels of self-paced physical exertion – during and after heat acclimatization.

These data provide a basis to understand how a state of partially complete, or temporarily undermined, heat adaptation may risk more serious forms of heat illness; they would also help to explain the increased hospitalization risk in heat illness cases (Stacey et al., 2015). One factor relevant to this interpretation may be the minimum exposure period recognized as fostering adequate acclimatization. Despite NATO recommendations stating that only a percentage of ultimate adaptation will be achieved with 7 days of protocolized exercise in the heat and United Kingdom guidance highlighting how some individuals will progress more slowly, commanders, and medical personnel alike may fail to appreciate that heat adaptation status has not been adequate to the task required in an individual subsequently affected by heat illness. Based on observations of improved cellular protection and increased organ efficiencies, Horowitz's group has advanced the case for long term heat acclimatization (LTHA) status taking at least 3 weeks to acquire (Schwimmer et al., 2006). The avoidance of strenuous physical activities until after 3 weeks of adaptation to a new hot environment has long been advocated in military medical practice, at least for deployments where circumstances permit a more graded introduction to heat (World, 2001).

Adaptations to heat exposure is never permanent. According to Givoni and Goldman (1973), heat adaptation is lost for every day spent without heat exposure at a rate that is twice as fast as the rate with which the heat adaptation was initially gained. The lasting effects of heat adaptation, upon removal of heat stimulus, and subsequent re-acclimation has relevance to the military undergoing short notice high intensity missions such as special forces personnel, but also personnel on more enduring operations. The United Kingdom Armed forces, in recent operations, granted its deployed service personnel a leave of rest and recuperation (R&R) for a period of 2 weeks midway through the operational tour. A meta-analysis of 12 studies reported adaptations in end-exercise heart rate decreased by 2.3% for every day of heat acclimation decay. For end-exercise core temperature, the daily decrease was 2.6% (Daanen et al., 2018).

There are several factors, environmental and logistical, which may attenuate or even reverse the physiological effects of heat acclimatization. Whilst some of these factors are specific to the military others are relevant to other occupational groups including firefighters and humanitarian organizations. Core physiological changes in heat adaptation include increased sweat rate, sweating occurring at a lower temperature (Sato et al., 1990) and decreased concentration of sodium and chloride (Conn and Johnston, 1944; Horvath and Shelley, 1946). More dilute sweat is more easily evaporated but only if the climate allows for evaporation (Taylor, 2014; Périard et al., 2015). Certain occupational professionals, particularly firefighters, police and military personnel have requirements for particular uniforms or personal protective equipment due to the nature of the work. These mandatory dress states may attenuate the physiological benefit from sweating adaptation (Smolander et al., 1984; Patton et al., 1995; Maynard et al., 2016) by limiting sweat evaporation even in the context of full acclimatization (Aoyagi et al., 1995). Body armor, having shown to substantially reduce fatal injury to the thorax and abdomen (Mabry et al., 1999; Masini et al., 2009; Belmont et al., 2010) has, over recent conflicts, become commonplace for military personnel, journalists, non-governmental organizations as well as some police units. Studies have indicated that the wearing of body armor increases temperature (Majumdar et al., 1997; Cheuvront et al., 2008; Caldwell et al., 2011) (both core and skin), heart rate (Majumdar et al., 1997; Cheuvront et al., 2008; Caldwell et al., 2011) and produces more sweat (Caldwell et al., 2011) whilst performing military activities (Chinevere et al., 2008; Caldwell et al., 2011). Several studies have outlined an increase in heat stress and thermoregulatory load to chemical warfare suits with a consequential degradation in human performance (Taylor and Orlansky, 1993; Aoyagi et al., 1995). The amount of protection (encapsulation), environmental conditions, physical condition of the soldiers, mission (including duration), amount of physical activity and work rest/cycles appear to be important co-factors (Pandolf et al., 1986; Taylor and Orlansky, 1993). Improved aerobic fitness and the degree of heat acclimation do little to improve the tolerance to CBRN suits during light and moderate exercise (McLellan and Frim, 1994).

Numerous studies have attempted to capture the potential risk factors implicated in heat illness. Commonly cited precipitants include: alcohol (Armstrong et al., 2007), medications (Epstein, 1990; Armstrong et al., 2007) (particularly psychiatric), recreational drugs (Epstein et al., 1999; Armstrong et al., 2007), sickle cell trait (Nelson et al., 2017; Singer et al., 2018), recent febrile illness/diarrheal illness (Epstein, 1990; Armstrong et al., 2007), sleep deprivation (Armstrong et al., 1990, 2007; Bolton et al., 2006; Stacey et al., 2015), sunburn (Armstrong et al., 2007), obesity (Gardner et al., 1996; Epstein et al., 1999; Wallace et al., 2006; Bedno et al., 2014), dehydration (Montain et al., 1998; Armstrong et al., 2007) as well as the known intrinsic factors such as environmental temperature and humidity, clothing and

activity levels (Kark et al., 1996; Howe and Boden, 2007). More militarily relevant studies have associated the increased risk of prior heat stroke (Armstrong et al., 2007), combat or healthcare occupational roles, reservists (Bricknell, 1996), subjects over 30 (Epstein, 1990), female sex (Fortney and Senay, 1979; Carter et al., 2005), lower levels of physical fitness (Shvartz et al., 1977b; Epstein, 1990; Gardner et al., 1996; Wallace et al., 2006; Bedno et al., 2014), lack of acclimatization (Epstein, 1990; Bricknell, 1996; Armstrong et al., 2007) and scarred skin areas (Epstein, 1990; Crandall and Davis, 2010; Ganio et al., 2013; Cramer et al., 2019). Caucasian soldiers appear to be at higher risk than African American (Carter et al., 2005). Militaries are drawn from the population of the nations they serve so these risk factors are not generalizable. Many factors can be controlled for using a command structure built on discipline, inherent in military organizations, coupled with careful occupational management. Both systems are fallible however (Cox et al., 2016b). How heat acclimation impacts or mitigates these exacerbating factors is unknown.

Hemorrhage remains the primary modality of battlefield death (Sauaia et al., 1995; Eastridge et al., 2012) commonly before the provision of surgical management (Lind et al., 1968; Crandall et al., 2010b) yet little is known of the effects of heat acclimation on hemorrhagic shock. This is of particular relevance to the military but also civilian aid workers and firefighters. Models, which typically use orthostasis to pool blood in the lower limbs, have demonstrated an inability to tolerate central hypovolemia during simulated hemorrhage when heat-stressed (Lind et al., 1968; Crandall et al., 2010b; Schlader et al., 2016b) although heat acclimation is reportedly protective (Greenleaf and Bosco, 1969; Shvartz and Meyerstein, 1970; Shvartz et al., 1975, 1977a). These findings are not consistent particularly when combining exercise with heat stress in the acclimated (Greenleaf et al., 1974). During heat stress skin blood flow increases significantly requiring a doubling of cardiac output to maintain arterial blood pressure (Schlader et al., 2016b) causing a reduction in central blood volume (Crandall et al., 2012), preload (Wilson et al., 2009), left ventricular end diastolic volume (Nelson et al., 2011) and stroke volume; the proposed mechanisms to how heat stress compromises tolerance to a simulated hemorrhagic insult (Crandall et al., 2019). Whilst heat adaptation, by increasing plasma volume, may improve orthostatic tolerance and reduce heat syncope this may have little effect on actual, rather than simulated, hemorrhage with volume expansion mitigated by dilution of endogenous clotting factors. Furthermore those with a better or worse tolerance to simulated hypovolemia in the heat (Schlader and Crandall, 2014) may not correspond to a better or worse tolerance to hemorrhagic hypovolemia. Heat stress is thought to confer a hyperadrenergic state (Niimi et al., 1997; Cui et al., 2002, 2004, 2010; Low et al., 2011), which potentially reduces the reserve which can be sympathetically activated to maintain blood pressure in the event of a hemorrhagic insult (Crandall et al., 2019). In the military this hyperadrenergic state could be enhanced by sleep deprivation, inadequate nutrition, dehydration (Morgan et al., 2004; Lucas et al., 2013) or mental stress (Greenleaf and Bosco, 1969; Shvartz and Meyerstein, 1970; Shvartz et al., 1975, 1977a, 1981; Yamazaki and Hamasaki, 2003).

Dehydration often used synonymously with hypohydration (Cheuvront and Kenefick, 2014; Kavouras, 2019) and is a common finding in deployed military personnel (Rogers and Cole, 2016; Kavouras, 2019). Heat acclimation potentially guards against dehydration during thermal stress and is associated with reduced markers of renal stress and AKI incidence (Omassoli et al., 2019). Whilst physical conditioning maintains physical work following dehydration, heat acclimatization did not appreciably supplement this effect (Buskirk et al., 2000). Dehydration following heat acclimation may attenuate the effect of heat acclimation in terms of reducing cardiac output during exercise (Montain et al., 1998) and attenuating improvements in cardiac stability. The acclimation derived alterations in core temperature appear to be maintained during dehydration being unaffected by exercise intensity (Montain et al., 1998). Partly due to hypohydration being so ubiquitous in a hot environment, it has been hypothesized that permissive dehydration drives the improved physiological response during heat acclimation (Garrett et al., 2011; Neal et al., 2016) although this remains disputed (Schleh et al., 2018). Permissive dehydration during exercise in the heat may increase the response of fluid regulatory hormones aldosterone, vasopressin, and cortisol (Kenefick et al., 2007) and potentiate acclimation by increased fluid retention resulting in plasma volume expansion (Garrett et al., 2011). Due to concerns regarding heat illness such a strategy would unlikely to gain traction in a military context without further research to address concerns on safety and efficacy.

That acclimatized soldiers succumb to severe heat illness, or heat stroke (Stacey et al., 2015), suggests a dissociation between thermoregulation and heat tolerance (Lim, 2018). There is a growing body of literature suggesting that exertional heat illness is an endotoxemia (Moseley and Gisolfi, 1993; Lim, 2018). A secondary function of the gastrointestinal tract is in the prevention of translocation of potentially harmful luminal antigens into the systemic circulation (Camilleri et al., 2012; Wells et al., 2016). Exercise can adversely disrupt the gastrointestinal barrier integrity (Lambert, 2004) in proportion to the amount of thermal stress (Costa et al., 2017b; Pires et al., 2017) so causing leaking of gram negative bacteria into the intravascular space (Lambert, 2004; Yeh et al., 2013) potentially causing exertional heat stroke (Armstrong et al., 2018; Lim, 2018). Mechanisms include a transient gut hypoxemia secondary to blood shunting from the viscera to exercising muscles causing an overwhelming efflux of endotoxins in circulation (Pyne et al., 2014) and systemic inflammatory responses. Immune stress may further potentiate this with military personnel and aid workers, through sleep deprivation (Day and Grimshaw, 2005; Stacey et al., 2015; Moore et al., 2016), psychological stress (Clow and Hucklebridge, 2001) or repeated (particularly gram negative) infection (Wale, 1989; Grainge and Heber, 2005; Howe and Boden, 2007; Cox et al., 2016a) causing a heightened inflammatory state. Attempts have been made to modulate exercise-induced gastrointestinal

permeability with nutritional countermeasures. These include carbohydrates which as well as improving exercise performance (Pöchmüller et al., 2016), immune function (Bermon et al., 2017) and recovery (McCartney et al., 2018) may have a beneficial effect on GI barrier integrity (Gentilcore et al., 2009; David et al., 2014; Edinburgh et al., 2018), although this effect has not been reproducible (Moncada-Jiménez et al., 2009; Sessions et al., 2016; Costa et al., 2017a; Trommelen et al., 2017). Glutamine, an energy substrate of GI enterocytes (Kim and Kim, 2017) is thought to be protective if consumed in higher doses prior to exercise (Zuhl et al., 2014, 2015; Pugh et al., 2017). Bovine Colostrum has been shown to reduce surrogate markers of bacterial translocation and gut permeability following short duration high intensity running (Antonio et al., 2001) but the effects appear attenuated in more demanding exercise protocols in the heat (Morrison et al., 2014; McKenna et al., 2017; March et al., 2019). Whilst multi-strain probiotic supplementation has been shown to reduce endotoxin concentrations in some studies (Shing et al., 2014; Roberts et al., 2016), but not others (Pugh et al., 2019), single strain probiotics may increase concentrations of endotoxin following running in the heat (Gill et al., 2016). Zinc-Carnosine whilst commonly used to treat gastric ulcers (Matsukura and Tanaka, 2000), may also be beneficial in improving gut barrier permiability (Davison et al., 2016). Studies have also assessed the effect of polyphenols such as quercetin during isothermic heat acclimation regimes (Kuennen et al., 2011) or curcumin, a constituent of turmeric (Szymanski et al., 2018), but the effects of these appear negligible (Sergent et al., 2010). Other studied agents include the NO precursors L-arginine (Costa et al., 2014) and L-citrulline (Van Wijck et al., 2014) which also lack objective evidence. Overall there is insufficient reproducibility to recommend the use of large scale nutritional supplementation to prevent heat stroke either during, or as an adjunct, to heat acclimation at present. From a nutrition perspective in terms of heat acclimatization, the military has previously had high salt intakes in their diet (Bannister, 1959) which has the beneficial effect of improving orthostatic tolerance (El-Sayed and Hainsworth, 1996; Cooper and Hainsworth, 2002; Claydon and Hainsworth, 2004). The effect of salt intake on the physiological adaptations following heat acclimation has been studied with the potential of accelerating plasma volume (Armstrong et al., 1993) expansion whilst decreasing heat rate and rectal temperature (Armstrong et al., 1987).

