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The Impact of Heat Acclimatization Induction and Intermittent Exercise-Heat Exposures on Physiological Adaptations

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Background: Heat acclimatization (HAz) or acclimation (HA) is one of the most beneficial, heat illness prevention and performance strategies used during physical activity. However, the optimal method to maintain these methods are unknown. Purpose: To test the efficacy of a novel dual heat acc (DHA) induction protocol and to examine if there is a dose response relationship related to the frequency of intermittent heat training following HA on aerobically training athletes. Methods: Twenty-seven male endurance athletes (mean[M]±standard deviation[SD]; age, 34±12 years; height, 178.44±6.31 cm; body mass, 72.56±8.81 kg; VO$_{2\text{max}}$ 57.65±6.79 ml·kg$^{-1}$·min$^{-1}$) completed five tests (Un-acclimatized [Test$^{#1}$], following HAz [Test$^{#2}$], following HA [Test$^{#3}$], the middle of heat training (HT) [Test$^{#4}$] and the end of HT [Test$^{#5}$]) following HA that involved sixty minutes of steady state exercise (59.12±1.74% vVO$_{2\text{max}}$Test$^{#1}$) in an artificial environmental laboratory (M±SD; ambient temperature [T$_{\text{amb}}$], 35.42±1.06°C; relative humidity [%RH], 46.35±2.48%; Wet Bulb Globe Temperature [WBGT] 29.62±1.37°C; wind speed, 3.98±0.30 mph) on a motorized treadmill. The study, in its entirety, was approximately six months in length. Following Test$^{#3}$, participants were randomly assigned to three groups: control group with no heat exposures (HT$^{\text{CON}}$), once per week heat exposure group (HT$^{\text{MIN}}$), and twice per week heat exposure group (HT$^{\text{MAX}}$). Repeated measures ANOVA were utilized to determine differences in physiological variables between trials. Results: DHA resulted in significant mean differences in maximal HR (p<0.001), average HR (p<0.001), ending T$_{\text{rec}}$ (p<0.001), average T$_{\text{rec}}$ (p=0.001), delta T$_{\text{rec}}$ (p=0.026), sweat rate (p=0.033), and T$_{sk}$ (p<0.001) between Test$^{#1}$, Test$^{#2}$,
and Test\textsuperscript{#3}. At Test\textsuperscript{#5}, the highest trial HR was significantly higher in HT\textsubscript{CON} compared to HT\textsubscript{MAX} (M±SD, HT\textsubscript{CON}, 173.88±22.22 bpm; HT\textsubscript{MAX}, 151.00±16.52 bpm, p<0.05), but was not different than HT\textsubscript{MIN} (M±SD, 159.33 bpm). There were statistical differences between HT\textsubscript{CON} and HT\textsubscript{MAX} % change of rectal temperature from Test\textsuperscript{#3} (HT\textsubscript{CON} vs HT\textsubscript{MAX}, [95%CI] 0.46%, 2.7%; ES=1.37; p=0.009), but not between HT\textsubscript{MIN} (HT\textsubscript{CON} vs HT\textsubscript{MIN}, [95%CI] -0.26%, 2.8%; ES=0.85; p=0.098) at Test\textsuperscript{#5}. Conclusions: HT\textsubscript{MAX} (twice weekly heat training) provides clear evidence for the ability to maintain and possibly improve physiological adaptations following DHA. HT\textsubscript{MIN} (once weekly heat training) may be sufficient for some individuals to maintain gains made from DHA.
The Impact of Heat Acclimatization Induction and Intermittent Exercise-Heat Exposures on Physiological Adaptations

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M.S., Florida State University, 2016

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2020
The Impact of Heat Acclimatization Induction and Intermittent Exercise-Heat Exposures on Physiological Adaptations

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2020

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To my Lord and Savior, Jesus Christ. For being the most complete and perfect guide throughout this journey. I will use this experience to glorify your name all of the days of my life.

To my husband, Amos. Self-sacrifice is a quality that I have always hoped for in a partner. The day after we said “I do”, you personified this characteristic by moving to a completely new part of the country, hundreds of miles away from family and friends, to support my dream of pursuing a PhD. I’m happy to soak up every second of forever with you. Sweet Home Alabama!

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Ch.1 Study Overview

Exertional heat stroke is among the top three leading causes of death in sport and other exertional heat illnesses, including heat exhaustion, heat syncope, and heat cramps, are a prevalent and recurring issues across all levels of sport.¹ Heat acclimatization (HAz) (occurring in a natural environment) or heat acclimation (HA) (occurring in an artificial environment) is one of the most beneficial, simple heat illness prevention strategies athletic trainers, coaches, and sport scientists can implement to improve safety for athletes. For simplicity, the term “HA” will be used to interchangeability describe both HA and HAz, unless otherwise specified. The process of HA, which refers to gradually and systematically introducing exercise in hot environments, will enhance performance and reduce the risk of exertional heat illness.² Most notably, research has found that states mandating HAz in youth sports reduced heat illness prevalence by 55%.³ The physiological adaptations that occur with HA, including a reduction of internal body temperature, heart rate, sweat electrolyte concentration, and decreased rating of perceived exertion and sweat electrolyte concentration, drastically improves the thermoregulatory systems of athletes during intense exercise in the heat.² Limiting the rise of heart rate and internal body temperature is crucial for reducing heat illness risk, sustaining exercise, and mitigating an earlier onset of fatigue.³,⁴

HA minimizes the loss of electrolytes, which is crucial for maintaining adequate safety and thermoregulatory benefits during training and competing in heat.² Minimizing sodium loss is important for limiting heat-related muscle cramps and for lowering the risk of exercise-associated hyponatremia.⁵ In addition to this positive adaptation, the expansion of blood plasma volume contributes to improved thermoregulation by increasing stroke volume, which increases cardiac output that allows a larger amount of blood to be distributed to working muscles, which
leads to optimal performance and safety of the athlete. This positive adaptation is critical to the initial enhancement of one’s thermoregulation via delivery of blood to the periphery for the purposes of sweating and evaporation all while continuing one’s exercise. As a result of these known adaptations to exercise in the heat, many have turn to heat acclimatization to enhance performance.

A note on assessing sweat electrolyte concentration: Sweat electrolyte (sodium, potassium, chloride) concentration was assessed at the end of the testing session. The whole-body wash down technique was utilized for this assessment, which is the gold standard method. Participants brought their testing clothes into the lab more than 24 hours prior to testing. The clothes and towels that were used for the test were first washed in an automatic washing machine with detergent and then again without soap or detergent to remove electrolyte content from the fibers of the clothing. The clothes were then dried in an automatic dryer without fabric softeners or any other fabric care products. Just before entering the environmental chamber for testing, participants were instructed to shower without the use of soap or any other product to remove all electrolyte content from the surface of the skin. The participants then dried off with the electrolyte free towel and donned the electrolyte free clothes. Participants then underwent the exercise test and were instructed to continuously capture sweat throughout the duration of the test by using towels. Gloved researchers also assisted in the collection of the sweat during the exercise. Upon cessation of exercise, participants stood in a tarp lined tub and were rinsed with a known amount (2 gallons) of distilled water using the process described by Armstrong et al. Participants then left the tub and proceeded to the bathroom where they provided the exercising clothes to the researchers to add to the towels and sweat mixture in the tub. The clothes were
then thoroughly mixed in the tub and three samples were collected from the tub for analysis. The electrolyte concentrations were assessed from these samples (Medica, EasyLyte Plus, MA). HA is an impactful strategy that can be used to optimize performance and safety when competing in the heat, however, strategies to sustain these benefits throughout a competitive season are not well understood.2,7 There is evidence demonstrating the effectiveness of HAz in team sports, such as soccer, as well as individual sports and activities.8–11 However, the environmental conditions that athletes may compete in can greatly fluctuate due to the timing of the sport season or the travel involved with a given sport.12 For example, the environmental conditions in the northeastern United States may not drive the internal body temperature up enough to elicit a large magnitude of positive physiological adaptations.2 Therefore, additional more extreme heat exposures following HAz may allow athletes to gain even greater adaptations. Several studies have demonstrated that biomarkers of HA (rectal temperature, heart rate, sweat rate) decay without sufficient heat exposure.2,13,14 and a recent meta-analysis by Daanan et al. demonstrated that internal body temperature and heart rate responses were reduced ~2.5% per day without continued heat exposure.15

Although the time course of the gain and deterioration of the many benefits of HA are well-established, one study investigated the effectiveness of intermittent exercise-heat exposures to sustain the adaptions over an extended period of time.16 This same study investigated the implementation of an exercising heat exposure once every five days following HA induction.16 With this protocol, the physiological variables measured (heart rate, internal body temperature, sweat rate, and plasma volume) did not deteriorate to the same magnitude as the control group who did not participate in any intermittent exercise-heat exposure.16
No study to date has attempted to sustain the performance benefits of HA of endurance athletes, who are known to have an advantage over recreational athletes during exercise in the heat. Upon successful completion of this study, coaches and sport medicine professional could implement this intermittent-exercise heat exposure plan with their respective teams and athletes to optimize performance and safety.

Figure 1. Overall research aim.

The overall aim of this study is to provide coaches, sport medicine professionals, and athletes with a practical solution to maintain the benefits of HA throughout a competitive season. Although the decay of sweat electrolyte losses is unknown, the improvements in heart rate, internal body temperature, skin temperature, and sweat rate from HA are typically diminished after one week to one month with no heat exposure. The findings from this research will provide a practical, novel method for maintaining the benefits of HA to the current scientific literature and physically active community.
Specific Aims

Aim 1: Assess the effectiveness of self-directed summer training of endurance athletes followed by short-term HA on physiological variables during steady-state exercise in the heat.

Research Question #1a:
Does self-directed summer training of endurance athletes lead to improvements in physiological variables during steady-state exercise in the heat?

H1a: Self-directed summer training will elicit improvements in physiological adaptations known to improve from HA during steady-state exercise in the heat.

Research Question #1b:
Does a 5-day short-term HA induction protocol elucidate positive physiological adaptations following self-directed summer training of endurance athletes?

H1b: HA induction following self-directed

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<td><strong>Independent Variables</strong></td>
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<td>Post-Heat Acclimatization Trial</td>
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<td>Post-Heat Acclimation</td>
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<td>Post-Heat Acclimation</td>
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<td>Skin Temperature</td>
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summer training will demonstrate improvements of physiological variables known to improve from HAz during steady-state exercise in the heat. The environmental conditions in the northeastern united states are not be warm enough to elicit full HAz benefits, therefore, the short-term HA induction will allow athletes to fully acclimate.

**Research Question #2:** Does two intermittent exercise-heat exposures per week sustain the positive physiological adaptations (Trec, HR, etc) yielded from HAz induction during steady-state exercise in the heat more than one intermittent exercise-heat exposure per week or no intermittent exercise-heat exposures per week?

**Aim 2:** To test the efficacy of two intermittent exercise-heat exposure protocols to sustain improved physiological variables yielded from HAz.

**H1:** There is a dose response relationship with the number of weekly intermittent exercise-heat exposures on sustaining positive physiological adaptations yielded from HAz during exercise in the heat over the course of 8 weeks.
**Research Question #3a:** Can factors known to influence heat tolerance differentiate individual changes in internal body temperature, heart rate, and sweat rate following HAz and HA?

**Aim 3a:** Determine distinguishing factors for individuals who demonstrated improved Trec, HR, and SR following HAz and HA.

**H1a:** We hypothesize that individuals with greater adaptations to HAz and HA will demonstrate greater physiological stress prior to HAz and HA than the individuals who do not adapt.

**Methods and Techniques**

To examine the research aims, 36 aerobically trained male participants (maximal oxygen consumption $[\text{VO}_{2\text{max}}] \geq 45 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) were recruited to participate in this study. Data collection was completed between May 2019 and November 2019. All participants completed three $\text{VO}_{2\text{max}}$ tests, five lab tests, 5 days of short-term HA sessions. The timeline can be seen below:

Figure 2. Study timeline

$\text{VO}_{2\text{max}}$ Test: Participants were asked to don a heart rate monitor (H10®, Polar Electro™, Kempele, Finland) and compete a self-selected 5-minute warm-up. Following warm-up, participants completed a graded maximal exercise test on a treadmill (T150; COSMED,
Traunstein, Germany) at 2% grade to volitional exhaustion (TueOne, ParvoMedica, Sandy UT, USA). The mL of oxygen recorded during the final completed stage will be recorded as the VO$_{2\text{max}}$.