OPTIMIZING THE RISK-PERFORMANCE PARADIGM – THE FUTURE OF MILITARY HEAT ACCLIMATIZATION

Organizing deployments to allow time for military personnel to acclimate not only improves heat tolerance but can pay dividends in terms of improved physical and cognitive performance (Muza et al., 1988; Chen et al., 1997; Cheuvront et al., 2008; Jovanović et al., 2014). However, mission specific requirements may not allow time for these physiological responses and swifter methods, such as those outlined by Charlot et al. (2017) remain attractive. Whilst, as outlined above, maintaining a high level of physical fitness improves physiological responses to exercise in the heat (Armstrong and Maresh, 1991) by 'priming' leading to more rapid acclimation (Pandolf et al., 1977; Garrett et al., 2011) trained individuals may have a lower adaptation response due to higher levels of background adaption secondary to the physical training (Convertino, 1991; Taylor, 2000; Garrett et al., 2011). In contrast whilst slower adaptation is seen in individuals with lower fitness levels the physiological manifestations are reportedly greater (Shvartz et al., 1977b; Garrett et al., 2011). Regardless the untrained, in the military context, appear to be more susceptible to heat illness (Stacey et al., 2016) but whether lower levels of physical fitness confer a risk of heat stroke, with fitter individuals being protected, remains challenging to study due to the numerous confounders.

Commonly an objective assessment of heat tolerance is used by assessing physiological responses to exercise in the heat (Bergeron et al., 2012). In athletes (Karlsen et al., 2015; Racinais et al., 2015) and military personnel (Taylor, 2014) this is commonly performed using a standardized heat tolerance test (HTT). The HTT was developed by the Israeli Defense Force (Epstein, 1990) as a means to occupationally manage their servicemen and women and is commonly used by militaries to differentiate between a temporary and permanent state of heat susceptibility (Moran et al., 2007). The HTT monitors heart rate and core temperature whilst walking for 120 min at 5 km/h at 2% elevation at 40°C and 40% relative humidity. Heat intolerance is confirmed with a core temperature $\geq$ 38.5°C or a heart rate $\geq$145 for >3 min (Moran et al., 2007). Whilst recommended by the American College of Sports and Medicine (O'Connor et al., 2013) the HTT, for the military and a multitude of other occupational groups, is a practically and economically impossible way of assessing the presence of heat adaptation. Other studies have attempted to find surrogate markers including early reductions in heart rate and cortisol in the short term followed by diminished excitability by heart rate variability and nephrine measures with respect to LTHA (Stacey et al., 2018b). Elevated copeptin, a surrogate for arginine vasopressin secretion, measured before and after heat stress exposure in military subjects correlated with a core temperature rise greater than 38°C in comparison with subjects where the core temperature rise was less than 38°C (Stacey et al., 2018a). Whilst copeptin may be a surrogate for integrated physiological strain during work in a field environment it is unknown how this is influenced by heat adaptation. The modification of activity in the heat arguably could be better matched to physiological data particularly in the military where optimal performance is not only mission critical but conceivably could mean the difference between life and death. These potentially could include heat acclimatization status which could be combined with fitness, hydration status, core temperature, biomarkers, clothing and equipment and even potentially cumulative heat stress (Hosokawa et al., 2019). With wearable technologies, perhaps in combination with a heart rate target approach (Delves and Buller, 2017;

Friedl, 2018), this could prove a safe, effected and targeted method to heat acclimatize military or humanitarian agencies in the future. As with the other occupations and sporting pursuits, a definitive marker of heat adaptation status is one of the Holy Grails of military acclimatization research. As individual responses to heat acclimation are variable (Racinais et al., 2012) reliable markers of adaptation could not only provide assurance but reduce heat illness and lead to more personalized approaches to adaptation (Bergeron et al., 2012; Racinais et al., 2015).

Several novel, often technological, solutions, largely through the reduction of exogenous or endogenous heat stress, have been developed for the purposes of preventing heat illness and potentially improving athletic performance (Walters et al., 2017). Such advances in cooling either improve on the heat adaptive state or bridge the gap prior to full heat acclimation. Skin cooling maintains orthostatic tolerance in heated subjects and reduces heat syncope (Wilson et al., 2002, 2011). As heat loss through sweat evaporation is the only way humans can dissipate heat from the skin these mechanisms commonly aim to enhance or bridge acclimative changes of increased sweat production and evaporation (Candas et al., 1979a,b). The main determinant of evaporation is the vapor pressure gradient between the saturated skin surface and the ambient air which is influenced by skin wettedness, air velocity, and body surface area (Cramer and Jay, 2017). Cooling mechanisms therefore largely exert effects by evaporative cooling, phase change materials, compressed air systems and/or thermoelectric systems (Jovanović et al., 2014). How these systems interact or attenuate a heat adaptive state is unknown. The role of novel cooling systems in protecting personnel from thermal stress would appear more prescient when a rapid robust response is required but there is minimal time to acclimatize, e.g., fighting wildfires or a CBRN scenario. Studies using varying methods often show a reduction in temperature (Cheuvront et al., 2008; Hadid et al., 2008), improved tolerance (Walters et al., 2017) and lower cardiovascular parameters (Muza et al., 1988; Chen et al., 1997). All studies are in small cohorts and none have shown a reduction in heat illness which, along with cost effective considerations, would be required for widespread use. Novel cooling solutions, such as carotid artery cooling (O'Hara et al., 2008), have been studied for the purposes of the acute management of heat stroke but have yet to be studied extensively as a preventative tool. Cooling may be best directed toward the face where studies which attempted to uncouple the psychological from physiological effects of heat acclimatization suggested that the reduction in perceived strain of exertion and thermal stress following acclimatization may be derived through greater reductions in facial temperature (Malgoyre et al., 2018). Whilst there has been significant research in this area, due to the obvious potential advantages, the efficacy of cooling devices, such as cooling vests, are reportedly, and somewhat disappointingly, only as effective as drinking ice cold water (Sawka et al., 1992; O'Hara et al., 2008). In a meta-analysis (Daanen et al., 2018), adaptations in core temperature were more sustained when daily heat exposure duration was increased but heat exposure intensity was decreased. For improvements in sweat rate longer heat acclimation periods are correlated. In contrast core temperature reduction and attenuation appears to be related to higher heat exposure intensity. Importantly, heat re-acclimation appears to induce changes at a faster rate than initial heat acclimation (Daanen et al., 2018). Whether a state of heat acclimation can be maintained for prolonged periods despite repeated loss of heat exposure through rapid heat re-acclimation protocols requires further study in the military context. Potential novel methods include the use of hot baths or saunas (Stanley et al., 2015; Casadio et al., 2017; Mee et al., 2018).

CONCLUSION

Heat stress remains a persistent problem for militaries and is likely, with climate change, to represent a challenge in years to come. Militaries are drawn from the populations they serve. The diverse constituents of militaries, coupled with the individual responses to heat acclimation and the varied environments in which militaries operate make generalizations difficult. Thermal stress in modern military training and operational deployments must be reviewed in this context. Heat adaptation remains a mainstay of augmenting physical and mental performance in the military. The ultimate singular goal of a military is to succeed in mission objectives whether in training, contingency operations, or warfighting. The consequences of not understanding what attenuates heat adaptation can pose an existential threat to militaries and their personnel regardless of the threat of heat illness. Heat adaptation in the military should not be reviewed in isolation but in the context of warfighting including wounding, the wearing of personal protective equipment, dehydration and febrile illnesses. Further work should ascertain the mechanisms by which physical fitness and acclimatization may protect against heat illness, over and above the heat syncope mitigated by plasma volume expansion. In the military this is challenging due to the obvious confounder that fitter individuals are more likely to be part of DCCC units and at increased risk of heat illness. Methods of being able to rapidly and safely heat adapt large numbers of varied military personnel, easily measure the adaptive changes and maintain, even manipulate, this adaptation remains a topic of ongoing research. The military remains a special population for thermal research.

AUTHOR CONTRIBUTIONS

IP and MS researched, drafted, and edited the manuscript. DW provided editing and overarching review of content.

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Could Heat Therapy Be an Effective Treatment for Alzheimer's and Parkinson's Diseases? A Narrative Review

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Neurodegenerative diseases involve the progressive deterioration of structures within the central nervous system responsible for motor control, cognition, and autonomic function. Alzheimer's disease and Parkinson's disease are among the most common neurodegenerative disease and have an increasing prevalence over the age of 50. Central in the pathophysiology of these neurodegenerative diseases is the loss of protein homeostasis, resulting in misfolding and aggregation of damaged proteins. An element of the protein homeostasis network that prevents the dysregulation associated with neurodegeneration is the role of molecular chaperones. Heat shock proteins (HSPs) are chaperones that regulate the aggregation and disaggregation of proteins in intracellular and extracellular spaces, and evidence supports their protective effect against protein aggregation common to neurodegenerative diseases. Consequently, upregulation of HSPs, such as HSP70, may be a target for therapeutic intervention for protection against neurodegeneration. A novel therapeutic intervention to increase the expression of HSP may be found in heat therapy and/or heat acclimation. In healthy populations, these interventions have been shown to increase HSP expression. Elevated HSP may have central therapeutic effects, preventing or reducing the toxicity of protein aggregation, and/or peripherally by enhancing neuromuscular function. Broader physiological responses to heat therapy have also been identified and include improvements in muscle function, cerebral blood flow, and markers of metabolic health. These outcomes may also have a significant benefit for people with neurodegenerative disease. While there is limited research into body warming in patient populations, regular passive heating (sauna bathing) has been associated with a reduced risk of developing neurodegenerative disease. Therefore, the emerging evidence is compelling and warrants further investigation of the potential benefits of heat acclimation and passive heat therapy for sufferers of neurodegenerative diseases.

Keywords: neurodegenerative disease, heat shock protein, passive heating, thermal therapy, body warming, alpha-synuclein

INTRODUCTION

Humans are homeothermic and as such regulate their core body temperature within a narrow range. Perturbations to this homeostasis, induced by external environmental thermal stress or internally generated metabolic heat, produces both autonomic and behavioral responses designed to elicit a return of core body temperature toward thermal balance (Schlader and Vargas, 2019). While in an acute sense this stress response is a defense mechanism, regularly challenging the thermal equilibrium via active or passive thermal stress results in positive physiological and perceptual adaptations (Tyler et al., 2016). Recent research has shown positive therapeutic effects of passive heating for people with peripheral arterial disease (Neff et al., 2016; Akerman et al., 2019), chronic heart failure (Kihara et al., 2002; Ohori et al., 2012), diabetes (Hooper, 1999), and depression (Janssen et al., 2016). Passive heating also improves a range of health markers, including cardiovascular health indices, such as vascular function, blood pressure, and arterial stiffness (Brunt et al., 2016a,b), as well as metabolic health and glycemic control (Janssen et al., 2016; Kimball et al., 2018; Ely et al., 2019; Maley et al., 2019). Several mechanistic pathways may underpin these adaptations, including improved cellular respiration (Hafen et al., 2018), circulating factors (Brunt et al., 2019), and vascular shear stress (Tinken et al., 2009; Thomas et al., 2016). The upregulation of heat shock proteins (HSPs) as a result of acute and/or chronic (repeated) exposure to passive heating is also an adaptive outcome, which may provide a specific mechanistic pathway for improving health and function within the body (Faulkner et al., 2017; Brunt et al., 2018).

Recent reviews have identified the upregulation of HSPs as therapeutic targets for the treatment of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease (Carman et al., 2013; Kalmar et al., 2014; Schapira et al., 2014; Ciechanover and Kwon, 2017; Webster et al., 2017; Klaips et al., 2018). Neurodegenerative diseases are characterized by the progressive deterioration of structures within the central nervous system responsible for motor control, cognition, and autonomic function. Alzheimer's and Parkinson's diseases are among the most common neurodegenerative diseases and have an increasing prevalence over the age of 50 (Pringsheim et al., 2014). Loss of protein homeostasis, due to protein mis-folding and aggregation of damaged proteins, is a hallmark of both Alzheimer's and Parkinson's diseases (Labbadia and Morimoto, 2015). HSPs function as chaperones to ensure appropriate cell function with distinct roles in the unfolded protein response, recognizing misfolded or mis-localized proteins that may be subsequently degraded by the proteasome, and are a key component of chaperone-mediated autophagy (Adachi et al., 2009; Stetler et al., 2010; Leak, 2014; Zarouchlioti et al., 2018). For their role in regulating protein homeostasis, HSP expression has been proposed as a therapeutic target for the treatment of these neurodegenerative diseases (Carman et al., 2013; Kalmar et al., 2014; Schapira et al., 2014; Ciechanover and Kwon, 2017; Webster et al., 2017; Klaips et al., 2018).