**Lab Exercise Test:** Following a 5-minute warm-up, participants were asked to run at 60% of their VO$_{2\text{max}}$ for one hour in hot environmental conditions (M±SD; ambient temperature [T$_{\text{amb}}$], 35.11±0.62°C; relative humidity [%RH], 47.61±0.38%; Wet Bulb Globe Temperature [WBGT] 29.53±0.63°C; wind speed, 4.02±0.12 mph). To ensure euhydration, upon arrival to the laboratory, urine specific gravity (Model TS400; Reichert Inc., Depew, NY, USA) (USG) (and urine color were assessed. If USG >1.020, the participant was asked to consume 500mL of water prior to the start of testing. If USG was > 1.025, the test was rescheduled for a different day. Fluid consumption was restricted during the testing session.

A description of the test can be seen in Figure 3.

Power Analysis: The sample size calculation was performed in G Power and is based on the variability of internal body temperature between two groups from a heat training intervention in a previous study examining the effectiveness of heat exposures every five days following HA. Internal temperature was 0.47°C with a 95% confidence interval of -0.24°C to 1.19°C and an effect size of 0.68 lower in the heat exposure group compared to the control group. For a two-sided test with 0.05 alpha level and desired power level of 0.8 the estimated sample size would be 24 participants total or 8 participants per group. Due to the nature of this study, participants were included in the statistical analysis if they complete all
maintenance sessions. Due to the time-line of this data collection, it was anticipated that some participants would have to drop out of the study due to illness, injury, time constraints, etc. To account for the dropout risk, 36 participants were consented and included in this study (~12 participants per group).

**Research Question #1**

**Research Question #1a:** Does self-directed summer training of endurance athletes lead to improvements in physiological variables during steady-state exercise in the heat?

**Research Question #1b:** Does a 5-day short-term HA induction protocol elucidate positive physiological adaptations following self-directed summer training of endurance athletes?

**Research Question #1 Methods**

*Self-Directed Summer Training:* Following an initial VO$_{2max}$ test and lab exercise test, participants were provided with a heart rate monitor (Polar H10) and instructed to download the Polar Flow and CoachMePlus phone application. Participants were instructed to continue their normal training plan throughout the entirety of the summer, tracking every training session with the heart rate monitor and phone application. Participants were also asked to enter their training rating of perceived exertion and clothing information worn during training into the CoachMePlus application.

*5 Day Short-Term HA:* To induce natural HA, participants exercised in an environmental lab (35°C ambient temperature, 50% RH) lab for five days. These sessions occurred within 8 days to ensure the full physiological adaptations of HA. A hyperthermic zone heat acclimation protocol was utilized. This protocol involves changes to exercise intensity to achieve an internal body temperature between 38.5°C and 39.75°C for 60 minutes.
Lab Testing: As described in Figures 2 and 3, the lab testing protocol was performed prior to the start of self-directed summer training (Test #1), after self-directed summer training (Test #2), and after the short-term HA protocol (Test #3).

Statistical Analysis: All data were reported as mean ± standard deviation, effect sizes, and 95% confidence intervals. The level of significance will be set at p<0.05. All statistical analysis was performed in a statistical software (SPSS, v.25. IBM Corporation, Armonk, NY). A repeated measures ANOVA was used to determine if there were differences between the testing time points for the dependent variables throughout the lab test.

Research Question #2

Research Question #2: Does two intermittent exercise-heat exposures per week sustain the positive physiological adaptations yielded from HAz induction during steady-state exercise in the heat more than one intermittent exercise-heat exposure per week or no intermittent exercise-heat exposures per week?

Research Question #2 Methods

Following lab test #3 (post short-term HA), participants were assigned to one of three groups. These groups were assigned and balanced based on VO$_{2\text{max}}$, age, and body mass. The three groups include: 1) One exercise heat exposure per week, 2) Two exercise heat exposures per week, 3) Control group without exercise heat exposure.
Heat Training: Participants in the one or two exercise heat exposure groups completed the same exercise protocol as during the short-term HA induction. All participants continued their self-directed training and will be instructed not to exercise in temperatures >72°F.

Lab Testing: As described in Figures 2 and 3, the lab testing protocol was performed after the short-term HA induction (Test³), 4 weeks after the short-term HA induction (Test⁴), and 8 weeks after the short-term HA induction (Test⁵).

Statistical Analysis: All data are reported as mean ± standard deviation, effect sizes, and 95% confidence intervals. The level of significance was set at p<0.05. All statistical analysis were performed in a statistical software (SPSS, v.25. IBM Corporation, Armonk, NY). A repeated measures ANOVA was used to determine if there were differences between the testing time points for the dependent variables throughout the lab test.

Research Question #3

Research Question #3: Are there distinguishing characteristics of individuals who demonstrate improved rectal temperature, heart rate, and sweat rate following HAz and HA?
Table 4. Factors examined to distinguish individuals who demonstrated improved rectal temperature, heart rate, and sweat rate following heat acclimatization and heat acclimation.

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Heat Acclimatization Training</th>
<th>Heat Acclimatization Environmental Conditions</th>
<th>Heat Acclimation Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2max</td>
<td>Total Training Time</td>
<td>Ambient Temperature</td>
<td>AUC</td>
</tr>
<tr>
<td>Body Fat Percentage</td>
<td>Average Heart Rate</td>
<td>Relative Humidity</td>
<td>AUC above 38.5°C</td>
</tr>
<tr>
<td>Age</td>
<td>Weekly Distance</td>
<td>Heat Index</td>
<td>Average Heart Rate</td>
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<tr>
<td>Body Mass</td>
<td>Total # of Sessions</td>
<td>Wet-Bulb Globe Temperature</td>
<td>Sweat Volume</td>
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<tr>
<td>Max Heart Rate</td>
<td>Running Training Time</td>
<td></td>
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<tr>
<td>Initial rectal temperature, heart rate, and sweat rate</td>
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**Statistical Analysis:** Based on positive vs negative absolute change, we distributed participants into two groups for each physiological response ($T_{rec}$, HR, and SR). Our approach is unique in that it considers the practical application and evaluates the physiological mechanisms that might distinguish success or failure in response to HAz or HA. The detriment to safety and performance during exercise in the heat with an elevated $T_{rec}$ has been well-established.\(^{24-26}\) Similarly, an elevated HR typically leads to earlier onset of fatigue and lower performance and this is exacerbated during exercise in the heat.\(^{27,28}\) Finally, one of the primary mechanism to achieve a lower $T_{rec}$ and HR following HAz or HA is an increase in SR.\(^{29,30}\) An increase in $T_{rec}$ or HR and a lower SR is not considered successful HAz or HA.

To ensure physiological differences between the groups, a repeated measures ANOVA with post-hoc comparisons were performed for each variable at each test. To examine differences in those that demonstrated improvement and no improvement of $T_{rec}$, HR, and SR, independent t-test were performed for each participant characteristic, training metric, and environmental
condition from HAz and for AUC, AUC$^{38.5}$, HA average HR, and HA sweat volume from HA. Cohen’s d effect sizes (ES) were calculated to quantify the magnitude of pairwise differences. ES was interpreted according to the following thresholds: < 0.2 = trivial, 0.2–0.6 = small, 0.7–1.1 = moderate, 1.2–2.0 = large, and > 2.0 = very large.$^{31}$ Statistical significance was set at p<0.05, a priori. Data are reported as M±SD, 95% confidence intervals (95%CI) and effect size (ES). All statistical analyses were completed using SPSS Statistics for Mac, version 25 (IBM Corp., Armonk N.Y., USA).

**Human Subjects Research**

Human subjects will be participating in this study. The institutional review board (IRB) has approved a version of this protocol. The IRB protocol number is H19-015.
Ch. 2 Literature Review

Purpose

Intense physical activity in the heat occurs often places athletic and military populations at risk for experiencing exertional heat illness and not performing at their best. Major sporting events, such as the 2020 Tokyo Olympics and the 2022 FIFA World Cup in Qatar, are of grave concern for elite athletes and spectators due to the extreme environmental conditions of these venues. Recreational athletes, youth athletes, and military personal are also severely impacted by exercise in the heat and are at risk for experiencing exertional heat illnesses. In an attempt to reduce this risk, several heat mitigation strategies have been investigated, including the use of cooling modalities, hydration strategies, and heat acclimation (HA).

HA or heat acclimatization (HAz) are terms used to describe systematic and repetitive exposures to hot/humid environmental conditions to induce positive physiological adaptations. “HA” typically refers to artificial heat exposure, such as that in a laboratory setting or sauna and “HAz” refers to heat exposures that occur outside in a natural environmental, such as during American football preseason. Throughout the remaining sections of this literature review, “HA” will be used as an all-encompassing term to describe both HA and HAz. The next sections of this literature review will cover topics specifically related to the research aims in Chapter 2, including 1) physiological adaptations that occur throughout HA, 2) factors that determine HA outcomes and 3) HA decay and prevention.

Physiological Adaptations of HA

Heat Balance Equation

The balance between the heat produced internally through metabolic functions and the environment determine the physiological outcomes observed during exercise in the heat. Heart
rate and internal body temperature rises with exercise intensity, which results in vasodilation, leading to an elevated skin temperature and initiation of the sweating response. Sweating, through evaporation into the environment, is the primary mechanism of heat dissipation for humans.\textsuperscript{37} In extremely hot/humid environments in which sweat cannot easily evaporate, metabolic heat production can be greater than heat dissipation through the evaporation of sweat, which will ultimately result in heat gain or a rise of internal body temperature. This phenomenon is demonstrated in the heat balance equation seen below, with these specific mechanisms highlighted in green:

\[
M - W = E + R + C + K + S
\]

\(M\) = metabolic heat production

\(W\) = Work

\(E\) = Evaporation

\(R\) = Radiation

\(C\) = Convection

\(K\) = Conduction

\(S\) = Internal body heat

Unlike heart rate, humans can reach extremely dangerous internal body temperatures without before reaching volitional fatigue. For this reason, exertional heat illness occurs in sport, military, and occupational settings. The most extreme heat illness is called exertional heat stroke, defined as an internal body temperature \( \geq 104.5 \) with central nervous system dysfunction can be deadly without the gold-standard treatment. Most notably, research has found that states that mandating HA reduced heat illness prevalence by 55\%.\textsuperscript{3} The most beneficial adaptation of HA
from a safety standpoint, involves the lowering of internal body temperature, through improved central and peripheral sweating mechanisms. While the importance of the prevention of heat illness should not be overstated, perhaps the most widely applicable concept of HA is to optimize performance. Time trial, time to exhaustion, mean power output, peak power output, and VO$_{2\text{max}}$ performance have all demonstrated improvements following HA, although several HA methodological factors influence these outcomes and will be discussed below.\textsuperscript{38,39} While elevated internal body temperature alone has resulted in decreased performance\textsuperscript{40} the expansion of plasma volume, lowering of heart rate, decreasing skin temperature, increasing sweat rate, and decreasing sweat electrolyte concentration are all positive adaptations that occur with HA that lead to enhanced performance outcomes. Limiting the rise of heart rate and internal body temperature is crucial for reducing heat illness risk, sustaining exercise, and mitigating an earlier onset of fatigue.\textsuperscript{3,4} These adaptations work in congruence with one another throughout HA and the mechanisms behind them are discussed in detail below.

*Adaptations of Internal Body Temperatures*

The most marked physiological adaptation of HA is a lower internal body temperature at a given exercise intensity.\textsuperscript{38} This response has been observed historically since 1938 by E.F. Adolph.\textsuperscript{41} The body’s response to an elevated internal body temperature is controlled through warm-sensitive neurons in the preoptic anterior hypothalamus,\textsuperscript{42} as afferent signals are sent there from the skin and blood. Homeostasis is the ultimate goal of this thermoregulatory system, therefore, when a certain “set-point” temperature is not met, attempts to achieve homeostasis in the body begin.\textsuperscript{43} Efferent signals are sent through vasodilation and sweating at the skin, which
will be discussed in detail in the next section. This adaptation is one of the first to complete, with approximately 95% of the adaptations occurring 5-8 days into a HA plan.