As physical and cognitive ability decline in Alzheimer's and Parkinson's diseases, passive heat therapy may yield an achievable alternative to the presently recommended exercise interventions in this population. Intriguingly, the incidence of Alzheimer's disease has recently been shown to be reduced in people who undertook moderate to frequent sauna bathing (Laukkanen et al., 2017). While the current evidence for heat therapy in neurodegenerative disease is associative and the mechanisms by which improved health outcomes are achieved have yet to be elucidated, the potential of passive heating in this population remains an alluring therapeutic option.

This review will examine pathophysiological determinants of common neurodegenerative disease, examine the evidence of an elevated HSP expression as a potential therapeutic intervention in common neurodegenerative diseases, and describe the role heat acclimation and passive heat therapy have in inducing HSP expression. In addition, central and peripheral adaptations to body warming in healthy adults, including improved muscular function, cerebral blood flow, and metabolic health, will be considered with their potential influence on neurodegenerative disease outcomes. Finally, considerations for undertaking heat acclimation and/or passive heating interventions in people with neurodegenerative diseases will be addressed.

NEURODEGENERATIVE DISEASES

Epidemiology and Pathophysiology

Central in the pathophysiology of neurodegenerative diseases is the loss of protein homeostasis and the progressive loss of selective neurons. Protein homeostasis involves a complex system of protein synthesis, folding, disaggregation, and degradation that ensures the correct function of the human body and particularly the central nervous system (Klaips et al., 2018). Loss of protein homeostasis, due to protein mis-folding and aggregation of damaged proteins, is a hallmark of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Labbadia and Morimoto, 2015). Alzheimer's and Parkinson's are the two most common degenerative neurological conditions and are more prevalent with advancing age. Both of these neurodegenerative diseases are progressive with pathological features demonstrating topographic distribution. The progressive loss of selective neurons includes amyloidosis, tauopathies, alpha-synucleinopathies, and proteinopathies, all of which have their own characteristic histopathological imaging features, as well as clinical symptomology. The diseases are incurable and result in long-term cognitive, psychological, motor, and non-motor impairments that have a profound impact on functional mobility, psychological well-being, independent living, and quality of life.

Alzheimer's Disease

Alzheimer's disease is the most prevalent neurodegenerative disease and is the most common form of dementia (Thies and Bleiler, 2012), which affects 40–50 million people worldwide (Prince et al., 2013; Nichols et al., 2019). Early stages of Alzheimer's presents with mild cognitive impairment involving memory loss and progresses with deficits in attention, language, and visuospatial abilities (Galton et al., 2000;

Wattmo et al., 2016). Social withdrawal accompanies disease progression, as symptoms include a reduced capacity to perform activates of daily living, impaired executive function and judgment, along with disorientation (Wattmo et al., 2016). These outcomes have a significant impact on independence, quality of life, and years of life with a disability (Martyr et al., 2019). Furthermore, the economic cost of dementia is $968 billion globally (Xu et al., 2017). These costs are born by individuals and their caregivers, social health services, as well as public and private health care providers (Castro et al., 2010). Due to the aging population, the prevalence and impact of Alzheimer's disease are anticipated to increase in the future (Prince et al., 2013; Nichols et al., 2019).

Neurodegenerative diseases such as Alzheimer's are marked by a loss of cellular protein homeostasis (Ciechanover and Kwon, 2017; Klaips et al., 2018). The pathophysiology of Alzheimer's is evidenced by intracellular and extracellular amyloid-β plaques as well as neurofibrillary tangles of hyperphosphorylated tau (Montine et al., 2012; Ciechanover and Kwon, 2017). Neurodegeneration occurs as a result of the accumulation of tau proteins and atrophy of cerebral cortices. Amyloid deposits occur in the neocortex and hippocampus (Phases 1 and 2), the striatum (Phase 3), the brainstem (Phase 4), and the cerebellum (Phase 5) (Montine et al., 2012). In concert with abnormal protein accumulation, the pathogenesis of Alzheimer's disease may also involve vascular impairments leading to chronic cerebral hypoperfusion (de la Torre, 2004; Akinyemi et al., 2013; Sweeney et al., 2018). To combat these pathophysiological progressions, therapeutic interventions to improve protein quality control and regulation or improve vascular health and function have been recommended (Akinyemi et al., 2013; Ciechanover and Kwon, 2017).

Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disease, after Alzheimer's disease. For Parkinson's disease, the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta results in severe motor (e.g., tremor, rigidity, bradykinesia, postural instability) and non-motor symptoms (e.g., sleep disturbances, apathy, cognitive dysfunction, anxiety, depression) (Politis et al., 2010; Asahina et al., 2013). Both motor and non-motor impairments contribute to reduced physical activity and consequently decreased fitness in people with Parkinson's disease (Speelman et al., 2011). This decreased fitness exacerbates both pre-existing and disease-specific conditions including cardiovascular disease, muscle weakness, postural instability, osteoporosis, sleep disruption, impaired cognitive function, depression and constipation (Speelman et al., 2011). Estimates suggest that Parkinson's disease affects between 5 and 7 million people worldwide (Dorsey et al., 2018). The annual economic cost of Parkinson's disease is estimated to be £3.3 billion in the United Kingdom (Findley, 2007), $23 billion in the United States (Findley, 2007), $6.3 billion in Australia (Access Economics (Firm) and Parkinson's Australia, 2007).

The degradation in neural function in Parkinson's disease is evidenced by degeneration of substantia nigral neurons, accumulation of alpha-synuclein and Lewy bodies, cortical atrophy and alteration in neural oscillatory activity between basal ganglia, thalamus, cortex, and brainstem areas. It has been proposed by Braak et al. (2003) that in Parkinson's disease, the topographic progress of neurodegeneration, follows patterns of alpha-synuclein aggregation expressed in Lewy neurites and Lewy bodies. This occurs first in the medulla and olfactory bulb, then progressively dorsally through the brainstem, mediobasal forebrain, limbic structures, higher-order sensory association and prefrontal areas, and finally to primary sensory and motor areas (Braak et al., 2003). A central tenet of this proposal is that the initial disease onset is a result of inflammatory processes in the enteric system that result in progression to the central nervous system via the vagal nerve (Breen et al., 2019). Additionally, deterioration in the vasculature of the brain resulting in abnormal cerebral perfusion patterns has been identified in people with Parkinson's disease, suggesting a role of cerebral blood flow in the pathophysiology of the disease (Melzer et al., 2011; Syrimi et al., 2017; Sweeney et al., 2018).

Heat Shock Proteins as a Therapeutic Target

Recent reviews have clearly identified the upregulation of HSPs as thermally activated therapeutic targets for the treatment of neurodegenerative diseases including Parkinson's and Alzheimer's (Carman et al., 2013; Kalmar et al., 2014; Schapira et al., 2014; Ciechanover and Kwon, 2017; Webster et al., 2017; Klaips et al., 2018). HSPs are a collective family of proteins, suffixed by their molecular weight (in kilodaltons; kDa), which are present in both constitutively expressed, and inducible isoforms across several intracellular tissue sites and in extracellular fluid following stress (Kampinga et al., 2009). Relative to increased intracellular HSP content (a necessary component for protective cellular adaptation), the presence of extracellular changes in HSP concentration reflects a less pertinent (in the context of adaptation) transient stress response which acts as an acute signaling response. The 70 kDa (HSPA) and 90 kDa (HSPC) family of HSPs, hereafter referred to as HSP70 and HSP90, are generally the most widely studied responders to thermal stressors and are likely of most relevance within the field of heat therapy and heat adaptation for neurodegenerative disease (Luo et al., 2010; Fontaine et al., 2016; Lackie et al., 2017). HSP70 and HSP90 function as chaperones to ensure appropriate cell function and have distinct roles in the unfolded protein response, e.g., recognizing misfolded or mis-localized proteins that may be subsequently degraded by the proteasome, and are a key component of chaperone-mediated autophagy (Adachi et al., 2009; Stetler et al., 2010; Leak, 2014; Kampinga and Bergink, 2016; Zarouchlioti et al., 2018). It is outside of the focus of this review to describe each of these roles, with the reader directed elsewhere to contextualize these actions (Adachi et al., 2009; Stetler et al., 2010; Leak, 2014; Kampinga and Bergink, 2016; Zarouchlioti et al., 2018).

As therapeutic targets, HSP70 and HSP90 may be considered to have a direct and indirect role in neurodegenerative diseases. Direct roles for HSPs on the nervous system arise

from the aforementioned notion that aggregation of misfolded proteins is characteristic of neurodegenerative diseases, including Parkinson's, Alzheimer's, and Huntington's (Stetler et al., 2010). In Parkinson's, HSP70 is reported as being of decreased gene expression (Mandel et al., 2005), while during proteomic profiling, reduced phosphorylation of HSP90 is also reported (Kulathingal et al., 2009). Pharmacological and animal models utilizing HSP expression (elevated HSP70 and reduced HSP90) have reduced the aggregation and toxicity of alpha-synuclein in Parkinson's disease (Auluck et al., 2002; Klucken et al., 2004; Danzer et al., 2011; Gao et al., 2015). In Alzheimer's, HSP70 may suppress the proteolysis of amyloid precursor protein (Hoshino et al., 2007) and in addition to HSP70, HSP90, and small HSPs reduce the formation of A-beta fibrils (Kudva et al., 1997; Lee et al., 2005; Wilhelmus et al., 2006a) and A-beta toxicity (Wilhelmus et al., 2006b) which subsequently form amyloid plaques. Tauopathy occurrence in Alzheimer's may also be positively impacted by HSP changes with HSP70 (Jinwal et al., 2009) and HSP90 (Evans et al., 2006; Luo et al., 2007). Further, HSPs have been found to regulate huntingtin via reduced cell aggregation in Huntington's disease (Muchowski et al., 2000; Sittler et al., 2001), and slows the muscle denervation of amyotrophic lateral sclerosis (Motor Neuron Disease) (Kieran et al., 2004; Kalmar et al., 2008, 2012).

Much of the literature describing these responses involve complex and isolated tissue/cell models to understand how HSP manipulation impacts upon neurodegenerative disease factors, thus direct application for humans remains unknown. However, with mechanistic support for the role of HSP augmentation to improve disease states, the application of heat therapy and/or heat adaptation in this context warrants further investigation.

RESPONSES TO ACTIVE (EXERCISE HEAT ACCLIMATION) AND PASSIVE HEAT THERAPY IN HEALTHY ADULTS

Physical activity and exercise have long been identified as mechanisms of inducing physiological stressors and subsequent positive adaptations in healthy (Tyler et al., 2016) and chronic disease (Hoffmann et al., 2016) populations. Unfortunately, those with increasing disease severity or diseases that challenge their motor control capabilities may be physically incapable of performing such beneficial exercise. Heat therapy has recently been targeted as a potential vehicle to evoke these positive thermal-induced adaptations in those precluded from undertaking exercise. Experimental investigations, large cohort surveys and reviews have expressed the potential for passive heating to improve physical and mental health in patients with cardiovascular disease (Brunt et al., 2016a,b; Maeda et al., 2018), diabetes (Kimball et al., 2018; Maley et al., 2019), peripheral arterial disease (Akerman et al., 2019), and depression (Janssen et al., 2016).

While there is a myriad of beneficial physiological and molecular effects of active and passive heating, this review will primarily focus specifically on the outcome of HSP expression, for its potential to influence proteostasis in neurodegenerative disease. For active and passive heating to be effective in increasing HSP expression, the minimum exposure requirements to elicit a desirable response in HSPs, from both acute and chronic (repeated) exposure, needs to be identified.

Acute Effects of Body Warming on Heat Shock Proteins

Transcription of HSP mRNA, an essential step before protein translation, is primarily regulated by Heat shock factor protein 1 (HSF-1) as part of the Heat Shock Response (Kregel, 2002). HSF-1 activation involves a complex series of regulatory events, including nuclear localization, oligomerization and acquisition of HSE–DNA binding, ultimately resulting in the transcription of HSP mRNA (Sarge et al., 1993) in response to the thermal and physiological challenge (McClung et al., 2008; Maloyan et al., 2011). Sufficient mRNA transcription then leads to increased protein within the stressed cell.

Precise parameters for intracellular increases, and thus cellular adaptation, have been less clearly defined. For example, mean core body temperature may not be the sole marker of an increase, rather the rate of change in core body temperature may be of greater importance to signal HSF-1 to HSP70 pathways. In the more common exercise-heat stress model, a recent analysis concluded that when transcription of the related HSP70 and HSP90α mRNA is important, protocols should rapidly induce large, prolonged changes in core body temperature (Gibson et al., 2016). This notion was supported by evidence that, when analyzed collectively, significant predictors of the post-exercise change in HSP70 and HSP90α mRNA were the change in mean and peak core body temperature, and the duration core body temperature was $\geq$38.5°C (Gibson et al., 2016). It should be acknowledged that these data describe responses to exercise-heat stress, an intervention that is likely to be challenging to implement in clinical populations. Accordingly, passive heating via body warming may prove to be a more efficacious intervention.

The HSP response to localized or whole body warming has also been investigated. In passive heating models, increases in HSP70 and HSP90 mRNA have been evidenced as peaking 30 min following 90 min of local heating to either the thigh or the whole leg of healthy human volunteers (Kuhlenhoelter et al., 2016). Regrettably, no intramuscular temperature data is available from this experiment to assist with identifying minimum exposure requirement. This increase following resting, local heat stress which does not alter core body temperature offers mechanistic insight as elevations in blood flow and shear stress provide a non-core body temperature dependent HSP response that parallels transcription of angiogenic markers (Kuhlenhoelter et al., 2016). Not all experimental work has observed changes in HSP following passive heating. Leg immersion in hot water at 45°C for 60 min, eliciting an increased intramuscular temperature of >39°C, did not affect muscle HSPs (HSP70, HSC70, HSP60, HSP27, alphaB-crystallin) in healthy young humans (Morton et al., 2007). It should be noted that this null-observation came 48 h following, rather than immediately after heating, which may provide a rationale

for the response. These data share commonality with responses observed elsewhere in relation to HSP70 and HSP27 stasis 24 h following ∼80 min of heating at ∼49°C (Vardiman et al., 2013) to increase intramuscular temperature also to >39°C, suggesting that the dose of heat therapy may be an important driver of HSP response or that the inconsistent timing of differential tissue sampling are experimental artifacts impacting current understanding.

Examination of extracellular changes in HSP70 during acute exercise-heat stress in humans has identified that the endogenous requirement for extracellular HSP70 release (at the cessation of exercise) may be a core body temperature mean of >38.5°C (peak of 39.2°C) for 56 min, alongside moderate exercising intensities (Gibson et al., 2014). Although changes may occur more rapidly (within 27 min) if exercise intensities are higher (Périard et al., 2012). Both the change in and final core body temperature attained are relevant to extracellular HSP70 release (Périard et al., 2012) and indicate achieving substantial elevations in thermal parameters is important when administering exercise-heat exposures to increase thermotolerance in whole-body models.

Chronic Effects of Body Warming on Heat Shock Proteins

The HSP responses to exercise-heat acclimation have been reviewed previously, with an acknowledgment that the intervention is an effective means to augment cellular thermotolerance, which may subsequently protect vital organs from deleterious effects of heat stress in humans (Amorim et al., 2015). An internal temperature threshold for intracellular HSP70 induction may exist, though it is also possible that this response occurs once a certain variation of internal, whole-body temperature is reached alongside additional stressors (Magalhães et al., 2010). For example, during a 10-day heat acclimation period, Magalhães et al. (2010) demonstrated the largest changes in post-exercise intracellular HSP70 when a core body temperature >39.0°C was achieved. In contrast, Yamada et al. (2007) and Hom et al. (2012) reported no change in HSP70 in response to a lower core body temperature (mean maximum of ∼38.5°C) following 10 days of heat acclimation. HSP70 and HSP90 mRNA transcription occur at a series of core body temperature thresholds during 90 min of exercise-heat stress (mean 37.6–38.2°C; peak 38.1–39.1°C) (Gibson et al., 2015a,b) therefore the dose of heat stress to elicit translation may be greater than that required to elicit transcription.

In human whole-body passive heating models, such as hot water immersion of >60 min whereby core body temperature is increased, higher extracellular HSP70 concentration (Faulkner et al., 2017), and intracellular HSP70 changes (Oehler et al., 2001), have been reported. The beneficial response once again is not unanimous, with others reporting chronic change (following 2 weeks of repeated therapy) in intracellular HSP70 after 45–60 min of passive heat therapy (Hoekstra et al., 2018). In a similar manner to that described for local heating, the dose of stress may be important given the lack of change in studies using 60 min heating (Hoekstra et al., 2018), in comparison to those who do report an acute increase in intracellular HSP70 following 120 min heating in 39°C water (Oehler et al., 2001).

Intracellular HSP70 and HSP90 levels in peripheral blood mononuclear cells (PBMC) are increased after 6–10 days of long term exercise-heat acclimation (Yamada et al., 2007; McClung et al., 2008). The two larger HSPs appear to be related with regards to exercise-heat stress changes, as an HSP70 increase of ∼21% was correlated with HSP90 increases of ∼18% (McClung et al., 2008). *In vitro* analysis of PBMC obtained from 10-day exercise-heat acclimated individuals exhibit greater blunting of the HSP response to heat shock of 43°C for 60 min (compared to unacclimated). This blunted pre-post response is indicative of increased thermotolerance and/or cellular protection from stress, likely due to increased basal intracellular HSP content and appears directly related to the degree of physiological heat acclimation (lower core temperature), thus the adapted individual/cell experiences lesser relative stress at the same absolute temperature (McClung et al., 2008).

The notion of individual differences in the responses to local heat therapy has been highlighted in a study observing that 24 h following 40 min of heat treatment (diathermy followed by heat packs), female subjects significantly increased HSP70 (+58%) and phosphorylation of HSP27 (+100%) content compared to the untreated leg (Touchberry et al., 2007). In comparison, male subjects had non-significant increases in HSP70 (+35%) and HSP27 phosphorylation (+32%) within skeletal muscle (Touchberry et al., 2007). These sex-specific responses are intriguing and warrant future investigation given no differences in Hsp70 mRNA have been reported during isothermic heat acclimation (Mee et al., 2016) and were not reported in the mixed-sex cohort undertaking passive heating described above (Kuhlenhoelter et al., 2016).

Central and Peripheral Effects of Body Warming

The administration of heat therapy and heat acclimation may provide additional benefits in the context of neurodegenerative diseases in relation to skeletal muscle function, cerebral blood flow, and metabolic health. Adverse reductions in strength and lean body mass are symptoms of neurodegenerative diseases, including Alzheimer's disease (Burns et al., 2010; Buchman and Bennett, 2011), Parkinson's disease (Berardelli et al., 2001; Petroni et al., 2003; Cano-de-la-Cuerda et al., 2010) and Amyotrophic Lateral Sclerosis (Gubbay et al., 1985; Munsat et al., 1988; Kiernan et al., 2011). While various mechanisms are at play, muscle atrophy and decreased strength likely owe to symptom-influenced reductions in physical activity, along with central and peripheral nervous system changes that limit muscle activation (Hass et al., 2007). Furthermore, reduced cerebral blood flow and poor metabolic health profiles may also be related to disease progression (de la Torre, 2004; Akinyemi et al., 2013; Bharadwaj et al., 2017; Sweeney et al., 2018). As there is a potential benefit to be gained, the below reviews the current

developing understanding of the acute and chronic effects of elevated temperature on skeletal muscle function, cerebral blood flow, and markers of metabolic health.

Skeletal Muscle Function

Increases in skeletal muscle temperature have long been accepted to improve acute muscle force, power and contractility (Bergh and Ekblom, 1979; Davies and Young, 1983; Bennett, 1984). Contrastingly, the inverse relationship between high core body temperature and muscle torque, muscle recruitment patterns, and voluntary activation is also documented (Morrison et al., 2004; Todd et al., 2005; Thomas et al., 2006). Importantly, however, these outcomes are most often viewed with a short-term lens, with limited understanding of the effects of time or repeated heat exposures on skeletal muscle in humans (Brazaitis and Skurvydas, 2010; Goto et al., 2011; Racinais et al., 2017).

Passive heating has been reported in experimental designs examining effects on exercise-induced muscle damage (Nosaka et al., 2007; Touchberry et al., 2012), recovery from muscle injury (Kojima et al., 2007; Oishi et al., 2009; Takeuchi et al., 2014) and immobilization (Selsby and Dodd, 2005; Senf et al., 2008; Dodd et al., 2009), and muscle hypertrophy (Uehara et al., 2004; Ohno et al., 2010) in animal models. The rationale for passive heat application relates to the altered cascade of inflammation and HSP expression that interact with mitochondrial biogenesis and muscle growth (Yoshihara et al., 2013; McGorm et al., 2018). Increases in wet muscle mass and protein content in rat soleus muscle have been described 7 days after a 60 min exposure to a 41–42°C heat chamber (Uehara et al., 2004; Ohno et al., 2010). Further, Kodesh and Horowitz (2010) observed higher muscle mass/body weight ratios in rats following 30 days of acclimation to 34°C environmental heat compared to a 24°C control. Similarly, in healthy men, Goto et al. (2011) saw an increased cross-sectional area of fibers in the vastus lateralis (8.3%) using a steam-generating sheet applied to the quadriceps muscle for 8 h·day^{-1} and 4 days·week^{-1} across a 10-week intervention. Collectively, it might be concluded that passive heating could support cell proliferation and facilitate muscle hypertrophy (Naito et al., 2000; Goto et al., 2004; Uehara et al., 2004). Such outcomes would be particularly beneficial to those experiencing neurodegenerative disease, particularly as passive heating appears also to attenuate human skeletal muscle atrophy (Hafen et al., 2019).

Most pertinent from a translational perspective, improved strength has been demonstrated to couple the increased skeletal muscle growth after passive heating (Goto et al., 2011; Racinais et al., 2017). Higher isometric knee extensor torque (5.8%) was achieved after 10 weeks of heat stress, which the authors explained as potentially relating to the increase of myonuclear number (Goto et al., 2011). Goto et al. (2011) also found a 4% increase in knee extensor strength in the non-heated leg. While the contralateral effects of unilateral resistance training are acknowledged (Lee and Carroll, 2007; Frazer et al., 2018), this phenomenon may imply that there are central nervous system effects of chronic passive heating. A potential role of circulating factors has also been proposed (Hendy and Lamon, 2017). The site(s) of possible neural adaptation explaining cross-education remain unclear. However, it is conceivable that adaptations could occur at the spinal and/or cortical level considering the noted decline in descending motor drive during acute episodes of hyperthermia (Todd et al., 2005; Périard et al., 2012; Ross et al., 2012). It seems that higher body temperature acutely impairs somatosensory processing (Nakata et al., 2015), though how adaptation to passive heating might affect neural activity in healthy or diseased states in thermoneutral conditions remains to be elucidated. Regardless, adaptation to passive heat therapy is promising, particularly in a rehabilitation setting and for those with neurodegenerative diseases, as acute stress may increase motor cortex excitability and augment motor skill acquisition (Littmann and Shields, 2016).

Cerebral Blood Flow

Another avenue by which passive heating may have a therapeutic effect is through improved cerebral blood flow. Reduced cerebral blood flow and dysfunction in the blood-brain barrier have been identified in neurodegenerative diseases, including Alzheimer's and Parkinson's (de la Torre, 2004; Akinyemi et al., 2013; Sweeney et al., 2018). Both motor and cognitive impairments have been associated with poor perfusion in several brain regions in Parkinson's disease (Melzer et al., 2011). Similarly, overall and regional cerebral blood flow reductions have been associated with cognitive decline in mild cognitive impairment and Alzheimer's disease (Leeuwis et al., 2017). For these reasons, the vasculature supplying blood to and across the brain are also relevant targets to examine the beneficial adaptations of passive heat therapy for neurodegenerative diseases.

Several vascular adaptations attributable to heat therapy have been reported. These have included improved flow-mediated dilation (Brunt et al., 2016a), increased pulse wave velocity (an index of arterial stiffness) (Brunt et al., 2016a), reduced carotid intima thickness (Brunt et al., 2016a) improved capillarization (Hesketh et al., 2019) and subsequently enhanced systemic blood pressure profiles (Brunt et al., 2016a; Akerman et al., 2019). Mechanistically, HSP27 has been shown to reduce intimal hyperplasia (Connolly et al., 2003), with greater carotid intima thickness associated with reduced cerebral blood flow (Sojkova et al., 2010). Undesirable vascular hypertrophy can also be mitigated against via HSP70 associated inhibition of Angiotension II (Zheng et al., 2006), with HSP90 conferring a more general adaptation aligned to the stabilization of the vascular endothelial growth factor (VEGF) upstream target hypoxia-inducible factor-1 (HIF-1α) (Maloyan et al., 2005), and elevated endothelial nitric oxide production and improved the stabilization and bioavailability of endothelial nitric oxide synthase (Averna et al., 2008). Collectively these vascular adaptations observed in passive heating interventions have the potential to maintain cerebral blood flow and blood-brain barrier function, outcomes which may have beneficial effects for cognitive function in Alzheimer's and Parkinson's diseases.