Adaptations of the Skin and Sweat Gland

When humans begin exercising, the working muscles produce heat through metabolism. The blood surrounding these muscles is then heated through convection and will travel to the skin, resulting in vasodilation. Depending on the relative humidity of the environment, the body dissipates between 30-100% of its heat through evaporative cooling.\textsuperscript{23} This mechanism works by increasing skin wettedness and evaporation of the fluid from the microclimate created on the skin's surface.\textsuperscript{37} A rise in internal body temperature is the primary signal that initiates sweating\textsuperscript{44} and through HA, the threshold temperature to induce the sweating response is lower than before HA.\textsuperscript{23,45} While internal body temperature is the primary signal to initiate sweating, central mechanisms, mean and local skin temperature changes and local changes at the sweat gland, are also known to modify this response.\textsuperscript{44,46}

Two neural pathways are responsible for acting on the vasomotor control of the skin.\textsuperscript{47,48} The sweat glands responsible for cooling the body during exercise in the heat are innervated by sympathetic adrenergic vasoconstrictor neurons and sympathetic cholinergic vasodilator neurons.\textsuperscript{47,48} The combination of the withdrawal of the vasoconstrictor nerves with the increase in the vasodilator nerves leads to rapid response to activity in the heat.\textsuperscript{44} There is not a complete understanding of the neurotransmitters that are responsible for this vasodilation but nitric oxide, histamine, prostaglandins, and others have been speculated to play a role.\textsuperscript{49,50} When examining the relationship between skin blood flow and internal body temperature, it appears that the slope is not effected from that at rest, meaning as internal temperature rises, so does skin blood flow.\textsuperscript{51}
As expected from their relationship to skin blood flow, eccrine sweat glands are activated by the release of the neurotransmitter, acetylcholine, from the sympathetic cholinergic nerves that binds to receptors on the glands. As previously mentioned, skin blood and local temperature are independently capable of modifying sweat rate, however, this has only been thoroughly investigated through passive heat at rest. Besides an elevated internal body temperature, the onset of sweating has been observed at the very beginning of exercise without any rise in internal body temperature. A CNS response has been proposed as a mechanism for this response. Additionally, local receptors that make up the sweat gland may also contribute to this onset of sweating. Normative sweat rate data was recently published, with male adult athletes reporting 1.24 L·hour\(^{-1}\) and female adult athletes reporting 0.92 L·hour\(^{-1}\). Although, HA was not taken into account for this data. The time course for increased sweat rate has historically been reported to occur between 8-14 days.

In addition to adaptations in fluid losses through sweat, HA also minimizes the loss of electrolytes, which is crucial for maintaining adequate safety and thermoregulatory benefits during training and competing in in heat. Minimizing sodium loss is important for limiting heat-related muscle cramps and for lowering the risk of exercise-associated hyponatremia. Normative data for sweat sodium concentrations was recently published, with male athletes reporting an average 37.4 mmol·L\(^{-1}\) of sodium losses and female athletes reporting on average 34.2 mmol·L\(^{-1}\) of sodium. Several previous research studies have examined and demonstrated that HA results in sodium and chloride preservation, however, most HA research thus far has only examined these changes using a sweat patch for collection. Due to regional body differences in sweating responses, the gold-standard method for determining sweat electrolyte concentration is the whole-body wash down technique.
sodium and chloride concentration following approximately 10 days of HA is 30-60%, although the method of sweat collection and the method of HA could contribute to the wide range of adaptations observed with this variable. Ninety-five percent of this adaptation will be seen in 5-10 days of HA, depending on several methodological factors.35,36

**Adaptations of the Cardiovascular System**

From an exercise performance perspective, the cardiovascular adaptations that occur from HA are perhaps some of the most valuable. Reaching a maximal heart rate or sustaining an elevated heart rate for a substantial period of time will eventually result in volitional fatigue and the cessation of exercise. HA results in adaptations that allow for the same amount of physical work to be done at a lower cardiovascular cost. One reason for this is the expansion of plasma volume. Plasma volume expands through the increase of total body water, as it is hypothesized that there is an increase in the production of albumin, which leads to water moving from the interstitial space and into the intravascular space.44 This adaptation results in greater cardiac filling and increased stroke volume, which will ultimately result in a lower heart rate at a given exercise intensity. Performance improvements have been observed in maximal aerobic performance, both in hot and cool environmental conditions, following HA.61 The authors postulate that one of the main mechanisms behind this performance improvement is the expansion of plasma volume.61 Unlike the skin and sweat adaptations, 95% of the cardiovascular adaptation, including both plasma volume expansion and heart rate, occurs 3-6 days into traditional HA.36
Factors that Determine HA Outcomes

Physiological Outcomes: A Case for Hyperthermic Zone HA

While there is clear evidence that HA results in several physiological adaptations, the wide range of the magnitude and time course of these physiological responses could be due to the wide range of variability in program designs. A plethora of external and internal factors that could explain the variability in these outcomes is examined in detail below. A review by Taylor et al. stated that the optimal HA approach is yet to be determined. While the optimal approach is still unknown, a concept known the area under the $T_{rec}$ curve could help explain some of the discrepancies.

The concept of the area under the curve in HA is demonstrated in Figure 4. This model demonstrates the calculations of internal body temperature area under the curve throughout a single HA session. Theoretically, the higher the area under curve, the greater the physiological outcomes to HA. This concept is based on the overload principle training, in which the body adapts to the stresses it is placed under.

The following factors have been proposed to contribute to discrepancies observed in HA literature and are considered variables one should consider when designing a HA program. While these factors are all important to consider, this theoretical model points to the possibility
of greater adaptation with as much area under the curve as safely possible in a HA regimen. Manipulating these factors to achieve the greatest possible area under the curve, practically, could be the optimal way to achieve HA.

Method Types: There are three commonly used methods of HA programs: 1) isothermal, 2) fixed-work rate, 3) self-paced. The concept of the isothermal method, also termed “controlled hyperthermia, was first introduced by Fox et al. in 1963. Since its introduction, this method has been developed into the concept of maintaining a critical core temperature of 38.5°C throughout a HA protocol by adjusting exercise intensity. Some argue that this critical threshold is arbitrary in nature and that 38.5°C was chosen for no reason other than it guarantees hyperthermia. A recent publication aimed to examine if there were differences in varying degrees of controlled hyperthermia by testing two critical temperature thresholds: 38.5°C and 39°C and observed no differences. However, the limitation to this method is that athletes experience internal body temperatures well above either of these critical thresholds and this method may not elicit the full HA adaptations that athletes need when performing intense exercise in extreme environments. The fixed-work rate method is defined as setting an exercise steady intensity (for example, 50% VO2max) for throughout a HA session. While this method can elicit a greater thermal stress internal body temperature, the limitation to this method is that if the workload is hard enough to drive internal body temperature, the session must stop when the laboratory cut-off point is reached. Alternatively, if the intensity is to low, the drive to reach elevated temperatures will take a long time, which is not practical for athletes. Self-paced exercise has also been examined in previous research, however, this method allows the athlete to self-select intensity which could result in a reduced thermal, cardiovascular, and relative work-load. Recently, a new method known as “clamped heart rate” was proposed as an alternative, practical
strategy for coaches and athletes to utilize. This method simply involves adjusting exercise intensity to maintain a fixed heart rate throughout exercise. Similar to both limitations of the isothermal method and the fixed-work rate method, clamped heart rate could result in lower or higher internal body temperatures than desired.

Length: The typical length of a HA program ranges from 3-14 days and has previously been categorized into short-term ("7 days), medium-term (8-14 days), and long term (≥14 days). While a meta-analysis has stated that short-term HA will not elicit sweating responses (the last adaptation to occur in HA), arguments have been made for this length in highly trained athletes. Keeping in mind the area under the curve concept, this study utilized the isothermal protocol in 40°C ambient temperature with 60% relative humidity to elicit these adaptations.

Session Duration: The session duration of HA protocols ranges from approximately 30-120 minutes, depending on the method utilized. Studies that have utilized 30 minute protocols utilized either isothermal, or high (>70%VO₂max) fixed-work rate protocols. Previous research has stated that the minimal thermal load is a temperature at 38.5°C for 60 minutes.

Frequency: HA programs have been completed on consecutive days and intermittently, with days off between sessions. Previous research has examined the impact of two HA sessions per day compared to one and concluded that there were no differences between the groups. While these findings are a start to answering these questions, the duration and method of HA should be more thoroughly examined and future research is needed in elite athletic populations.
Mode: The mode of exercise of a HA sessions is strongly dependent on the ultimate goal of HA and the feasibility of specific protocols must be examined. For example, American football players may experience HA during practice throughout pre-season.

Internal Factors:

Age: Early literature investigating age impacts on HA reported that young participants had a better response compared to older participants. However, this study did not control for important factors known to influence heat tolerance, such as aerobic fitness level. In 1988, Pandolf et al. matched middle age participants to young participants with VO$_{2\text{max}}$ and this seemed to level out the differences post HA. However, there were differences prior to HA between the groups with the middle age men outperforming their younger counterparts. Pandolf noted that the middle age men had a much higher training volume than the young group and that training volume could impact heat tolerance, independent of VO$_{2\text{max}}$. In 2014, a study was released that assessed young and older cyclists pre and post HA. These highly trained participants were matched and the authors concluded that the older well-trained individuals did achieve the same cardiovascular adaptations as their younger counterparts, however, internal body temperature and sweat loss was not impacted by age.

Aerobic Fitness: It is well-established that aerobic fitness also contributes to thermal tolerance and this concept remains true in terms of HA. In 1977, Pandolf et al. demonstrated that aerobic fitness and time for rectal temperature to plateau throughout HA were significantly related ($r = -0.68$).
Sex: While the purpose of this literature review is not investigating sex on HA, it is important to note that little is known about this topic. Due to the impacts of menstrual cycle status on internal body temperature, it is difficult to investigate a female population in the state of current literature. Because HA involves acute repeated heat exposures, it is difficult to test females, put them through a HA protocol, and test them again all while in the same phase of the menstrual cycle. A few studies have attempted to study females throughout HA, however, the authors note this limitation. ⁸⁷⁻⁹⁰

Understanding the interplay of all of these factors is critical in conceptualizing HA outcomes. The conceptualization of maximal thermal load is presented in a hypothetical example below (Figure 5). The concept of this figure was derived by Gibson et al.⁴

Figure 5. AUC of various HA protocols.

The three methods presented above describe isothermal (maintain a temperature at 38.5°C), fixed exercise intensity (%VO₂max), and the proposed method, maximal hyperthermia. In this hypothetical example, all three methods begin with an exercise intensity and
environmental conditions that elicit 0.5°C rise in internal body temperature every 5 minutes. In the isothermal method, when the temperature reaches 38.5°C, the exercise intensity is continuously adjusted to maintain that internal body temperature. In the fixed-exercise intensity method, the rate of rise continues and the exercise stops at 35 minutes due to the laboratory cut-off point for internal body temperature being reached (40°C). The maximal hyperthermia method involves allowing an individual to reach a substantially elevated body temperature (39.0-39.75) and adjusting the exercise intensity to maintain throughout a designated time (in this case, 90 minutes). Based on the area under the curve calculations described above, these three methods of HA elicit substantially different thermal loads. To put this theory into context, the area under the curve calculations for one HA session of these three examples are as follows:

Isothermal=3453.75
Fixed-Exercise Intensity=9560.63
Maximal Hyperthermia=41,908.13

When these calculations are carried out across 5 days (short-term HA), the thermal load using the maximal hyperthermia method is 209,540.63. In order to achieve the same thermal load as maximal hyperthermia with the fixed-exercise intensity method, it would take 21.92 days. It would take 60.67 days of completing the isothermal HA protocol to achieve the same thermal load as maximal hyperthermia.

These methods of HA are useful in a lab setting, but the question of external validity remains. While several studies have investigated the impacts of HAz, none have attempted to control internal body temperature throughout the process and were simply examining changes
following a bout of sport specific training, such as pre-season.\textsuperscript{8,11,12} The driving forces of internal body temperature are exercise intensity and environmental conditions.\textsuperscript{63} For practitioners attempting to utilize HAz in the field, future research is needed to determine exactly how that may be achieved the most effectively and safely. Because the activities during many sport sessions are decided by a coach and are somewhat self-controlled by the athletes, the environmental conditions may play a large role to increase internal body temperature. It is expected that internal body temperature will be elevated in warmer environmental conditions when exercise intensity is held constant, therefore, the magnitude of adaptations will be greater in locations with these environments. Future research is needed to determine how these findings can be utilized in a field setting.