Metabolic Health

Markers of metabolic health may also play a role in the pathogenesis of neurodegeneration in Alzheimer's disease. The neurodegeneration seen in Alzheimer's disease has been linked

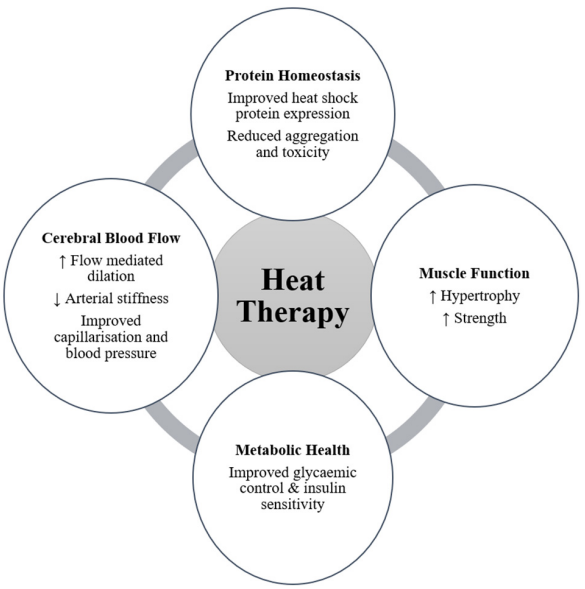

FIGURE 1 | Potential benefits arising from heat therapy for people with neurodegenerative disease (↑ symbolizes an increasing effect; ↓ symbolizes a decreasing effect).

with impaired cerebral insulin signaling and glucose metabolism (Bharadwaj et al., 2017). The loss of protein homeostasis (Aβ accumulation and tau hyperphosphorylation), synaptic degeneration, and neural dysfunction have been associated with these impairments to normal metabolic processes (Bharadwaj et al., 2017). Recent research has therefore proposed therapeutic interventions to improve insulin signaling and reduce insulin resistance. Interestingly, heat therapy has been proposed for people with type II diabetes, and early studies have highlighted a reduction in fasting plasma glucose following repeated hot water bath immersions over 3 weeks (Hooper, 1999). Furthermore, chronic heat therapy interventions have improved glucose tolerance and insulin sensitivity in women with polycystic ovary syndrome who experience obesity and metabolic dysfunction (Ely et al., 2019). Therefore, the effects of heat therapy on metabolic health are another potential avenue of therapeutic benefit for people with neurodegenerative disease.

Summary

For individuals who do not experience these important physiological stressors through habitual activity and exercise, heat therapy may provide a vehicle to achieve a range of health and physiological benefits (**Figure 1**). Much remains to be understood with regards to the mechanisms and stimuli required to elicit the desired increases in relevant HSPs following heat therapy, in addition to further quantifying the magnitude of importance of these responses. Additionally, the potential to improve muscular function, cerebral blood flow, and markers of metabolic health offer significant benefit for people with a neurodegenerative disease by improving their quality of life and reducing disease severity. Part of the present ambiguity results from the utilization of different methods, e.g., heating technique, heating duration and magnitude, tissue sample site and time points, across experimental studies. Despite the need for further clarity regarding the mechanistic underpinnings and best practice implementation, the opportunities to investigate a tolerable heat therapy model in a relevant target population exists and should be encouraged.

BODY WARMING IN PEOPLE WITH NEURODEGENERATIVE DISEASES

Evidence for a Potential Benefit

At present, the authors are not aware of any studies that have directly assessed the effects of active or passive body warming on HSP release, and disease severity or progression, in people with neurodegenerative diseases. Therefore, this review will draw on findings from epidemiological studies, and indirect studies of other interventions such as exercise, that have shown benefits among people with neurodegenerative diseases.

Habitual body warming, through sauna bathing or exercise, has been shown to reduce the risk of developing neurodegenerative diseases. Regular passive heating has been associated with a reduced risk of developing neurodegenerative diseases, including Alzheimer's (Heinonen and Laukkanen, 2018). Men participating in sauna bathing 2–3 or 4–7 times per week had a 0.80 and 0.35 hazard ratio for developing Alzheimer's disease compared to men who sauna once per week or less (Laukkanen et al., 2017). Regular exercise also has a protective effect on the risk of developing Alzheimer's and Parkinson's diseases (Paillard et al., 2015). Consistent and frequent participation in moderate to vigorous physical activity was found to reduce the risk of Parkinson's disease by up to 40% (Xu et al., 2010). Similarly, exercising three or more times per week is associated with a lower incidence rate of dementia and Alzheimer's (Larson et al., 2006). While these studies do not elucidate the potential mechanisms by which a protective effect is elicited, it does lend anecdotal evidence toward the expression of HSP through regular body warming as a distinct possibility.

Further to a preventative effect, exercise has been described as having a restorative effect on the neurodegeneration observed in Alzheimer's and Parkinson's diseases (Loprinzi et al., 2013; Paillard et al., 2015). Clinical and epidemiological studies have provided evidence supporting the conclusion that exercise has therapeutic value by reducing the symptoms and slowing disease progression (Ransmayr, 2011; Hou et al., 2017; Liu et al., 2019). Moderate to high-intensity aerobic exercises, such as treadmill walking or assisted cycling, are recommended for improved motor and cognitive function in Parkinson's disease (Ahlskog, 2011; Salgado et al., 2013; Evens and Clark, 2017). High-intensity treadmill exercise of 30 min at a target heart rate prevented Parkinson's disease progression, compared to moderate intensity and control (Schenkman et al., 2018). Furthermore, interval exercises (alternating periods of low and high intensity assisted cycling) have shown positive improvements in functional ability in Parkinson's patients (Uygur et al., 2015, 2017). Immersion

in warm water (33°C) for 50 min while performing dual-task exercises (combining physical movements with cognitive tasks) was found to improve functional mobility (timed up and go, and five-time sit-to-stand) among people with Parkinson's disease following 3 months of twice-weekly exposures (Silva and Israel, 2019).

Exercise interventions have also had significant effects on slowing the progression of Alzheimer's disease. Over 1 year, people with Alzheimer's disease who participated in twice-weekly 1-h exercise sessions showed reduced rates of decline in measures of functional independence and physical performance, compared to control participants (Pitkala et al., 2013). In a similar intervention, the ability to perform activities of daily living declined significantly slower in people with Alzheimer's disease performing twice-weekly exercise programs (Rolland et al., 2007). A 6-month walking program has also shown people with Alzheimer's disease to be able to maintain cognitive function on the Mini Mental State Exam (MMSE), compared to significant declines in those not exercising (Venturelli et al., 2011). Overall, there is compelling evidence that exercise improves motor and cognitive function in neurodegenerative disease and it is therefore recommended by clinicians (Ransmayr, 2011; Hou et al., 2017; Liu et al., 2019). However, exercise and body warming interventions should consider the difficulties in performing such activities for these populations (section "Considerations for Heat Therapy for People With Neurodegenerative Disease").

The studies of moderate to high-intensity exercise among neurodegenerative disease populations provide circumstantial evidence that the assumed body warming experienced may be contributing to the beneficial effects observed. While there may be many avenues by which exercise and body warming promote improved health and function, the role of body temperature elevation, thermoregulatory responses, and HSP expression have been overlooked in these experiments. Given the growing body of evidence that supports the expression of HSPs as therapeutic targets for Alzheimer's and Parkinson's diseases (section "Heat Shock Proteins As a Therapeutic Target"), there is a clear need for future investigations of passive heating to monitor thermoregulation and HSP responses in people with these neurodegenerative diseases.

Considerations for Heat Therapy for People With Neurodegenerative Disease

An important consideration in conducting heat acclimation and heat therapy for older adults and clinical populations will be how their impairments or any co-morbidities may affect their ability to perform and tolerate these interventions. Firstly, their disease severity may impair their physical ability to perform movements effectively and safely (Kerr et al., 2010; Buchman and Bennett, 2011). Secondly, neurodegeneration may cause deficits in thermoregulatory processes. In Parkinson's disease, neural degeneration in higher-order brain centers including the hypothalamus is associated with impaired sudomotor function which in turn may influence their tolerance to body warming (LeDoux, 2013).

Neurodegenerative diseases such as Parkinson's and Motor Neuron Disease primarily affect motor control. As the diseases progress, motor function deteriorates resulting in impaired gait and balance and an increased risk of falls (Pickering et al., 2007). While Alzheimer's is usually associated with cognitive impairments, significant motor impairments are also associated with this disease (Buchman and Bennett, 2011). Therefore, exercise and heat acclimation interventions should consider the level of impairment of their target population and how the risk of falls and injury may be managed. As such, in these populations where movement is limited, passive heat therapy may be an achievable alternative to exercise interventions.

The autonomic nervous system, responsible for thermoregulation, can exhibit deficits in neurodegenerative diseases, specifically in the thermoeffector responses of sweating and skin blood flow. Abnormalities in the sweating response, hyperhidrosis and/or hypohidrosis, are commonly reported in Parkinson's disease (De Marinis et al., 1991; Swinn et al., 2003; Schestatsky et al., 2006) and may be more prominent with increasing age and disease severity of patients (Saito and Kogure, 1989). Hypohidrosis, an absence of the sweating or reduced sweat output, may reduce effective body cooling during exercise and body warming. However, the sweating response is highly individualized, and compromised sweating in one body region may be compensated by increased sweating in other body regions (Schestatsky et al., 2006). Careful attention should, therefore, be given to the rate of body warming people with Parkinson's disease may experience, and ensuring appropriate cooling strategies are available.

Cardiovascular regulation of blood pressure is also influenced by neurodegenerative disease. The expected elevation in heart rate and blood pressure may be blunted in people with Parkinson's disease, and they may also experience post-exercise hypotension (Asahina et al., 2013). Orthostatic intolerance is also reported in 10–60% of people with Parkinson's disease (Senard et al., 1997; Wüllner et al., 2007). These cardiovascular impairments may influence their ability to tolerate exercise and body warming and should be considered in determining individual suitability for heat therapy and heat acclimation. The modality of passive heating may therefore also be an important factor in determining appropriate therapeutic techniques. Infrared sauna bathing has been reported to promote lower cardiovascular strain than traditional sauna techniques (Mero et al., 2015) and may be one avenue that could be investigated for suitability in these at-risk populations.

FUTURE DIRECTIONS

Overall, there are three key points from the scientific literature that support a proposal for a therapeutic effect of heat therapy or heat acclimation to promote HSP expression in people with neurodegenerative disease. These include, (1) Exercise, sufficient to raise core body temperature, is currently recommended for people with neurodegenerative disease (e.g., Parkinson's and Alzheimer's diseases) as it has been shown to improve their symptoms; (2) Elevated HSP levels have been identified as a therapeutic target to reduce protein aggregation and toxicity; and (3) Exercise and body warming have been shown to elevate HSP

expression in healthy adults. Furthermore, heat therapy may have additional benefits for muscle function, vasculature health and cerebral blood flow, and indicators of metabolic health, which have also been implicated in the pathophysiological presentation of neurodegenerative diseases. These findings from the current scientific literature support the proposal for further investigation into the potentially beneficial adaptations for people with neurodegenerative diseases to heat therapy and heat acclimation.

Initial research is required to establish the acute effects of heat therapy and/or heat acclimation in people with neurodegenerative diseases. As these diseases may involve impairment of autonomic pathways involved in thermoregulation, research is required to elucidate the thermoeffector responses, sweating and skin blood flow, to acute heat stress and how these may affect tolerance to body warming. Alongside these outcomes HSP expression, muscular adaptations, and vasculature function responses to body warming should be measured in neurodegenerative disease populations, to determine the presence of a similar response to body warming as seen in healthy adults, and the magnitude of the response in relation to the tolerable limits of body warming. Consequent to acute observations that tolerable heat exposures promote the desirable HSP response and vascular adaptations, further investigation should then pursue the effects of chronic or repeated heat therapy and/or heat acclimation on these responses and indicators of disease severity and progression. Finally, an understanding of the dose-response relationship between frequency and intensity of body warming and improved symptomology should be determined. In concert with these research efforts, investigations of the underlying mechanisms by which HSP expression and body warming may improve neuromuscular function are warranted.

CONCLUSION

While the current evidence for heat therapy in neurodegenerative disease is associative and the mechanisms of improved health outcomes have yet to be elucidated, the potential of passive heating in this population remains an alluring therapeutic option. Heat acclimation and heat therapy have been shown to improve HSP responses in healthy adults, as well as induce additional benefits for skeletal muscle function and cerebral blood flow. Furthermore, HSPs have been identified as therapeutic targets to restore protein homeostasis and reduce protein toxicity in several neurodegenerative diseases. While circumstantial evidence exists that body warming may promote improved health and function in neurodegenerative disease, through exercise or sauna bathing, there is yet to be any direct studies of body warming, thermal tolerance, and HSP responses in these populations. For individuals who do not experience these important physiological stressors through habitual activity and exercise, heat therapy may provide a vehicle to achieve improved health and slowed disease progression in people with neurodegenerative disease, and, therefore, warrants further investigation.

AUTHOR CONTRIBUTIONS

All authors contributed to the planning and writing of the manuscript. Specifically, AH contributed to the design, writing, and review of the manuscript and was the primary contributor to section "Body Warming in People With Neurodegenerative Diseases." GM contributed to the design, writing, and review of the manuscript and was the primary contributor to section "Central and Peripheral Effects of Body Warming." OG contributed to the writing and review of the manuscript and primarily contributed to sections "Heat Shock Proteins As a Therapeutic Target," "Acute Effects of Body Warming on Heat Shock Proteins," and "Chronic Effects of Body Warming on Heat Shock Proteins." GK contributed to writing section "Epidemiology and Pathophysiology." IS contributed to the overall plan and design of the manuscript and writing sections "Introduction" and "Future Directions."