**Heat Acclimation Decay**

While, HA is evidently an impactful strategy that can be used to optimize performance and safety when competing in the heat, strategies to sustain these benefits throughout a competitive season are not as well understood.\textsuperscript{2,7} There is evidence demonstrating the effectiveness of HA in team sports, such as soccer, as well as individual sports and activities.\textsuperscript{8–11} However, the environmental conditions that athletes may compete in can greatly fluctuate due to the timing of the sport season or the travel involved with a given sport.\textsuperscript{12,91} Several studies have demonstrated that biomarkers of HA (rectal temperature, heart rate, sweat rate) decay without sufficient heat exposure.\textsuperscript{2,13,14} A recent meta-analysis by Daanan et al. demonstrated that internal temperature and heart rate responses that were improved by HA reduced ~2.5% per day without continued heat exposure.\textsuperscript{15}

Heart rate values are highly dependent on the adaptations that occur through skin blood flow because when exercise begins in a hot environment, the skin and the working muscles are
competing for more blood (O₂) to continue metabolism.⁹² HA improves the ability to sustain cardiac output, however, the effectiveness of this adaptation (as with all of the other adaptations) depends on the exercise intensity.⁹² A lower heart rate and increased stroke volume is likely linked to improvements in myocardial autonomic tone through the improvements in central and local (arterial baroreflexes and chemoreflexes) commands.⁹²,⁹³ In fit soldiers, Pandolph et al. showed a 2-29% decay in heart rate after 12 and 18 days of HA decay.⁸⁶ Improved heart rate is also most likely related to expanded plasma volume that occurs with HA. Plasma volume expands with HA through the expansion of total body water, as it is hypothesized that there is an increase in the production of albumin, which leads to water moving from the interstitial space and into the intravascular space.⁴⁴ Very few studies have assessed the decay of plasma volume following HA induction.⁹⁴,⁹⁵ Garrett et al. did not observe changes in plasma volume during HA so the decay couldn’t really be assessed.⁹⁴ Neal et al. did not show any changes in plasma volume after 7 days without heat exposure.⁹⁵

In a maintenance study, they observed ~50% decay in heart rate following a maintenance protocol compared to the control who lost more than 150%.⁹⁶ Due to the stimulation of sweating and skin blood flow,¹⁸ improved evaporative cooling,¹⁹ greater cardiac stability,²⁰ changes in fluid dynamics,²¹ earlier onset of sweating,²²,²³ and greater sweat sensitivity,²⁴ aerobically trained individuals appear to show partial HA benefits.⁸ While there are several variables to consider (such as length of HA, heat stress of HA, and testing methods before, during, and after maintenance), the timeline of decay of rectal temperature appears to be happening later and aerobic training status might play a role.⁴⁵,⁸¹,⁸⁶ A review of the literature pointed to the study that demonstrated the smallest delay (<20%) after 20 days.⁹⁷,⁹⁸ The average VO₂max of these participants was 55 ml·kg⁻¹·min⁻¹ and they were considered “active”.⁹⁸ A study that has shown
one of the fastest decays for this variable (40%) after 7 days, recruited individuals who were not aerobically trained and their VO$_{2\text{max}}$ was 51 ml·kg$^{-1}$·min$^{-1}$. When a 10-day, isothermal method of HA induction was used, decay in core temperature was minimal after 26 days. These data, along with the fact that the isothermal method of HA demonstrates the strongest data for the slowest decay, indicates that heat exposure every 7th day might be enough to maintain the majority of benefits of HA. In the single maintenance study that has been published, two 60 minutes bouts of exercise at 45% VO$_{2\text{max}}$ with a ten minute break in between was elicited every 5 days (in the heat for the intervention group and in room temperature for the control group) resulted in ~15-20% decay in rectal temperature, but was much better than the control group who lost ~70% of their rectal temperature adaptations. These participants were deemed recreationally active and their VO$_{2\text{max}}$ was 55 ml·kg$^{-1}$·min$^{-1}$. Aerobic fitness may also play a role in one’s ability to continue exercise at higher internal body temperatures than untrained individuals.

The positive sweating response seen from HA is mainly driven by an earlier onset of sweating at lower skin and internal body, temperatures which assists with evaporative cooling earlier during exercise, however, there are other central and local (metabaroreceptors) mechanisms that might contribute to the discrepancy in the maintenance of this variable at rest. Due to the many day-to-day and individual variations (training status, age, sex, ethnicity) in individual sweat rates, this variable is hard to quantify in HA induction, which makes it even harder to quantify for decay studies and limited research has been done in this area. The improved conservation of sodium through adaptations at the sweat gland will result in more dilute sweat following HA, however, no research has investigated how this specific adaptation is maintained following HA.
The results on the decay of skin temperature following HA are conflicting. The slowest decay in this variable was seen following 10 days of isothermal HA induction. The fastest decay seen in this variable was observed in a paper by Stephens and Hoag who used 10 days of a fixed exercise intensity for 100 minutes. Similarly, little research has been done investigating skin blood flow adaptations following HA induction, and very little on its decay. Maintenance of adaptations to skin blood flow would be expected to respond in a similar way as sweating since both are linked through the sympathetic nervous system. The few studies that have investigated skin blood flow throughout HA found that an increase in skin blood flow was initiated earlier, which would be the mechanism behind the decreased skin and internal body temperature since the body is more effectively cooling through evaporative heat loss.

Although the time course of the gain and deterioration of the many benefits of HAz are well-established, limited research has investigated the effectiveness of intermittent exercise-heat exposures to sustain the adaptations over an extended period of time. One study investigated the implementation of an exercising heat exposure once every five days following HA induction. With this protocol, the physiological variables measured in this study (including heart rate, internal body temperature, sweat rate, and plasma volume expansion) did not deteriorate to the same magnitude as the control group who did not participate in any intermittent exercise-heat exposures.
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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$_{2\text{max}}$), 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$_{2\text{max}}$ ($n=204$), 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$_{2\text{max}}$ (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
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), typically occurs within 5–10 days of HA and increase in sweat rate and plasma volume are all positive adaptations that occur throughout HA (Périard et al., 2015; Casado 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\textsubscript{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\textsubscript{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 1h 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, sport 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$_{2\text{max}}$, 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$_{2\text{max}}$" - 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 $>40^\circ$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$_{2\text{max}}$, (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$_{2\text{max}}$ 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$_{2\text{max}}$, "moderate" between 55 and 70% VO$_{2\text{max}}$ or isothermal, and "high" being greater that 70% VO$_{2\text{max}}$ 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 $38.5^\circ$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 ($\geq 25^\circ$C) or thermoneutral ($<25^\circ$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$_{2\text{max}}$ tests, nine were performed in thermoneutral...
FIGURE 1 | Flow chart summary of the study selection process.
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_{2\text{max}} (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_{2\text{max}}, 53.7 ± 6.8 ml·kg^{-1}·min^{-1}; time to exhaustion 49.2 ± 8.1 ml·kg^{-1}·min^{-1}; time trial, 52.3 ± 9.0 ml·kg^{-1}·min^{-1}; mean power, 53.8 ± 9.2 ml·kg^{-1}·min^{-1}; peak power, 53.1 ± 7.4 ml·kg^{-1}·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]; 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, 0.57 [0.05, 0.68], p < 0.001; VO_{2\text{max}}, 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 1 | VO_{2\text{max}} descriptive table.

<table>
<thead>
<tr>
<th>References</th>
<th>n</th>
<th>Induction method*</th>
<th>Induction length (Days)</th>
<th>Session duration (min)</th>
<th>Exercise intensity*</th>
<th>Heat index induction</th>
<th>Heat index testing</th>
<th>Baseline fitness (ml·kg^{-1}·min^{-1})</th>
<th>PEDro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horstman and Christensen (1982)</td>
<td>A &amp; B</td>
<td>Controlled work rate</td>
<td>11</td>
<td>68</td>
<td>Low</td>
<td>54</td>
<td>N/A</td>
<td>5.6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>King et al. (1985)</td>
<td>10</td>
<td>Controlled work rate</td>
<td>8</td>
<td>90</td>
<td>Low</td>
<td>43</td>
<td>43</td>
<td>46.1</td>
<td></td>
</tr>
<tr>
<td>Piknerik et al. (1987)</td>
<td>16</td>
<td>Controlled work rate</td>
<td>6</td>
<td>90</td>
<td>Moderate</td>
<td>43</td>
<td>43</td>
<td>44.2</td>
<td></td>
</tr>
<tr>
<td>Fobbrho et al. (1994)</td>
<td>13</td>
<td>Controlled work rate</td>
<td>7</td>
<td>90</td>
<td>Low</td>
<td>43</td>
<td>39</td>
<td>68.1</td>
<td></td>
</tr>
<tr>
<td>Aoyagi et al. (1998)</td>
<td>A, B, C, &amp; D</td>
<td>Controlled work rate</td>
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<td>60</td>
<td>Low</td>
<td>43</td>
<td>43</td>
<td>47.4</td>
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<tr>
<td>Nunez et al. (2013)</td>
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<td>5</td>
<td>35</td>
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<td>53.0</td>
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<tr>
<td>Molloy et al. (2013)</td>
<td>A &amp; B</td>
<td>Controlled work rate</td>
<td>14</td>
<td>30</td>
<td>High</td>
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<td>22</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Kalsor et al. (2015)</td>
<td>8</td>
<td>Controlled work rate</td>
<td>10</td>
<td>90</td>
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<td>39</td>
<td>39</td>
<td>58.1</td>
<td></td>
</tr>
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<td>Dlisu et al. (2016)</td>
<td>10</td>
<td>Controlled work rate</td>
<td>5</td>
<td>90</td>
<td>Low</td>
<td>47</td>
<td>47</td>
<td>50.0</td>
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<tr>
<td>Neal et al. (2016a)</td>
<td>Isothermal</td>
<td>5</td>
<td>90</td>
<td>Moderate</td>
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<td>Neal et al. (2016b)</td>
<td>A &amp; B</td>
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<td>90</td>
<td>Moderate</td>
<td>55</td>
<td>20</td>
<td>56.9</td>
<td></td>
</tr>
<tr>
<td>James et al. (2017)</td>
<td>10*</td>
<td>Isothermal</td>
<td>5</td>
<td>90</td>
<td>Moderate</td>
<td>50</td>
<td>37</td>
<td>58.9</td>
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<tr>
<td>Randell et al. (2017)</td>
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<td>11</td>
<td>90</td>
<td>Moderate</td>
<td>55</td>
<td>22</td>
<td>58.5</td>
<td></td>
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<tr>
<td>Willmott et al. (2018)</td>
<td>A &amp; B</td>
<td>Isothermal</td>
<td>10</td>
<td>60</td>
<td>Moderate</td>
<td>47</td>
<td>21</td>
<td>48.7</td>
<td></td>
</tr>
</tbody>
</table>

*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_{2\text{max}}, “moderate,” which was isothermal or 55–70% VO_{2\text{max}}, and “high,” which was >70% VO_{2\text{max}}.

* Included four females.

* Included one female.
defined by a pre-determined internal body temperature. The magnitude of change seen in time to exhaustion following HA induction method significantly impacted the magnitude of change seen in time to exhaustion following HA induction, however, no variance in the results was explained by this model (coefficient [95% CI]; exercise intensity, 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 = 0.28; moderate intensity, −0.25 [−0.65, 0.18], p = 0.26; induction method, −0.08 [−0.53, 0.37], p = 0.74; 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 2 | Time to exhaustion descriptive table.**