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Risks of Heat Illness in Athletes With Spinal Cord Injury: Current Evidence and Needs

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It has been well-documented that spinal cord injury (SCI), especially with resultant tetraplegia or high level paraplegia (T6 and above) (Price and Campbell, 2003), leads to disrupted somatic, sensory, and autonomic functions below the level of lesion (Cruz and Blauwet, 2018; Walter and Krassioukov, 2018), thereby compromising the heat dissipation mechanisms (Price and Trbovich, 2018). The current consensus when it comes to exercise and sports in the heat is that athletes with SCI, multiple sclerosis, or cerebral palsy (another neuromuscular disorder affecting thermoregulation) are more susceptible to hyperthermia than able-bodied athletes (Lepretre et al., 2016). The purpose of this review was to examine the published evidence to assess the risk of heat injury during competition and training in SCI athletes and use this information to provide a basis for improved protection for these sports.

Empirical evidence to date, however, is notably limited regarding the assumed risks of heat injury among this cohort (Price, 2016), which could be due to a very small incidence count, lack of resources, or failures to collect data (Trbovich et al., 2019). Grobler et al. (2019), for the first time, reported accurate medical records (level III evidence) during the 2015 IPC Athletics World Championships held in the heat (venue wet-bulb globe temperature 24.6–36.0°C). According to their field data, not only was the incidence rate of all illnesses (37.6 per 1,000 para athletes) low in comparison to that from outdoor IAAF World Championships between 2009 and 2017 (50.2 per 1,000 able-bodied athletes) (Edouard et al., 2019), but it was also explicitly low in heat illness (seven recorded cases of heat illness, of which there was only one case of athlete with SCI).

The discrepancy between hypothesis and observation is that the cause of the elevated body temperature (which ultimately leads to heat illness) during the time course of exercise and sports is not solely attributed to the body's heat dissipation mechanisms. Rather, the absolute heat production rates and the capacity of the body to transport heat and the environment to absorb the heat generated by the body are the real issues among this cohort. This is akin to what economists call an "identification problem." For instance, Olympic-caliber marathoners reached a VO_{2max} of 79.6 $ml \cdot kg^{-1} \cdot min^{-1}$, and they ran at an average of 89.7% VO_{2max} (resultant oxygen consumption ~71 $ml \cdot kg^{-1} \cdot min^{-1}$) during a 10-km time trial (Billat et al., 2001). In contrast, Paralympic-caliber wheelchair racers with SCI showed considerably lower VO_{2max}, 46.4 $ml \cdot kg^{-1} \cdot min^{-1}$ as well as a substantially lower percentage of VO_{2max}, 73.7% (resultant oxygen consumption ~34 $ml \cdot kg^{-1} \cdot min^{-1}$) during a 25-km time trial (Edwards et al., 2018). Furthermore, due to the difference in race velocity, the duration of endurance sports is typically longer among able-bodied events; for example, the average time of Rio 2016 Olympics and Paralympics marathon finishers

was Men, 02:22:22 vs. T54 Men, 01:32:14 and Women, 02:43:34 vs. T54 Women, 01:42:04[1]. Therefore, given the same body mass, the metabolic energy expenditure, which is a proximate cause of heat illness, and thereby the cumulative heat production is relatively lower among para athletes.

In terms of competition environments, many para sports, such as wheelchair basketball, are held at indoor stadiums where the venue temperature is typically controlled at 19–21°C. Such temperate environments could partially offset the deleterious effects of the autonomic nervous system related dysfunction in thermoregulation. Certain para athletes participating in outdoor endurance events (e.g., T54 wheelchair athletes competing in 5,000 m, 10,000 m, marathon, and H1–5 para cyclists) could be subject to the negative influence of heat on performance and health when competitions are held in hot and humid environments. However, the nature of these sports allows para athletes to get some thermoregulatory relief in the form of enhanced convection and evaporation from air movement. While Olympic-caliber marathoners ran at a 18.8 $km \cdot h^{-1}$ pace (Billat et al., 2001), Paralympic-caliber wheelchair racers with SCI showed an average race speed of 30.7 $km \cdot h^{-1}$ (Edwards et al., 2018), which resulted in significantly faster air motion from the wheelchair movement relative to the environment.

In the able-bodied population, manipulating air velocities of 10–33.5 $km \cdot h^{-1}$ during 2-h submaximal exercise in the heat (33°C ambient temperature, 59% relative humidity) reduced heat storage by 47–60% compared with a no-wind condition (Saunders et al., 2005). Similarly, combining an air velocity of 9.2 $km \cdot h^{-1}$ and rehydrating 100% of sweat losses during 1-h submaximal exercise in the heat (36°C ambient temperature, 29% relative humidity) reduced heat storage by 41% whilst also maintaining cardiac output compared with control conditions in a group of heat-acclimated healthy males (Mora-Rodriguez et al., 2007). Although these findings from able-bodied persons cannot be directly extrapolated to SCI athletes who show compromised sweating responses (Price and Trbovich, 2018), in particular among athletes with tetraplegia (e.g., >50% reduction in estimated whole body sweat rate for tetraplegia vs. paraplegia) (Price and Campbell, 2003), SCI athletes competing in the outdoor wheelchair 10,000 m, marathon, and paratriathlon could still get thermoregulatory relief from greater air flow, which results in better evaporation. This has been demonstrated in a field study showing that wheelchair athletes (comprised mostly of SCI athletes) displayed significantly a smaller increase in core temperature compared with athletes with a visual impairment (i.e., +0.26°C vs. +1.03°C) during the running segment of a paratriathlon (Stephenson et al., 2019). Therefore, despite the compromised heat dissipation mechanisms, SCI athletes generally carry less than expected accumulated thermal burden as a result of both a relatively lower heat production rate and higher evaporative heat transfer to the environment.

It is worth noting that the ability to maintain core body temperature within normothermia while exercising in the heat would be affected by the varying degrees of sympathetic integrity in this cohort. There is consistent reporting of continual increases in core body temperature during rest (Griggs et al., 2019a) and exercise (Price and Trbovich, 2018) among persons with tetraplegia. For example, competitive wheelchair rugby match play (4 × 8-min quarter; ambient temperature, 18.4–20.9°C; relative humidity, 31.1–45.1%) resulted in 39.3°C core body temperature in elite players with a cervical SCI (C5/6–C7) (Griggs et al., 2017). A popular perception is therefore that athletes with tetraplegia, who exhibit greater disruption of evaporation (sweating) and convection (cutaneous vasodilation), should be prepared with appropriate cooling strategies during exercise in the heat provided they demonstrate heightened thermal strain (Griggs et al., 2019b; Trbovich et al., 2019). While this increase in core body temperature, especially if >39.0°C, could be materially alarming, trained tetraplegic athletes who participate in indoor events (e.g., wheelchair basketball, rugby, and fencing) or outdoor sports (e.g., H1 para-cycling), exhibit no expected higher incidence rate of heat injury. True exertional heat injury is primarily triggered by a pathological elevation of the core body temperature, usually >40.5°C (Casa et al., 2015). Additionally, severe heat injury, including exertional heat stroke, could have resulted from excessive endogenous thermogenesis, leading to widespread muscle necrosis and even organ failure (Rae et al., 2008). To date, none of these unfortunate medical comorbidities have been documented in this cohort. Furthermore, another important catalyst for exertional heat injury is prolonged duration of continuous exercise. Athletes with tetraplegia usually compete in much shorter durations compared with able-bodied endurance events, such as marathons and triathlons, that incur more frequent cases of heat injury. Therefore, despite severe interruption of effector sympathetic pathways in athletes with tetraplegia, the hypothesized higher risks of heat injury (Price, 2016) is not supported by the available epidemiological data.

However, the low incidence rate of illness, including heat-related illness, among para athletes (Grobler et al., 2019) should not be celebrated. No one is immune to heat illness, and para athletes are no exception. Preventive measures and post-incident medical strategies are still needed to ensure overall health, especially if events are held in hot and humid environments. Thermoregulatory data recorded at a paratriathlon (time to completion 54.6–103.9 min) in the heat (33°C ambient temperature, 35–41% relative humidity, 25–27°C water temperature) have revealed that 78.6% of studied para athletes displaying a core body temperature >39.5°C, including 28.6% of para athletes showing a core body temperature >40.0°C (Stephenson et al., 2019). This exertional hyperthermia was further tied to 57% of studied para athletes experiencing self-reported symptoms of heat illness (Stephenson et al., 2019). The field study by Stephenson et al. (2019) also indicated that, although the incidence rate of heat illness may not be officially documented, para athletes participating in endurance

[1]Results retrieved from Rio 2016 Summer Olympics Men's and Women's Marathon and Rio 2016 Paralympic Games Men's and Women's Marathon—T54. Available online at: https://www.olympic.org/rio-2016/athletics/marathon-men, https://www.olympic.org/rio-2016/athletics/marathon-women, https://www.paralympic.org/rio-2016/schedule-results/info-live-results/rio-2016/eng/at/engat_athletics-results-men-s-marathon-t54-1-01.htm, https://www.paralympic.org/rio-2016/schedule-results/info-live-results/rio-2016/eng/at/engat_athletics-results-women-s-marathon-t54-1-01.htm (accessed October 9, 2019).

sports may very well be experiencing much greater deep body temperatures, and suspected symptoms of heat illness and their consequences may therefore be overlooked by these athletes. Proper education of athletes, coaches, and event organizers about heat injury is thus warranted. Without a well-prepared framework for early recognition, diagnosis, and treatment of heat illness, persistent exertional hyperthermia could lead to thermoregulatory collapse, resulting in heat injury in any type of athlete.

Across a variety of organized sports that SCI athletes are eligible to compete in, wheelchair marathons, triathlons, and tennis usually last over 60 min, making these SCI athletes vulnerable to heat illness in warm/hot weather. Despite this potential health risk, policies regarding safe competition under heat exposure for SCI athletes have only been implemented by the International Tennis Federation (Regulations for Wheelchair Tennis 2019, September 2019)[2], which still lacks specific guidelines for the risk management of heat illness. In comparison, governing bodies of able-bodied sports, such as National Collegiate Athletic Association[3] and Australian Open[4], have established specific heat policies to maximize the safety for sport competition in the heat.

Moreover, it is crucial to understand that SCI athletes display unique patterns of thermoregulatory responses as a consequence of their underlying physiology. Cutaneous temperature sensation has been studied extensively, including in persons with SCI (Price and Trbovich, 2018). In response to the stimulus, nerve impulses regarding the thermal state of the body and the environment are sent to the spinal cord, the major bidirectional connection between the body and the brain, where neural signals are transmitted upwards until they are blocked at the level of the lesion. Reduced afferent input from the insensate portion of the body, especially at those regions (i.e., chest, forearm, hand, finger, and thigh) rich in warm thermoreceptors (Arens and Zhang, 2006), results in altered thermal sensation. Griggs et al. (2019a) presented data showing that active persons with SCI undergoing passive heat exposure (37°C ambient temperature; initially 20% relative humidity, with an increase by 5% every 7 min thereafter), despite displaying warmer mean skin temperature, were not able to perceive the magnitude of thermal strain as measured using thermal sensation. Should athletes present higher levels (tetraplegia vs. paraplegia) of SCI, or completeness (vs. incompleteness) of SCI, the resultant thermal sensation in the heat is expected to be further disrupted.

In summary, abnormal somatic, sensory, and autonomic functions after SCI present significant challenges for these individuals participating in competitive sports. Despite the fact that thermal dysfunction exists, the circumstances of SCI sports competition may largely mitigate any compromise of heat dissipation in these athletes, leading to the low incidence rate of heat illness. For marathon, tennis, and paratriathlon, the nature and distinct risks of heat illness of any para athlete competing in warm to hot environments underline the need for specific guidelines aimed at improving knowledge and medical support for athletes with SCI in particular. Continued research to evaluate heat tolerance among all athletes in all sports is vital to safe sports competition. For the upcoming Tokyo 2020 Paralympics, the environmental conditions are expected to pose a challenge to para athletes' performance and health (Kakamu et al., 2017). Accordingly, a systematic heat policy addressing appropriate preventive measures (Griggs et al., 2015, 2019b), early recognition and correct diagnosis (Epstein and Yanovich, 2019), and effective treatment strategies (Belval et al., 2018) of heat illness would be essential to uphold safe and successful sports participation for this special population.

[2]Regulations for Wheelchair Tennis 2019 (2019). Available online at: https://www.itftennis.com/media/309868/309868.pdf (accessed September 26, 2019).

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AUTHOR CONTRIBUTIONS

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ORIGINAL RESEARCH
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Long Term Adaptation to Heat Stress: Shifts in the Minimum Mortality Temperature in the Netherlands

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It is essentially unknown how humans adapt or will adapt to heat stress caused by climate change over a long-term interval. A possible indicator of adaptation may be the minimum mortality temperature (MMT), which is defined as the mean daily temperature at which the lowest mortality occurs. Another possible indicator may be the heat sensitivity, i.e., the percentage change in mortality per 1°C above the MMT threshold, or heat attributable fraction (AF), i.e., the percentage relative excess mortality above MMT. We estimated MMT and heat sensitivity/AF over a period of 23 years for older adults (≥65 years) in the Netherlands using three commonly used methods. These methods are segmented Poisson regression (SEG), constrained segmented distributed lag models (CSDL), and distributed lag non-linear models (DLNM). The mean ambient temperature increased by 0.03°C/year over the 23 year period. The calculated mean MMT over the 23-year period differed considerably between methods [16.4 ± 1.2°C (SE) (SEG), 18.9 ± 0.5°C (CSDL), and 15.3 ± 0.4°C DLNM]. MMT increased during the observed period according to CSDL (0.11 ± 0.05°C/year) and DLNM (0.15 ± 0.02°C/year), but not with SEG. The heat sensitivity, however, decreased for the latter method (0.06%/°C/year) and did not change for CSDL. Heat AF was calculated for the DLNM method and decreased with 0.07%/year. Based on these results we conclude that the susceptibility of humans to heat decreases over time, regardless which method was used, because human adaptation is shown by either an increase in MMT (CSDL and DLNM) or a decrease in heat sensitivity for unchanged MMT (SEG). Future studies should focus on what factors (e.g., physiological, behavioral, technological, or infrastructural adaptations) influence human adaptation the most, so it can be promoted through adaptation policies. Furthermore, future studies should keep in mind that the employed method influences the calculated MMT, which hampers comparability between studies.

Keywords: mortality, temperature, climate change, human adaptation, older adults, minimum mortality temperature

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INTRODUCTION

Humans possess a great capacity to acclimatize to heat (Periard et al., 2016). Over a period of approximately 10 days, cardiovascular, thermoregulatory and fluid control mechanisms are optimized so that heat strain has a reduced effect on human well-being and performance. These acute adaptations are well documented (Périard et al., 2015; Periard et al., 2016), but adaptations to long term exposure (i.e., several years) are essentially unknown. This is problematic for accurate estimations of future morbidity and mortality in the face of climate change, with numerous scientific papers making a disclaimer for the unknown effects of the "human adaptation" (Sanderson et al., 2017). When adaptation to heat is assumed, it has a considerable impact on predicted mortality and associated societal costs (Díaz et al., 2019).

Heat related excess mortality is mainly observed in elderly subjects (Baccini et al., 2008). Older adults (>65 years) are most at risk for temperature related mortality due to intrinsic changes in the thermoregulatory system, like a reduced sweat response and thirst sensation (Foster et al., 1976; Kenney and Chiu, 2001). In addition, older adults are often less physically fit and have more illnesses and disabilities what makes them also more susceptible to heat-related morbidity and mortality (Koppe et al., 2004). However, older adults are able to acclimatize to the heat (Inoue et al., 1999; Best et al., 2014) when a sufficient number of days for adaptation is allowed (Daanen and Herweijer, 2015). Furthermore, they may be more resilient to heat in hot cities than in colder cities (Worfolk, 2000). This increased resilience may be due to better housing, behavioral adaptations, increased use of air conditioners (JRAIA, 2018), improved awareness of heat impact due to public campaigns, but also due physiological adaptations of the human body to the heat.

Mortality data, especially in the older population, often exhibit a U- or V-shaped relationship with temperature (Kunst et al., 1993), with the number of deaths increasing for temperatures below or above the so-called minimum mortality temperature (MMT). MMT is the mean daily temperature at which the lowest mortality occurs and quantifies the threshold between the cold and heat mortality slope. The term MMT was first used to illustrate the considerable differences in the temperature-mortality relationship in the United States (Curriero et al., 2002): MMT for Boston was 21°C and 27°C for Miami. Cities show higher MMT values when located closer to the equator (Hajat and Kosatky, 2010). This is observed for European cities (Baccini et al., 2008), but also within countries. Tobias et al. (2017), for instance, showed that hotter cities have a higher MMT in Spain.

Recent studies have proposed the use of the MMT as a potential indicator of human long-term adaptation to heat in case the MMT shifts to a higher temperature (Barrett, 2015; Todd and Valleron, 2015; Åström et al., 2016). If humans become less susceptible to heat, an increase in MMT can be expected over time, similar to higher MMT values in warmer cities due to geographic differences. Todd and Valleron (2015) found an increase in MMT from 17.5°C in 1968–1981 to 17.8°C in 1982–1995 and 18.2°C in 1996–2009 in France. An increase in ambient temperature of 1.6°C over these years was accompanied by an increase in MMT of 0.8°C. For comparison: a temperature difference of 1.6°C between geographical areas was accompanied by a 1.1°C increase in MMT.

There are several approaches to calculate MMT from temperature-mortality time-series data and different methods have been used in the studies mentioned above. One simple statistical model predicting the logarithm of the death counts by actual temperature is the segmented Poisson regression model (SEG) providing estimates of the breakpoint (MMT) as well as of the negative temperature slope in the cold and positive slope in the heat, while accounting for covariates, e.g., day of week (Muggeo, 2003, 2008a, 2017). Only focusing on the temperature influence on the same day, SEG neglects the time series structure, and especially does not consider lagged effects of temperature on mortality. However, it is easily applicable to separate one-year periods allowing for assessing the development of MMT over the whole observation period, as well as of heat and cold sensitivity from the respective slopes. The constrained-segmented distributed lag model (CSDL) also includes MMT as estimated parameter, but extends the simple V-shape model by considering non-linear lagged effects as well as long-term and seasonal trends in the time series (Muggeo, 2008b, 2010). By additionally relaxing the linear V-shape assumption, so-called distributed lag non-linear models (DLNM) allow to fit more flexible temperature-mortality relationships to such time series data (Gasparrini, 2011; Gasparrini et al., 2017). DLNM requires an extra step applying a search algorithm for finding the MMT (Tobias et al., 2017). Both the CSDL and DLNM models usually rely on longer observation periods covering at least 10 – 20 years. Thus, in order to assess the development of MMT over time, recent studies fitted the data to the observations from non-overlapping (Carson et al., 2006; Petkova et al., 2014; Todd and Valleron, 2015) or partly overlapping (Åström et al., 2016; Chung et al., 2017) sub-periods.

It is unknown if the variation in MMT is due to human adaptation or due to the methods used to calculate MMT as outlined above. Therefore, the aim of the current study was twofold: we investigated changes in MMT in the Netherlands over a period of 23 years, from 1995 to 2017, for older adults (≥65 years), whilst comparing the three previously mentioned models (SEG, CSDL, and DLNM).

MATERIALS AND METHODS

Database

The daily number of deaths and population size in the Netherlands, obtained from Statistics the Netherlands (CBS), and temperature data, obtained from the Royal the Netherlands Meteorological Institute (KNMI) from January 01, 1995 to December 31, 2017 were used for the calculations in this study. Only mortality in the age group of 65 years and older was processed, because this group is reportedly the most vulnerable to extreme ambient temperatures (Koppe et al., 2004).

Hourly ambient temperature was obtained from five weather stations representative for the Netherlands: Station De Bilt (in the center of the Netherlands), Station Eelde (rural area, farmland, northern part of the Netherlands), Maastricht (average sized

city, southern part of the Netherlands), Rotterdam (large city near the coast, western part of the Netherlands), and Schiphol airport (industrial area, amid densely populated areas, western part of the Netherlands). Daily temperature used in this study was obtained by averaging the hourly values over the five weather stations and time.

Model Calculations

Calculations were performed using R version 3.6.1 (R Core Team, 2019). For the entire time series, a segmented Poisson regression model (SEG) allowing for over dispersion (Wood, 2006) was fitted to daily mortality with daily mean temperature as predictor and day-of-week as only covariate using the R package *segmented* (Muggeo, 2003, 2008a, 2017). Estimates and SE were provided for MMT and for the cold and heat slope parameters. Relative risks (RR) with mortality at MMT as reference were calculated by exponentiation of the slope parameters multiplied with the difference of MMT to temperature. Sensitivities to cold and heat calculated by exponentiation of the slope estimates were expressed as percentage change per degree decrease or increase in temperature from MMT, respectively.

Similarly, we also obtained MMT and sensitivities to heat and cold by fitting constrained segmented distributed lag (CSDL) models using the package *modTempEff* (Muggeo, 2008b, 2010) controlling for the day-of-week, while considering lag temperature effects and adjusting for annual and seasonal trends with spline functions as suggested by the software manual (Muggeo, 2010).

Distributed lag non-linear models (DLNM) were fitted using the *dlnm* package (Gasparrini, 2011), also including day-of-week covariate while considering lag temperature effects up to 25 days and adjusting for long-term and seasonal trends with spline functions using eight degrees-of-freedom per year. MMT with SE was estimated by a search algorithm over the fitted response function (Tobias et al., 2017). The sensitivities to heat and cold were calculated using the attributable fraction (AF), expressed as percentage relative excess mortality integrated over the lag periods and temperatures above and below MMT, respectively (Gasparrini and Leone, 2014).

Shift in MMT and Sensitivity to Heat and Cold

The shift in MMT over time in the SEG method was calculated by fitting the SEG model for every year separately. For CSDL and DLNM, the models were repeatedly fitted to reduced series from a sliding 15-year window, which was shifted by 1-year increments throughout the entire observation period.

A shift of MMT and heat and cold sensitivities (AF for DLNM) was assessed by performing linear regression analyses for changes in the parameters weighted by their inversed SE over time, while statistical significance was assumed for p-values < 0.05.

RESULTS

The average number of deaths in the Netherlands over the investigated 23-year time span was 382 ± 40 (SE) individuals per day. The mean daily temperature in the Netherlands was 10.5 ± 6.3°C. The mean temperature increase over the observed period was 0.03°C/year. The number of people over 65 years increased from 2.0 million to 3.2 million between 1995 and 2017, but their mean age was stable at 74.5 ± 0.1 years over the investigated period (CBS, 2019a).

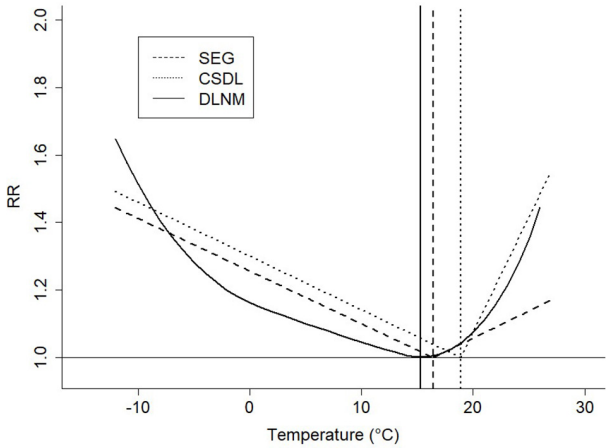

FIGURE 1 | Temperature related to the relative risk (RR) for mortality of older adults (≥65 years) for three different methods: Segmented Poisson regression (SEG), constrained segmented distributed lag models (CSDL) and distributed lag non-linear models (DLNM), during the 23-year period from January 01, 1995 to December 31, 2017 in the Netherlands. The minimum mortality temperature (MMT) estimated by the three different methods is shown with the vertical lines. The slopes of the lines represent the cold/heat sensitivity of the SEG and CSDL method, whereas the cold/heat attributable fraction (AF) of the DLNM method is determined as relative excess mortality integrated over the temperatures above and below MMT, respectively.

Figure 1 shows the relative risk (RR) of mortality at different daily mean temperatures over the entire 23-year period according to the three different methods. The SEG and CSDL methods assume a linear relation and, therefore, have a V-shaped estimation of the temperature-mortality curve. The DLNM method assumes a non-linear relation and, therefore, has a more U-shaped curve. The mean calculated MMT, and cold and heat sensitivity/AF are shown in **Table 1**. Large differences are shown for the calculated MMT between methods with values between 15.3 ± 0.4°C for the DLNM method and 18.9 ± 0.5°C for the CSDL method. Cold sensitivity was similar with 1.3 ± 0.2% and 1.3 ± 0.3% for, respectively, the CSDL and SEG methods, but a large difference was found for the heat sensitivity with 5.6 ± 0.6% for the CSDL method and 1.5 ± 0.8% for the SEG method. The AF to the cold calculated with the DLNM method was higher than the AF to the heat, with, respectively, 5.0 ± 0.3% and 1.1 ± 0.2%.

Figure 2 shows the calculated MMT per year for the SEG method and with a sliding 15-year window for the CSDL and DLNM methods. Cold and heat sensitivity are reported for the SEG and CSDL methods and cold and heat AF for the DLNM method. A significant increase in MMT of 0.11 ± 0.05°C and 0.15 ± 0.02°C per year was observed for the CSDL ($p < 0.001$) and DLNM ($p < 0.05$) methods, respectively. However, no significant increase in MMT was found with the SEG method

TABLE 1 | The minimum mortality temperature (MMT) (Mean ± SD), cold and heat sensitivity (SEG and CSDL models) and attributable fraction (AF) (DLNM model) (%) calculated for the 23-year period from January 01, 1995 to December 31, 2017 in the Netherlands with the three different methods: Segmented Poisson regression (SEG), constrained segmented distributed lag (CSDL) model, and distributed lag non-linear models (DLNM).