<table>
<thead>
<tr>
<th>References</th>
<th>n</th>
<th>Induction method*</th>
<th>Induction length (Days)</th>
<th>Session duration (min)</th>
<th>Exercise intensitya</th>
<th>Heat index induction</th>
<th>Heat index testing</th>
<th>Baseline fitness (ml·kg⁻¹·min⁻¹)</th>
<th>PEDro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horstman and Christensen (1982)</td>
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<td>Controlled work rate</td>
<td>11</td>
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<td>Low</td>
<td>44</td>
<td>44</td>
<td>51.4</td>
<td>5</td>
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<td>Pandolf et al. (1988)</td>
<td>4</td>
<td>Controlled work rate</td>
<td>11</td>
<td>108</td>
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<td>44</td>
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<td>47.2</td>
<td>5</td>
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<td>Nielsen et al. (1993) A &amp; B</td>
<td>13</td>
<td>Controlled work rate</td>
<td>10.5</td>
<td>61</td>
<td>Moderate</td>
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<td>37</td>
<td>59.0</td>
<td>6</td>
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<td>Nielsen et al. (1997)</td>
<td>12</td>
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<td>48.3</td>
<td>Low</td>
<td>61</td>
<td>61</td>
<td>62.0</td>
<td>4</td>
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<tr>
<td>Ayogil et al. (1998)</td>
<td>6</td>
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<td>6</td>
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<td>Low</td>
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<td>43</td>
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<td>Inoue et al. (1999) A, B, &amp; C</td>
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<td>Controlled work rate</td>
<td>8</td>
<td>90</td>
<td>Low</td>
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<td>48.0</td>
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<td></td>
<td>5</td>
<td>Controlled work rate</td>
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<td>49</td>
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<td>57.1</td>
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<td>53.8</td>
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<td>Chen et al. (2013) A &amp; B</td>
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<td>Controlled work rate</td>
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<td>38</td>
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<td>51</td>
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<td>53.0</td>
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<td>Controlled work rate</td>
<td>5</td>
<td>38</td>
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<td>51</td>
<td>51</td>
<td>53.0</td>
<td>7</td>
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<td>Kallar et al. (2014)</td>
<td>21</td>
<td>Controlled work rate</td>
<td>10</td>
<td>100</td>
<td>Low</td>
<td>42</td>
<td>42</td>
<td>53.8</td>
<td>4</td>
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<tr>
<td>Oltip et al. (2014)</td>
<td>20</td>
<td>Controlled work rate</td>
<td>10</td>
<td>100</td>
<td>Low</td>
<td>57</td>
<td>57</td>
<td>58.9</td>
<td>6</td>
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<tr>
<td>Ashley et al. (2015)</td>
<td>10</td>
<td>Controlled work rate</td>
<td>10</td>
<td>120</td>
<td>Low</td>
<td>57</td>
<td>57</td>
<td>29.2</td>
<td>7</td>
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<tr>
<td></td>
<td>8</td>
<td>Controlled work rate</td>
<td>10</td>
<td>120</td>
<td>Low</td>
<td>48</td>
<td>48</td>
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<td>Gibson et al. (2015) A, B, &amp; C</td>
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<td>James et al. (2017)</td>
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<td>Isothermal</td>
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<td>Moderate</td>
<td>50</td>
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<tr>
<td>Willmot et al. (2018) A &amp; B</td>
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<td>Isothermal</td>
<td>10</td>
<td>150</td>
<td>Moderate</td>
<td>47</td>
<td>21</td>
<td>48.7</td>
<td>6</td>
</tr>
</tbody>
</table>

*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.

aExercise intensity was defined as either “low,” which was −55% VO₂max, “moderate,” which was isothermal or 55–70% VO₂max, and “high,” which was >70% VO₂max.

*bIncludes four females.

cIncludes five females.

for time to exhaustion (Figure 3), time trial (Figure 4), mean power (Figure 5), VO₂max (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], r² = 0.26, p < 0.001) (Figure 8A). Fitness level also significantly impacted the magnitude of 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], r² = 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], r² = 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 [−0.84, 0.15], 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 analyzed 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, −0.25 [−0.65, 0.18], p = 0.26; induction method, −0.08 [−0.53, 0.37], p = 0.74; 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.
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$_{2\text{max}}$ Meta-Regression

When all of the covariates were run as individual models, they did not significantly impact the magnitude of change seen in VO$_{2\text{max}}$ 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$_{2\text{max}}$ 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], $p = 0.54$).
on average 23% (demonstrated in a meta-analysis that exercise capacity improved following HA induction. The largest performance improvement was observed in time to exhaustion with an average improvement of 144.30 s. Tyler et al. willmott et al. (2018) demonstrated 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). The current 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), VO2max (MD, 1.32 ml kg−1·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, VO2max 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

### 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

### TABLE 5 | Peak power descriptive table.

<table>
<thead>
<tr>
<th>References</th>
<th>n</th>
<th>Induction method</th>
<th>Induction length (Days)</th>
<th>Session duration (min)</th>
<th>Exercise intensity</th>
<th>Heat Index induction</th>
<th>Heat index testing</th>
<th>Baseline fitness (ml·kg⁻¹·min⁻¹)</th>
<th>PEDro</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Castle et al., 2011) A &amp; B</td>
<td>8</td>
<td>Controlled work rate</td>
<td>10</td>
<td>60</td>
<td>Low</td>
<td>37</td>
<td>38</td>
<td>43.3</td>
<td>6</td>
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<td></td>
<td>8</td>
<td>Controlled work rate</td>
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<tr>
<td>Brade et al. (2013)</td>
<td>10</td>
<td>Controlled work rate</td>
<td>5</td>
<td>40</td>
<td>High</td>
<td>45</td>
<td>45</td>
<td>55.3</td>
<td>7</td>
</tr>
<tr>
<td>Kosier et al. (2015)</td>
<td>8</td>
<td>Controlled work rate</td>
<td>10</td>
<td>90</td>
<td>Low</td>
<td>30</td>
<td>39</td>
<td>61.2</td>
<td>7</td>
</tr>
<tr>
<td>Neal et al. (2016a)</td>
<td>10</td>
<td>Isothermal</td>
<td>5</td>
<td>90</td>
<td>Moderate</td>
<td>55</td>
<td>22</td>
<td>63.3</td>
<td>7</td>
</tr>
<tr>
<td>Neal et al., 2016b A &amp; B</td>
<td>8</td>
<td>Isothermal</td>
<td>11</td>
<td>90</td>
<td>Moderate</td>
<td>55</td>
<td>20</td>
<td>56.9</td>
<td>6</td>
</tr>
<tr>
<td>Isom &amp; Kestler et al. (2017)</td>
<td>8</td>
<td>Isothermal</td>
<td>11</td>
<td>90</td>
<td>Moderate</td>
<td>55</td>
<td>20</td>
<td>56.9</td>
<td>6</td>
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<tr>
<td>Willmott et al. (2018) A &amp; B</td>
<td>10</td>
<td>Isothermal</td>
<td>10</td>
<td>60</td>
<td>Moderate</td>
<td>47</td>
<td>43</td>
<td>48.7</td>
<td>6</td>
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<td>60</td>
<td>Moderate</td>
<td>47</td>
<td>43</td>
<td>48.7</td>
<td>6</td>
</tr>
</tbody>
</table>

*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 = 0.99; 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).
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\textsubscript{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\textsubscript{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\textsubscript{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
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\(_{2\text{max}}\) (62.0 ml·kg\(^{-1}\)·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\(_{2\text{max}}\)) and in the most extreme environmental conditions (ambient temperature, 35.4 ± 0.04°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\(_{2\text{max}}\) = 52.9 ml·kg\(^{-1}\)·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·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 (Bards 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...
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 VO2max values appeared to have greater improvement in this type of performance test. For example, an individual with a VO2max of 60 ml·kg⁻¹·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·kg⁻¹·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), and 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 VO2max (65.0 ml·kg⁻¹·min⁻¹) in the most extreme environmental conditions (ambient temperature, 39.5°C; relative humidity, 60%). A previous review by Périard 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 (VO2max = 50.7 ml·kg⁻¹·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.

$VO_{2\text{max}}$

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 \( V_{O2\max} \) following HA induction. Keiser et al. observed a 9.6% improvement in \( V_{O2\max} \) following 10 days of 90 min low intensity HA sessions (Keiser et al., 2015). Lorenzo et al. also demonstrated large improvements in \( V_{O2\max} \) 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). \( V_{O2\max} \) 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 \( V_{O2\max} \) 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 \( V_{O2\max} \) 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 \( V_{O2\max} \).

**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 Manson, 2010). HA induction took place with 90 min of low intensity, controlled work rate for 10 days at 40\(^\circ\)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\(^\circ\)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\(^\circ\)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\(^\circ\)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 (Petrick 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 (Horsman 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, VO₂\text{max}, 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 a higher baseline fitness levels, as indicated by VO₂\text{max}. 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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Ch. 4 The Impact of Short-Term Heat Acclimation Following Heat Acclimatization

**Background:** Heat acclimatization (HAz), which occurs in an outdoor setting, and heat acclimation (HA), which occurs in an artificial environment, have been investigated for many years. The physiological impacts of combining these two methods (dual heat acc [DHA]) is currently unknown. **Purpose:** To assess the effectiveness of HAz followed by short-term HA on physiological variables. **Methods:** 25 endurance athletes (mean[M]±standard deviation[SD]; age, 36±12 years; height, 178.81±6.39 cm; body mass, 73.03±8.97 kg; VO$_{2\text{max}}$ 57.48±7.03 ml·kg$^{-1}$·min$^{-1}$) completed testing trials. These trials involved 60 minutes of exercise (59.31±1.73% vVO$_{2\text{max}}$) in an artificial environmental laboratory (M±SD; ambient temperature [T$_{\text{amb}}$], 35.11±0.62°C; relative humidity [%RH], 47.61±0.38%; Wet Bulb Globe Temperature [WBGT] 29.53±0.63°C; wind speed, 4.02±0.12 mph) at three time points: 1) baseline (Test$^1$), 2) post-HAz (Test$^2$), 3) post-Dual Heat Acc (DHA) (Test$^3$). Throughout the tests, internal body temperature (T$_{\text{rec}}$), heart rate (HR), and sweat rate (SR) were collected. HAz involved free-living summer training in the summer and HA utilized a novel hyperthermic zone (HZ) approach for five days. HZHA involved internal temperature between 38.50 and 39.75°C for sixty minutes. Repeated measure ANOVAs were utilized to determine differences in physiological outcomes between testing time points. Statistical significance was set at 0.05, a priori. **Results:** HR was significantly lower in Test$^2$ compared to Test$^1$ (M±SD; Test$^1$, 142.81±12.43 bpm; Test$^2$, 138.04±13.71 bpm, p=0.002;) and in Test$^3$ compared to Test$^1$ (M±SD, Test$^3$ 134.39±11.27 bpm, p<0.001). HR was significantly lower in Test$^3$ compared to Test$^2$ (p=0.013). T$_{\text{rec}}$ was significantly lower in Test$^3$ compared to Test$^2$ (M±SD; Test$^3$, 58
38.03±0.39°C; Test\(^2\), 38.25±0.42°C, p=0.009) and Test\(^1\) (M±SD; Test\(^1\), 38.29±0.37°C, p=0.005), however, there were no differences in Test\(^1\) and Test\(^2\) (p=0.479). SR differences were observed between the tests (p=0.029). SR was significantly higher in Test\(^3\) compared to Test\(^2\) (M±SD, Test\(^3\), 1.93±0.47 L·h\(^{-1}\); Test\(^2\), 1.76±0.43 L·h\(^{-1}\), p=0.027), however, no differences were observed between Test\(^1\) (M±SD; 1.79±0.36 L·h\(^{-1}\)) and Test\(^2\) (p=0.533) and between Test\(^1\) and Test\(^3\) (p=0.061)

**Conclusions:** HAz resulted in some improved physiological outcomes that indicate positive thermoregulatory benefits and a short-term HZHA protocol lead to additional benefits. DHA is an efficient and effective method to optimize performance and safety in the heat.

**Introduction:** During intense exercise in the heat, individuals experience reduced physiological and perceptual outcomes when compared to similar exercise in a thermoneutral environment.\(^1\) Elevated heart rate, increased internal body temperature, dehydration, and poor perceptual measures are all factors that can contribute to poor performance and safety outcomes.\(^2,3\) Several heat mitigation strategies, including aerobic performance enhancement, heat acclimation (HA), hydration, and body cooling, have been extensively examined.\(^4\) A recent meta-analysis reported that HA was the second most impactful heat mitigation strategy, following the improvement of aerobic fitness.\(^4\)

HA is the systematic process of repeated exposures to the heat that elicits positive physiological and perceptual adaptations.\(^5\) Typical responses following HA include lower heart rate, internal body temperature, skin temperature, and sweat electrolyte concentration and increased plasma volume and sweat rate.\(^6\) The term “HA” refers to a protocol that is completed in an artificial environment and heat acclimatization (HAz) refers to training in a natural
environment, such as exercise outside in the summer months. Some studies have examined changes in physiological adaptations known to occur with HAz.\textsuperscript{7–9} In a soccer cohort, Buchheit et al. demonstrated that pre-season training in Qatar led to plasma volume expansion (a known physiological adaptation to HA) and increased performance in the yo-yo intermittent recovery test level 1 in temperate (22°C ambient temperature) conditions.\textsuperscript{9} Alternatively, Armstrong et. al did not observe changes in heart rate, internal body temperature, sweat sodium and potassium, or plasma volume during exercise in the heat (30.3°C ambient temperature) following the summer training of endurance runners in the northeastern United States.\textsuperscript{7} While HAz literature exists, the majority of studies related to this heat mitigation strategy have investigated various HA protocols.