Method	MMT (°C)	Cold sensitivity (%/°C)/AF (%)	Heat sensitivity (%/°C)/AF (%)
SEG	16.4 ± 1.2	1.3 ± 0.3[a]	1.5 ± 0.8[a]
CSDL	18.9 ± 0.5	1.3 ± 0.2[a]	5.6 ± 0.6[a]
DLNM	15.3 ± 0.4	5.0 ± 0.3[b]	1.1 ± 0.2[b]

[a]*Sensitivity.* [b]*AF.*

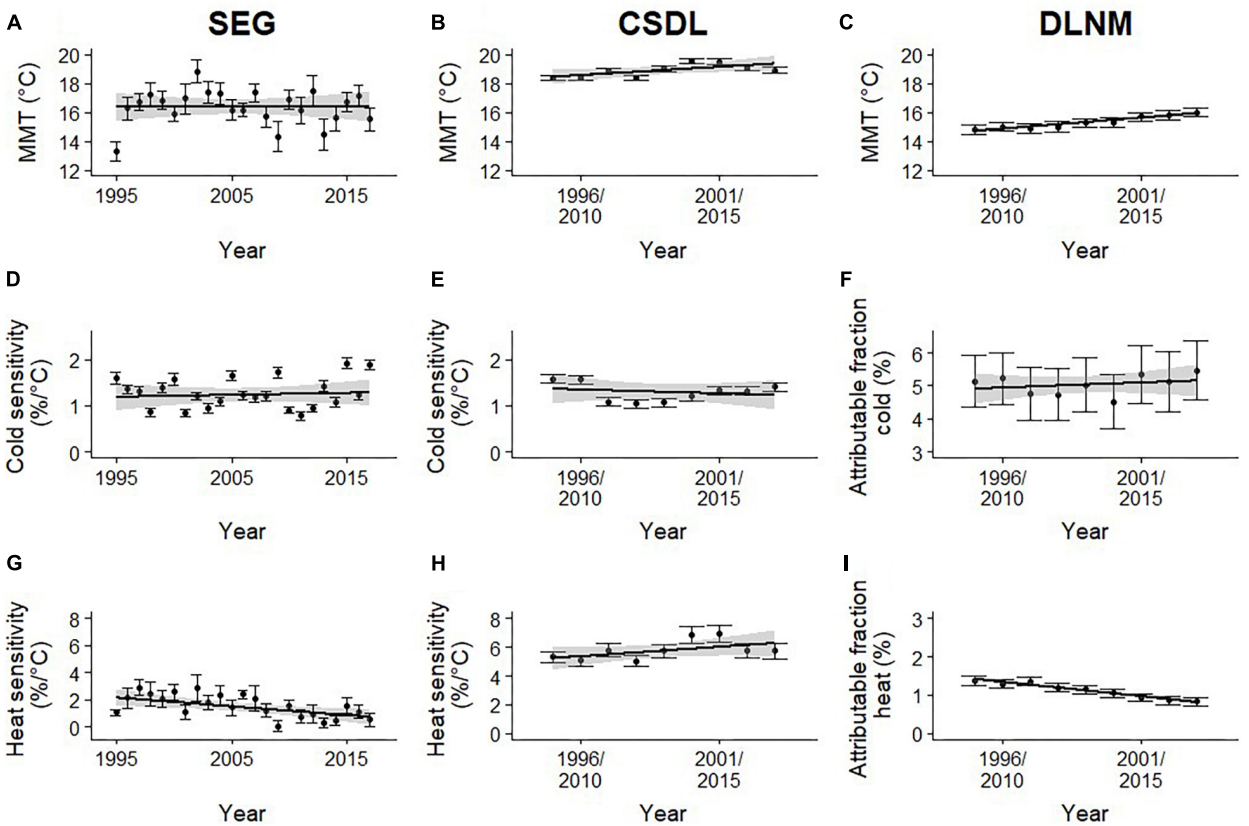

FIGURE 2 | Changes in minimum mortality temperature (MMT) **(A–C)**, cold sensitivity/attributable fraction (AF) **(D–F)**, and heat sensitivity/AF **(G–I)** with standard errors and gray shaded 95% confidence bands estimated by three different methods: Segmented Poisson regression (SEG) (left panel), constrained segmented distributed lag model (CSDL) (middle panel), and distributed lag non-linear models (DLNM) (right panel) of daily death counts for older adults (≥65 years) related to daily mean temperature for the 23-year period from January 01, 1995 to December 31, 2017 in the Netherlands.

(p = 0.96). Cold sensitivity did not change over time in both the CSDL (p = 0.57) and the SEG methods (p = 0.69). Heat sensitivity did not change significantly in the CSDL method (p = 0.12), but did decrease significantly with the SEG method (p = 0.01) with 0.06%/°C/year. No significant difference in cold AF is shown (p = 0.511), however, there was a significant decrease of 0.07%/year in heat AF ($p < 0.001$).

DISCUSSION

The aim of the current study was to investigate the change in MMT, and cold and heat sensitivity/AF over a 23-year period for older adults (≥65 years) in the Netherlands using three different methods (SEG, CSDL, and DLNM). Furthermore, the differences between the three methods were also analyzed to investigate the influence of the employed method on the results.

The calculated mean MMT and heat sensitivity over the 23-year period differed considerably between methods. The CSDL method resulted in the highest MMT (18.9 ± 0.45°C) and heat sensitivity (5.6 ± 0.6%). The high heat sensitivity is a result of the higher MMT, as only days with a higher temperature than the MMT are included in the calculation of the heat sensitivity. In other words, the data is refined to the steepest part of the mortality curve (**Figure 1**) resulting in a high heat sensitivity. The SEG method has a lower MMT (16.4 ± 1.2°C) and thus

mortality data is included of more moderate temperatures above this low MMT threshold. The MMT calculated with the DLNM method was the lowest of all three methods with 15.3 ± 0.4°C. The cold AF is higher than the heat AF with 5.0 ± 0.3% and 1.1 ± 0.2%, respectively, which can be explained with the fact that the average daily temperature in the Netherlands (10.5 ± 6.3°C) is below MMT and thus cold days are more prominent in the Netherlands. The differences in calculated MMT between methods are most likely due to the way the MMT is calculated in the models. Both the CSDL and DLNM method control for the day-of-week, annual and seasonal trends and consider lag temperature, while this is not the case in the SEG method. In addition, the DLNM method uses a non-linear approach, while both the SEG and CSDL method are linear. These results show that the used method has a large effect on the calculated MMT and the accompanying cold and heat sensitivity/AF. Comparability between studies employing different methods is therefore hampered.

The results of all three methods indicate that the susceptibility to heat in the Netherlands is declining over time. Two of the three methods (DLNM and CSDL) show an increase in MMT for adults of 65 years and older over the 23-year period (see **Figures 2A–C**). The SEG method does not show an increase in MMT, but does show a decrease in heat sensitivity over time from about 2% to 1% per degree Celsius. This indicates that less people die at similar heat exposure suggesting a gradual adaptation to heat. The CSDL method shows a slight increase in heat sensitivity, although not significant. This has to be considered in relation with the increasing MMT as the dataset for heat sensitivity will contain less moderate temperature days and increasingly more hot days. Therefore, it does not mean people are getting more susceptible to the heat based on the CSDL method. The same explanation accounts for the decrease in heat AF calculated with the DLNM method. As the MMT increases over time there are less days with a mean ambient temperature higher than the MMT and therefore less deaths are attributed to the heat. Cold sensitivity and AF does not change over the years for stable MMT (SEG) or for increasing MMT (CSDL and DLNM).

The observed increase of the MMT from 0.11 to 0.15°C/year, accompanied with mean daily temperature increases of about 0.03°C/year, is in line with previous studies (Todd and Valleron, 2015; Åström et al., 2016; Chung et al., 2018). In France the observed shift in MMT was lower than in the current study with 0.025°C/year for adults over 65 years old and an increase in summer temperature of 0.057°C/year (Todd and Valleron, 2015). In Sweden and Japan the shift in MMT was more comparable with the current study with, respectively, 0.08°C/year and about 0.12°C/year for the whole population (Åström et al., 2016; Chung et al., 2018). In Sweden the mean ambient temperature increased with 0.018°C/year over the observed period and in the study of Chung et al. (2018) the increase in mean ambient temperature was not reported. In the study of Todd and Valleron (2015) Generalized Additive Models were used and in the studies of Åström et al. (2016) and Chung et al. (2018) the DLNM method was used similar to our study. The difference in applied methods may explain the smaller observed shift in MMT reported for France. However, all studies, including the current study for the Netherlands, suggest human adaptation to climate change.

These human adaptations to the increasing ambient temperatures can be attributed to multiple factors, such as physiological, behavioral, technological adaptations or changes in infrastructure (Hondula et al., 2015). Repeated heat exposures lead to physiological adaptations in heart rate, body core temperature and sweat rate that slowly decay (Daanen et al., 2018), and thus may lead to a more or less permanent state of heat acclimation (Casadio et al., 2016). In line with this, it has been shown that mortality is considerably higher in the heat waves early in summer when compared to successive heat waves, probably partly due to heat acclimation in the subjects that survived the initial heat waves (Kysely and Kriz, 2008). Further, people born and raised in warm areas show reduced excess mortality in the heat when moved to relatively cold areas (Vigotti et al., 2006). Behavioral changes may occur because people become more aware of the impact of high ambient temperatures and raised awareness from the government. For example, since 2007 in the Netherlands, a heat health warning systems (HHWS) is activated if there is a high chance of five consecutive days with an ambient temperature exceeding 27°C (Lowe et al., 2011). The aim of the HHWS is to warn people when extremely high temperatures are expected and to give behavioral recommendations (e.g., drink more, reduce physical activity) during these days. Technological and infrastructural changes over the years include improved building insulation that reduces heat loss in the cold and prevents heating of the house in hot periods. Air conditioning is an effective way of reducing heat strain. In the Netherlands the air conditioner demand already increased with about 24% between 2012 and 2017 according to the Japan Refrigeration and Air Conditioning Industry Association (JRAIA, 2018), probably contributing to the observed reduction in heat susceptibility over the years. A long term adaptation to climate change observed in endotherms is an increase in the body surface to mass ratio to enhance heat loss (Gardner et al., 2011). In humans the body surface to mass ratio is higher in tropical than in cold areas (Katzmarzyk and Leonard, 1998). However, the body surface to mass ratio of the Dutch population shows a consistent linear decline over the investigated period (CBS, 2019b), so no signs of climate change related morphological changes are observed.

It has to be noted that the decreased susceptibility to heat over time may not only be related to climate change as suggested by Todd and Valleron (2015). Arbuthnott et al. (2016) showed that decreased heat susceptibility is a process that is not only visible in the last decades, but already started a century ago, when climate change was still negligible. Ten out of eleven included papers in their study found some evidence of decreasing susceptibility for heat over time. Cold susceptibility changes were negligible. It was argued that both planned adaptive measures, such as HHWS and improved buildings as well as adaptive behavior, improved health and treatment of heat casualties could explain the changes. Still, climate change may accelerate the adaptations as Todd and Valleron (2015) indicated.

Kinney (2018) argued that human adaptation should be better quantified and included in methods used for predicting

the effects of climate change on human survival. With ongoing climate change and associated adaptive processes, the temperature-mortality relationships on both sides of MMT may change, with the magnitude and direction of the change being uncertain. Future studies should focus on what particular factors, like the physiological, behavioral, technological or infrastructural changes mentioned before, are influencing the reduced susceptibility to the heat the most. Once the most effective factors are identified adaptation policies may be proposed accordingly.

In this study for the CSDL and DLNM method a 15-year sliding window was chosen, which covers quite an extensive part of the in total 23 years. Using sliding windows with fewer years resulted in larger volatility accompanied with a greater standard error, indicating a less precise MMT. However, previous studies like Åström et al. (2016) used a large sliding window as well of 30 years, although we are aware that they used a much larger total time period covering more than 100 years. Furthermore, data from the KNMI shows that an increase in ambient temperature is mostly present in the previous three decades, making it less relevant to use a dataset containing a longer time period than used in the current study (KNMI, 2018).

CONCLUSION

The susceptibility of humans to the heat decreases over time in the Netherlands, regardless which method was used, as human adaptation was shown by either an increase in MMT (CSDL and DLNM) or a decrease in heat sensitivity for unchanged MMT (SEG). Underlying factors for the reduced heat susceptibility may be due to physiological, behavioral, technological or infrastructural adaptations. Future studies should focus on what factor influences the human adaptation the most, so it can be promoted through adaptation policies. Further, future studies should keep into mind that the employed method influences the calculated MMT and, therefore, reduces comparability between studies using different methods.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

HD, MF, WB, and MM devised the study. HD, MF, and PB designed and conceived the analyses. CH, MF, and MM collected and organized the data. MF and PB performed the statistical analysis. MF and HD wrote the first draft. All authors interpreted the data, and, after critically reviewing and providing significant editing of its content, approved the final manuscript.

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