The length of HA typically ranges and can include short-term (5 days), medium term (6-14 days), and long term (>14 days) protocols.\textsuperscript{10} While variations exist in the literature, the two most common methods of HA are isothermal and fixed-exercise intensity.\textsuperscript{11} The isothermal method involves a protocol that continuously adjusts exercise intensity to elicit a specific internal body temperature response (usually 38.5°C) for a pre-determined duration.\textsuperscript{12} The limitation of this method is that some athletes can safely tolerate internal body temperatures well above the temperature utilized in this protocol (up to 41.5°C), therefore, temperatures of 38.5°C might not provide enough of a stimulus for some athletes.\textsuperscript{13} Fixed-exercise intensity involves the selection of intensity based on a known parameter (for example, 55%VO\textsubscript{2max}) and can result in too low or too high of an internal body temperature if the balance between intensity and environmental conditions are not seamless for each participant. The general timeline of adaptations has been established from previous literature, with changes in internal body temperature and heart rate occurring earlier in HA (~3-6 days) and changes in sweat rate occurring later in the process.
(10-14 days). While several variations of protocols are present in the literature, no optimal protocol has been determined and the timeline of these adaptations are dependent on the protocol.10

From a physiological perspective, HAz holds some advantages to HA, including the presence of radiant heat from the sun, however, HAz may not provide adequate thermal load (depending on location) to induce the complete physiological responses for optimal thermoregulation.14 As such, HA may produce greater outcomes than HAz, however, an effective short-term HA protocol to elicit optimal physiological and perceptual responses has yet to be determined. No study to date has examined the impact of a short-term HA protocol following HAz to achieve complete physiological and perceptual adaptations during exercise in the heat. Therefore, the aim of this study was to assess the effectiveness of HAz followed by short-term HA on physiological and perceptual variables during steady-state exercise in the heat.

**Methods:** Twenty-five endurance athletes were included in this study (mean[M]±standard deviation[SD]; age, 36±12 years; height, 178.81±6.39 cm; body mass, 73.03±8.97 kg; VO2max 57.48±7.03 ml·kg⁻¹·min⁻¹). This study was approved by the institutional review board at <removed for review> and all participants provided written informed consent. A within-participant longitudinal study design was utilized, with the participants completing two VO2max tests, three treadmill running exercise tests, HAz, and five days of HA.

**Testing:** Testing trials involved 60 minutes of steady state exercise (59.31±1.73% vVO2max) in an artificial environmental laboratory (M±SD; ambient temperature [Tamb], 35.11±0.62°C; relative humidity [%RH], 47.61±0.38%; Wet Bulb Globe Temperature [WBGT] 29.53±0.63°C; wind speed, 4.02±0.12 mph) at three time points: 1) baseline (Test #1), 2) post-HAz (Test #2), 3)
post-Dual Heat Acc (DHA) (Test\textsuperscript{#3}) (Table 1). All testing sessions were performed on a motorized treadmill (T150; COSMED, Traunstein, Germany). Test\textsuperscript{#1} occurred in May and early June, Test\textsuperscript{#2} occurred following HAz in August and September, and Test\textsuperscript{#3} occurred following five days of HA. The days between Test\textsuperscript{#1} and Test\textsuperscript{#2} were recorded (M±SD; Test\textsuperscript{#1} and Test\textsuperscript{#2}, 109±9 days). It was assumed that participants were un-acclimatized at Test\textsuperscript{#1}, as all participants resided in New England, USA (Figure 1). Throughout testing, physiological (heart rate [HR], rectal temperature [T\textsubscript{rec}], and skin temperature (T\textsubscript{sk})) and perceptual (rating of perceived exertion [RPE], thermal sensation [TS], thirst, and fatigue) measures were recorded every five minutes. HR was measured with a chest strap (H10®, Polar Electro™, Kempele, Finland) and participants were instructed to insert a rectal probe 10cm passed the anal sphincter and for internal body temperature to be recorded (MP160; BIOPAC Systems Inc., Goleta, CA, USA). T\textsubscript{sk} was measured on four-sites (iButton; iButton Link LLC., Whitewater, WI, USA), including the thigh, chest, upper arm, and calf and mean T\textsubscript{sk} was calculated\textsuperscript{15}. Sweat rate (SR) was estimated by taking the difference in nude body mass measurements assessed before and immediately post exercise. Sweat electrolyte concentration (sodium [Na\textsuperscript{+}], potassium [K\textsuperscript{+}], and chloride [Cl\textsuperscript{-}] was also assessed via the whole-body wash-down technique\textsuperscript{16}. Participants were instructed to arrive to the laboratory euhydrated and this was confirmed with urine indices (M±SD; urine specific gravity, 1.010±0.008; and urine color, 2±0).\textsuperscript{17} No fluid was provided throughout the 60-minute exercise.

\textit{VO\textsubscript{2max}}: Due to longitudinal nature of this study, VO\textsubscript{2max} changes were assessed to ensure that there were no changes in aerobic fitness that could influence the physiological variables observed in the tests. VO\textsubscript{2max} was assessed prior to Test\textsuperscript{#1} and Test\textsuperscript{#2}. Participants were asked to
don a heart rate monitor (H10®, Polar Electro™, Kempele, Finland) and compete a self-selected 5-minute warm-up. Following warm-up, participants completed a graded maximal exercise test on a treadmill (T150; COSMED, Traunstein, Germany) at 2% grade to volitional exhaustion (TueOne, ParvoMedica, Sandy UT, USA). The mL of oxygen recorded during the final completed stage will be recorded as the VO\textsubscript{2max}.

HAz: Following baseline testing, participants completed and recorded self-directed summer training (~June-August) between Test\textsuperscript{#1} and Test\textsuperscript{#2}, utilizing their own training devices (Garmin, n=21 [Forerunner® Fenix® Vivoactive® Garmin™ Ltd., Olathe, Kansas, USA]; Polar H10 and Polar Beat application, n=3 [H10®, Polar Electro™, Kempele, Finland]).\textsuperscript{18} In addition to these devices, three participants also utilized cycling computers to track their cycling training (Wahoo ELEMNT Bolt, n=1 [ELEMNT Bolt, Wahoo Fitness®, Atlanta, GA, USA], Garmin Edge, n=1 [Edge®, Garmin Ltd., Olathe, Kansas, USA], Bryton Rider 15 [Rider 15®, Bryton™ Inc., Taipei City, Taiwan]). No training instruction was given during this period. Meteorological data from training sessions that were performed outside (with the exception of swimming) were extracted from the nearest available automated surface observing station (ASOS), with a mean distance of 16±11 km. The location of training was determined by the GPS device and the latitude/longitude of that training session location was utilized to determine the closest weather station. Daytime WBGTs (7 a.m. - 7 p.m.) were modeled using Heat Stress Advisor software package (version 2005; Zunis Foundation, Tulsa, OK; Coyle 2000)\textsuperscript{19,20}, which is designed to work with weather station data; nighttime WBGTs were computed using the Liljegren model with solar radiation set to zero.\textsuperscript{21} Total distance, average HR, session duration, $T_{\text{amb}}$, %RH, and WBGT were reported.
**DHA:** Following Test\(^2\), participants completed a five-day HA protocol in an artificial environmental laboratory (M±SD; T\(_{\text{amb}}\), 38.67±1.03°C; %RH, 51.34±2.42%; WBGT, 33.82±1.20°C; wind speed, 0±0 mph). Five HA sessions were completed within eight days and the days between each HA session and tests were recorded (M±SD; Test\(^2\) and HA\(^1\), 4±2 days; HA\(^1\) and HA\(^2\), 1±1 day; HA\(^2\) and HA\(^3\), 2±1 days; HA\(^3\) and HA\(^4\), 2±1 days; HA\(^4\) and HA\(^5\), 1±1 days; total number of HA days, 6±1 days; HA\(^5\) and Test\(^3\), 3±1 days). The HA sessions involved exercise to induce hyperthermia for 60 minutes, which is defined as hyperthermic zone HA (HZHA). Hyperthermia was defined as temperatures between 38.50°C and 39.75°C. In general, the exercise sessions began with a higher intensity exercise (70% \(\text{vVO}_{2\text{max}}\)) and the intensity was adjusted throughout the session to allow the participant to experience hyperthermia for 60 minutes. Total \(T_{\text{rec}}\) and \(T_{\text{rec}}\) above 38.50°C integral area under the curve was calculated for each HA session.

**Statistical Analysis:** Repeated measure ANOVAs were utilized to determine differences in physiological and perceptual outcomes between testing time points. For all analyses, in the presence of a significant Mauchly’s Test of Sphericity, Greenhouse-Geisser correction was used. Pairwise differences were assessed post-hoc using LSD. Cohen’s d effect sizes (ES) were calculated to quantify the magnitude of pairwise differences. ES was interpreted according to the following thresholds: < 0.2 = trivial, 0.2–0.6 = small, 0.7–1.1 = moderate, 1.2–2.0 = large, and > 2.0 = very large.\(^{22}\) Statistical significance was set at \(p<0.05\), a priori. Data are reported as mean±standard deviation (M±SD), mean differences (MD) with 95% confidence intervals (CI) and ES. All statistical analyses were completed using SPSS Statistics for Mac, version 25 (IBM Corp., Armonk N.Y., USA).
Results: There were no differences in VO$_{2\text{max}}$	extsuperscript{#1} and VO$_{2\text{max}}$	extsuperscript{#2} (M±SD; VO$_{2\text{max}}$	extsuperscript{#1}, 57.92±6.82 ml·kg$^{-1}$·min$^{-1}$; VO$_{2\text{max}}$	extsuperscript{#2}, 59.65±8.24, p=0.67). Free-living summer training was recorded and descriptive training and environmental data can be seen in Table 2. The average T$_{\text{rec}}$ area under the curve experienced during HA was 15,639.24±882.51 (°C·min). Descriptive data from HA can be seen in Table 3.

Heart Rate: Differences in physiological outcomes between Test$^{\#1}$, Test$^{\#2}$, and Test$^{\#3}$ can be seen in Table 4. Differences were observed in average (p<0.001) and max HR (p<0.001), but not resting (p=0.67). Pairwise comparisons demonstrated that average HR was significantly lower in Test$^{\#2}$ compared to Test$^{\#1}$ (M±SD; Test$^{\#1}$, 142.81±12.43 bpm; Test$^{\#2}$, 138.04±13.71 bpm, p=0.002;) and in Test$^{\#3}$ compared to Test$^{\#1}$ (M±SD, Test$^{\#3}$ 134.39±11.27 bpm, p<0.001).

Additionally, average HR was significantly lower in Test$^{\#3}$ compared to Test$^{\#2}$ (p=0.013). Max HR was significantly lower in Test$^{\#2}$ compared to Test$^{\#1}$ (M±SD; Test$^{\#1}$, 163.16±15.07 bpm; Test$^{\#2}$, 155.44±17.22 bpm, p=0.002) and in Test$^{\#3}$ compared to Test$^{\#1}$ (M±SD; Test$^{\#3}$ 149.60±15.07 bpm, p<0.001) (Figure 2). Max HR was significantly lower in Test$^{\#3}$ compared to Test$^{\#2}$ (p=0.006).

Trec and Delta Trec: Differences were observed in average (p=0.003), resting (p=0.023), max (p<0.001), and delta (p=0.017) T$_{\text{rec}}$. Pairwise comparisons demonstrated that average T$_{\text{rec}}$ was significantly lower in Test$^{\#3}$ compared to Test$^{\#2}$ (M±SD; Test$^{\#3}$, 38.03±0.39°C; Test$^{\#2}$, 38.25±0.42°C, p=0.009) and Test$^{\#1}$ (M±SD; Test$^{\#1}$, 38.29±0.37°C, p=0.005), however, there were no differences in Test$^{\#1}$ and Test$^{\#2}$ (p=0.479). Minimum T$_{\text{rec}}$ was lower in Test$^{\#3}$ compared to Test$^{\#2}$ (M±SD; Test$^{\#3}$ 37.00±0.37°C; Test$^{\#2}$ 37.22±0.37°C, p=0.016), however, there were no
differences in Test\textsuperscript{#1} (M±SD; Test\textsuperscript{#1} 37.19±0.40\degree C) and Test\textsuperscript{#2} (p=0.577) and between Test\textsuperscript{#1} and Test\textsuperscript{#3} (p=0.067). Although approaching statistical difference, max $T_{rec}$ was not different between Test\textsuperscript{#1} and Test\textsuperscript{#2} (M±SD; Test\textsuperscript{#1}, 39.15±0.57\degree C; Test\textsuperscript{#2}, 39.00±0.54\degree C, p=0.059), however, Test\textsuperscript{#3} (M±SD, 38.73±0.5\degree C) was lower than Test\textsuperscript{#1} (p=0.001) and Test\textsuperscript{#2} (p=0.009) (Figure 2). Delta $T_{rec}$ was significantly lower in Test\textsuperscript{#2} and Test\textsuperscript{#3} compared to Test\textsuperscript{#1} (M±SD; Test\textsuperscript{#1}, 1.96±0.60\degree C; Test\textsuperscript{#2}, 1.78±0.45\degree C, p=0.025; Test\textsuperscript{#3}, 1.73±0.49\degree C, p=0.02). There were no differences between Test\textsuperscript{#2} and Test\textsuperscript{#3} (p=0.337).

\textit{Skin Temperature:} $T_{sk}$ differences were observed between tests (p<0.001). $T_{sk}$ was significantly lower in Test\textsuperscript{#3} compared to Test\textsuperscript{#2} (M±SD; Test\textsuperscript{#3}, 35.49±0.62; Test\textsuperscript{#2}, 35.86±0.55, p=0.005) and compared to Test\textsuperscript{#1} (M±SD; Test\textsuperscript{#1}, 36.3±0.46, p<0.001). $T_{sk}$ was also significantly lower in Test\textsuperscript{#2} compared to Test\textsuperscript{#1} (p=0.001). SR differences were observed between the tests (p=0.029). SR was significantly higher in Test\textsuperscript{#3} compared to Test\textsuperscript{#2} (M±SD, Test\textsuperscript{#3}, 1.93±0.47 L·h\textsuperscript{-1}; Test\textsuperscript{#2}, 1.76±0.43 L·h\textsuperscript{-1}, p=0.027), however, no differences were observed between Test\textsuperscript{#1} (M±SD; 1.79±0.36 L·h\textsuperscript{-1}) and Test\textsuperscript{#2} (p=0.533) and between Test\textsuperscript{#1} and Test\textsuperscript{#3} (p=0.061), although the difference was approaching statistical significance (Figure 2).

\textit{Sweat Concentration:} Differences in sweat [Na\textsuperscript{+}] (p<0.001) and [Cl\textsuperscript{-}] were observed (p<0.001), however, no differences were observed in sweat [K\textsuperscript{+}] (p=0.208) between tests. [Na\textsuperscript{+}] was lower in Test\textsuperscript{#3} compared to Test\textsuperscript{#2} (M±SD; Test\textsuperscript{#3}, 800.26±227.23 mEq·L\textsuperscript{-1}; Test\textsuperscript{#2}, 1067.17±437.97 mEq·L\textsuperscript{-1}, p=0.001) and compared to Test\textsuperscript{#1} (M±SD; Test\textsuperscript{#1} 1055.94±386.34 mEq·L\textsuperscript{-1}, p<0.001). There were no observed differences in [Na\textsuperscript{+}] between Test\textsuperscript{#1} and Test\textsuperscript{#2} (0.867). [Cl\textsuperscript{-}] was lower in Test\textsuperscript{#3} compared to Test\textsuperscript{#2} (M±SD; Test\textsuperscript{#3}, 1186.67±368.90 mEq·L\textsuperscript{-1}; Test\textsuperscript{#2}, 1529.89±648.49
mEq·L⁻¹, p=0.002) and compared to Test¹ (M±SD; Test¹, 1565.56±537.95 mEq·L⁻¹, p<0.001).
There were no differences in [Cl⁻] between Test¹ and Test² (p=0.747).

*Ratings of Perceived Exertion, Thirst, and Fatigue:* Differences in RPE (p=0.001), TS (p=0.001), Thirst (p=0.001), and Fatigue (p=0.027) were observed between tests. RPE was significantly lower in Test³ compared to Test² (M±SD; Test³, 10±2; Test², 11±2, p=0.001) and compared to Test¹ (M±SD; 11±2, p=0.007). No differences were seen between Test¹ and Test² (p=0.579). TS was significantly lower in Test³ compared to Test² (M±SD; Test³, 5.1±0.6; Test², 5.5±0.5, p=0.003) and compared to Test¹ (M±SD; Test¹, 5.6±0.6, p=0.001). There was no difference between Test¹ and Test² (p=0.633). Thirst was significantly lower in Test³ compared to Test² (M±SD; Test³, 3±1; Test², 4±1, p=0.001) and compared to Test¹ (M±SD; Test¹, 4±1, p=0.007). There was no difference in Thirst between Test¹ and Test² (p=0.304). Perceived fatigue was lower in Test³ compared to Test² (M±SD; Test³, 2±1; Test², 3±1, p=0.009, however no differences were observed between Test¹ (M±SD, 3±1) and Test² (p=0.137) and Test³ (p=0.232).

**Discussion:** Our findings point to a novel, effective DHA strategy that involves both summer HAz training and a short-term HA protocol (5 days) to elicit positive physiological and perceptual adaptations during exercise in the heat (Figure 3). Our unique protocol, that involved completing 5 days of HA following HAz, is useful to athletes, warfighters, and laborers who aim to complete physical activity in the heat safely and effectively and optimize the physiological adaptations and benefits of HA. Endurance athletes who trained throughout the summer months presented some physiological and perceptual improvements, however the short-term HA
protocol following HAZ elicited additional thermoregulatory benefits, including improvements in sweat rate, which is typically the latest adaptation to occur. Additionally, this optimal HA protocol is a novel approach that achieves high levels of thermal load that are often not achieved in traditional HA protocols.

Few studies have investigated the impacts of HAZ on aerobically trained athletes. HAZ resulted in small improvements in HR measures and moderate improvements in Tsk measurements, but not Trec or SR. In contrast to the current findings, previous research did not report changes in HR, Trec, or sweat electrolyte concentration following summer training in a similar sample, although, the testing conditions were lower than the current study (30°C Tamb, 35% RH), which could have resulted in difficulty observing changes in physiological variables. However, another study found that SR and sweat electrolyte concentration, but not HR, Trec, or Tsk improved following HAZ in a soccer cohort. These discrepancies are most likely due to the varying HAZ protocols and the factors known to influence thermoregulatory responses.

While a review of short-term HA has been previously published, the novel protocol of HA in the current study produced meaningful changes that expands the current HA literature. Two reasons this specific protocol was unique was that; 1) it followed HAZ, and 2) utilized the HZHA approach. A study by Daanen et al. recently examined a two-part HA protocol that involved participants completing nine consecutive days in moderate conditions (35°C Tamb, 29% RH) followed by three days of severe conditions (41°C Tamb, 33% RH). The author’s conclusion was that a two-stage acclimation program did not result in enhanced physiological adaptations and that the short length of exposure (3 days) to the severe environment may have contributed to these findings. This hypothesis appears to be true with findings from this study, as a longer (5-day) HA protocol following HAZ elicited further adaptations.
While improvements in several physiological variables were seen from Test #1 to Test #2 (classified as long-term HA), improvements were also observed from Test #2 to Test #3 (classified as short-term HA). A review of short-term HA protocols states that HR lowers by approximately 6-10% and resting and exercise Trec lowers by 0.2°C following short-term HA.26 These cardiovascular responses are consistent with our 9% improvement of HR following HAz and an additional 4% following HA. Max Trec improved by ~0.3°C in the present study following HA and 0.4°C following HAz + HA, which is slightly higher than previously reported data.26 Unlike many previous studies, SR also increased by ~9% following HA.10 Few studies have observed SR improvements following short-term HA,26 however, those that did demonstrate improvement in this thermoregulatory benefit were completed in some of the more extreme environments (38-40°C Tamb; 12-60% RH) with intense protocols23 or with the additional stress of nuclear, biological, and chemical suits.27,28 We propose that the mechanism behind many improvements following HA in the present study are a direct result of the HZHA protocol used.

While there is clear evidence that HA results in several physiological adaptations, the wide range of the magnitude and time course of these physiological responses could be due to the wide range of variability in program designs. A review by Taylor et al. stated that the optimal HA approach is yet to be determined, however, the three most common HA methods include isothermal fixed-work rate, and self-paced.10 A recent publication aimed to examine if there were differences in varying degrees of controlled hyperthermia by testing two critical temperature thresholds: 38.5°C and 39°C and observed no differences in outcomes.12,29 However, the limitation to this method is that during competitions or training, athletes experience internal body temperatures well above either of these critical thresholds13 and this method may not elicit the HA adaptations that athletes need when performing intense exercise in extreme environments.
The fixed-work rate method can elicit a greater thermal stress through an elevated internal body temperature, the limitation to this method is that if the workload is hard enough to drive internal body temperature, the session must stop when the laboratory cut-off point is reached. Alternatively, if the intensity is too low, the drive to reach elevated internal body temperature will lead to a long session duration, which is not practical for athletes.

Self-paced exercise has also been examined in previous research however, this method allows the athlete to self-select intensity which could result in a reduced thermal, cardiovascular, and relative work-load. The improvements demonstrated from the HA method in this study and the concept of maximizing the area under the $T_{rec}$ curve safely could help explain some of the discrepancies seen in previous HA literature. This concept is based on the overload principle training, in which the body adapts to the stresses it is placed under. This concept is most commonly observed in strength and conditioning research, however, this could also be applied to HA. Unlike previously reported HA protocols, the HZHA may be effective at eliciting greater positive physiological adaptations because this protocol leads to elevated internal body temperature and sweating.

One limitation of the present study was that there was no control group to account for training effect. While a control group could have improved this study design, the high aerobic fitness of the participants of this study reduces the chance that training alone would have impacted these results. Another limitation of the current study is that internal body temperature was not captured throughout HAz. Future research should aim to examine internal body temperature periodically throughout HAz to assess internal thermal load during summer training, which would allow researchers to understand the AUC that occurs during this time. Finally, due to the nature of this study, the days between testing were not identical across participants, although, the variations were limited and likely did not impact these outcomes.
**Conclusion:** The HAz of aerobically trained individuals resulted in some improved physiological and perceptual outcomes that indicate positive thermoregulatory benefits, however a short-term HZHA protocol following the HAz also lead to additional benefits, including improvements in SR. While future research is needed, it appears that DHA is an efficient and effective method to optimize performance and safety in the heat. Following DHA, average HR was ~8 bpm lower (moderate effect) and maximal HR was ~14 bpm lower (large effect) compared to baseline. Additionally, average $T_{rec}$ was ~0.26°C lower (moderate effect) and maximal $T_{rec}$ was ~0.42°C lower (moderate effect) compared to baseline. Finally, [Na+] was ~256 mEq·L⁻¹ lower (large effect) following DHA. This novel, effective method of obtaining the benefits of HA could be useful for athletes, warfighters, and physically active individuals who are at risk for exertional heat illness and are looking to reach peak performance during exercise in the heat.
References


Table 1. Complete description of trials throughout the study

<table>
<thead>
<tr>
<th>Test Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test #1</td>
<td>Baseline trial in an un-acclimated state; pre-summer</td>
</tr>
<tr>
<td>Test #2</td>
<td>Trial following heat acclimatization through free-living summer training; post summer; pre-heat acclimation</td>
</tr>
<tr>
<td>Test #3</td>
<td>Trial following 5-day short term heat acclimation; post dual heat acc</td>
</tr>
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</table>
Figure 1. Average maximum monthly ambient temperature in New England by climate division for April-May (un-acclimatized) (a) and June-August (acclimatized) (b) 2019.
Table 2. Free-living summer training (HAz) training and environmental data. Data are describing averages for each training session.

<table>
<thead>
<tr>
<th>Exercise Type</th>
<th>Distance (km)</th>
<th>Heart Rate (bpm)</th>
<th>Duration (minutes)</th>
<th>Ambient Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Heat Index (°C)</th>
<th>WBGT (°C)</th>
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<tbody>
<tr>
<td>Outdoor Running (1692)</td>
<td>10.28±8.43</td>
<td>139.82±14.63</td>
<td>56.38±72.66</td>
<td>22.35±4.89</td>
<td>66.10±19.44</td>
<td>29.89±2.42</td>
<td>22.31±4.23</td>
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<tr>
<td>Outdoor Cycling (364)</td>
<td>32.74±26.21</td>
<td>127.87±15.52</td>
<td>91.67±69.27</td>
<td>24.31±4.67</td>
<td>59.29±18.57</td>
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<td>23.68±3.96</td>
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<td>Multi-Sport (18)</td>
<td>27.88±15.43</td>
<td>125.00±6.27</td>
<td>90.71±31.78</td>
<td>22.58±6.69</td>
<td>68.17±17.83</td>
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<td>Hiking (19)</td>
<td>8.50±8.95</td>
<td>93.83±17.54</td>
<td>161.58±170.58</td>
<td>20.21±7.21</td>
<td>58.63±20.16</td>
<td>30.77±4.61</td>
<td>19.39±6.84</td>
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Data are reported as mean±standard deviation
Table 3. Physiological variables collected throughout the 5-day heat acclimation protocol.

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<tr>
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<th>HA #1</th>
<th>HA #2</th>
<th>HA #3</th>
<th>HA #4</th>
<th>HA #5</th>
<th>Overall</th>
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<tr>
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<td>M±SD</td>
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<td>Duration (min)</td>
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<td>Average HR (bpm)</td>
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<td>Average T&lt;sub&gt;rec&lt;/sub&gt; (°C)</td>
<td>38.85±0.42</td>
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<td>Max HR (bpm)</td>
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<td>Max T&lt;sub&gt;rec&lt;/sub&gt; (°C)</td>
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<td>39.46±0.30</td>
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<td>AUC (°C-min)</td>
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<td>3088±209.51</td>
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<td>3144.01±263.86</td>
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<td>Perceived Exertion</td>
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<td>Thermal Sensation</td>
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<td>Sweat Volume (L)</td>
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<td>2.47±0.59</td>
<td>2.72±0.60</td>
<td>2.74±0.56</td>
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<td><strong>Session after 38.5°C</strong></td>
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<tr>
<td>Average T&lt;sub&gt;rec&lt;/sub&gt; (°C)</td>
<td>39.16±0.42</td>
<td>39.24±0.22</td>
<td>39.16±0.36</td>
<td>39.16±0.30</td>
<td>39.11±0.22</td>
<td>39.17±0.17</td>
</tr>
<tr>
<td>Average HR (bpm)</td>
<td>138±14</td>
<td>132±14</td>
<td>131±14</td>
<td>131±14</td>
<td>128±12</td>
<td>132±12</td>
</tr>
<tr>
<td>AUC (°C-min)</td>
<td>46.31±15.60</td>
<td>46.04±12.38</td>
<td>42.29±15.70</td>
<td>41.67±14.72</td>
<td>38.15±9.86</td>
<td>42.89±13.65</td>
</tr>
</tbody>
</table>

*The HA protocol called for sixty minutes above 38.5°C

HA #*: Day of heat acclimation session

T<sub>rec</sub>: Internal body temperature

HR: Heart rate

AUC: Area under the curve

Data are presented as mean ± standard deviation (M±SD)
Table 4. Physiological outcomes from heat acclimatization, heat acclimation, and dual heat acc.

<table>
<thead>
<tr>
<th>Heart Rate (bpm)</th>
<th>1 vs 2</th>
<th>2 vs 3</th>
<th>1 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average</strong></td>
<td>MD±SE</td>
<td>95%CI</td>
<td>ES</td>
</tr>
<tr>
<td></td>
<td>-4.76±1.39</td>
<td>L: -7.62 U: -1.90</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td>-7.72±2.20</td>
<td>L: -12.27 U: -3.17</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Internal Temperature (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>-0.04±0.01</td>
<td>L: -0.15 U: 0.07</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td>-0.15±0.07</td>
<td>L: -0.34 U: 0.03</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td>0.04±0.06</td>
<td>L: -0.10 U: 0.17</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td>-0.18±0.08</td>
<td>L: -0.34 U: 0.03</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Skin Temperature (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>-0.45±0.11</td>
<td>L: -0.68 U: -0.22</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Sweat Rate (L.h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.03±0.05</td>
<td>L: -0.07 U: 0.13</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Test #1: Baseline-Un-acclimated; Test #2: Post Heat Acclimatization; Test #3: Post Dual Heat Acc

Negative values indicate later test is lower than earlier test.

Data are presented as mean difference ± standard error (MD±SE), 95% confidence intervals (95%CI), and effect sizes (ES). *Indicates statistical significance, p<0.05
Figure 2. Highest heart rate (a), highest internal body temperature (b), and sweat rate (c) during 60-minute exercise in the heat during Test#1, Test#2, and Test#3.
Test #1: Baseline-Un-acclimated

Test #2: Post Heat Acclimatization

Test #3: Post Dual Heat Acc

* Indicates significant mean differences from Test #2

^ Indicates significant mean differences from Test #2

Statistical significance, p<0.05
Figure 3. Percent change (%) of physiological adaptations from a baseline un-acclimatized state
Ch. 5 The Impacts of Minimal and Maximal Heat Training Following Heat Acclimatization and Heat Acclimation on Physiological Adaptations During Exercise in the Heat

Background: Heat acclimatization (HAz) or acclimation (HA) is one of the most beneficial, heat illness prevention and performance strategies used during physical activity. However, the optimal method to maintain these methods are unknown. Purpose: To test the efficacy of a novel dual heat acc (DHA) induction protocol and to examine if there is a dose response relationship related to the frequency of intermittent heat training following HA on aerobically training athletes. Methods: Twenty-seven male endurance athletes (mean[M]±standard deviation[SD]; age, 34±12 years; height, 178.44±6.31 cm; body mass, 72.56±8.81 kg; VO2max 57.65±6.79 ml·kg⁻¹·min⁻¹) completed five tests (Un-acclimatized [Test #1], following HAz [Test #2], following HA [Test #3], the middle of heat training (HT) [Test #4] and the end of HT [Test #5]) following HA that involved sixty minutes of steady state exercise (59.12±1.74% vVO2maxTest #1) in an artificial environmental laboratory (M±SD; ambient temperature [Tamb], 35.42±1.06°C; relative humidity [%RH], 46.35±2.48%; Wet Bulb Globe Temperature [WBGT] 29.62±1.37°C; wind speed, 3.98±0.30 mph) on a motorized treadmill. The study, in its entirety, was approximately six months in length. Following Test #3, participants were randomly assigned to three groups: control group with no heat exposures (HTCON), once per week heat exposure group (HTMIN), and twice per week heat exposure group (HTMAX). Repeated measures ANOVA were utilized to determine differences in physiological variables between trials. Results: DHA resulted in significant mean differences in maximal HR (p<0.001), average HR (p<0.001), ending Trec (p<0.001), average Trec (p=0.001), delta Trec (p=0.026), sweat rate (p=0.033), and Tsk (p<0.001) between Test #1, Test #2, and Test #3. At Test #5, the highest trial HR was significantly higher in HTCON compared to HTMAX.
(M±SD, HT_{CON}, 173.88±22.22 bpm; HT_{MAX}, 151.00±16.52 bpm, p<0.05), but was not different than HT_{MIN} (M±SD, 159.33 bpm). There were statistical differences between HT_{CON} and HT_{MAX} % change of rectal temperature from Test^{3} (HT_{CON} vs HT_{MAX}, [95%CI] 0.46%, 2.7%; ES=1.37; p=0.009), but not between HT_{MIN} (HT_{CON} vs HT_{MIN}, [95%CI] -0.26%, 2.8%; ES=0.85; p=0.098) at Test^{5}. Conclusions: HT_{MAX} (twice weekly heat training) provides clear evidence for the ability to maintain and possibly improve physiological adaptations following DHA. HT_{MIN} (once weekly heat training) may be sufficient for some individuals to maintain gains made from DHA.

Introduction: Intense physical activity in the heat occurs often placing athletic and military populations at risk for experiencing exertional heat illness and not performing at their best. Major sporting events, such as the 2020 Tokyo Olympics and the 2022 FIFA World Cup in Qatar are of grave concern for elite athletes and spectators due to the extreme environmental conditions of these venues.^{1} Heat acclimation (HA), the process of systematic heat exposures in an artificial environment to develop improved cardiovascular and thermoregulatory benefits, has been deemed an effective heat mitigation strategy.^{2} Heat acclimatization (HAz) refers to the same process as HA, although this type of training occurs in a natural environment. Throughout this manuscript, these terms may be used interchangeably, unless otherwise noted. While research has demonstrated that HA is an impactful strategy to optimize performance and safety when competing in the heat, strategies to sustain HA benefits throughout a competitive season are not as well understood.^{3,4}

There is evidence demonstrating the effectiveness of HA in team sports, such as soccer, as well as individual sports and activities.^{5–8} However, the environmental conditions that athletes may compete in can greatly fluctuate due to the timing of the sport season or the travel involved.
with a given sport. Several studies have demonstrated that biomarkers of HA (rectal temperature \([T_{\text{rec}}]\), heart rate \([HR]\), sweat rate \([SR]\)) decay without sufficient heat exposure. A recent meta-analysis by Daanan et al. demonstrated that \(T_{\text{rec}}\) and HR responses that were improved by HA reduced \(\sim 2.5\%\) per day without continued heat exposure.

While the evidence for decay is strong, the stimulation of sweating and skin blood flow, improved evaporative cooling, greater cardiac stability, changes in fluid dynamics, earlier onset of sweating, and greater sweat sensitivity of aerobically trained individuals, could make the timeline of this phenomenon delayed compared to untrained individuals. While there are several variables to consider (e.g. length of HA, the level of heat stress of HA, and testing methods before, during, and after maintenance), the timeline of decay of \(T_{\text{rec}}\) appears to be happening later and findings suggest that aerobic training status could play a role. Sweating responses are known to adapt to HA, however, the many day-to-day and individual variations (e.g. training status, age, sex, ethnicity) in individual SR, makes this variable hard to quantify during the induction of HA and even harder to quantify for decay. Furthermore, the improved conservation of sodium through adaptations at the sweat gland will result in more dilute sweat following HA, however, limited research exists on the outcomes of this specific adaptation with the cessation of HA.

Changes in HR are highly dependent on the adaptations that enhance skin blood flow when exercise begins in a hot environment. The skin and the working muscles are competing for more oxygen and substrate rich blood to maintain metabolism and the subsequent contractile muscle forces. While HA certainly improves the ability to sustain cardiac output, the effectiveness of this adaptation (as with all of the other adaptations) depends on the exercise intensity with higher intensity yielding greater physiological adaptation and lower resulting in
less adaption. A lower HR and increased stroke volume are likely linked to improvements in myocardial autonomic tone through improvements in central and local (arterial baroreflexes and chemoreflexes) commands.\textsuperscript{26,27} In fit soldiers, Pandolph et al. observed a 2-29\% decay in HR after 12 and 18 days of HA decay.\textsuperscript{22} Improvements in HR are also closely linked to the expansion of plasma volume that occurs with HA. Plasma volume has been observed to expand with HA through the expansion of total body water, as it is hypothesized that there is an increase in the production of albumin, which leads to water moving from the interstitial space and into the intravascular space.\textsuperscript{28} Very few studies have assessed the decay of plasma volume following HA induction.\textsuperscript{11,12} Garrett et al. did not observe changes in plasma volume during HA so the decay could not be assessed.\textsuperscript{11} Neal et al. did not show any changes in plasma volume after 7 days without heat exposure.\textsuperscript{12}

Although the time course of the gain and deterioration of the many benefits of HA are well-established, limited research has investigated the effectiveness of intermittent exercise-heat exposures, or heat training (HT), to sustain the adaptions over an extended period of time.\textsuperscript{29} One study investigated the implementation of an exercising heat exposure once every five days following HA.\textsuperscript{29} With this protocol, the physiological variables measured in this study (including HR, T_{rec}, SR, and plasma volume expansion) did not deteriorate to the same magnitude as the control group who did not participate in any HT.\textsuperscript{29} Throughout the course of many major sport seasons, the environmental conditions transition from hot to temperate and then cold, such as observed in American fall sports. To address the need for balancing sport-specific training, strength training, and recovery prior to a major competition, an effective HT strategy that will assist athletes in performing optimally in the heat is needed.\textsuperscript{30,31} Therefore, the purpose of this study was to examine the efficacy of minimal (~once per week) and maximal (~twice per week)
HT for four and eight weeks following dual heat acc (DHA). We hypothesize that there is a dose-response relationship with the frequency of HT sessions on physiological adaptations following DHA.

**Methods:** Twenty-seven male endurance athletes (mean[M]±standard deviation[SD]; age, 34±12 years; height, 178.44±6.31 cm; body mass, 72.56±8.81 kg; VO$_{2\max}$ 57.65±6.79 ml·kg$^{-1}$·min$^{-1}$) provided written informed consent to participate in this study, which was approved by the Institutional Review Board. In general, participants completed five tests that involved sixty minutes of steady state exercise (59.12±1.74% vVO$_2$max Test$^\#1$) in an artificial environmental laboratory (M±SD; ambient temperature [$T_{amb}$], 35.42±1.06°C; relative humidity [%RH], 46.35±2.48%; Wet Bulb Globe Temperature [WBGT] 29.62±1.37°C; wind speed, 3.98±0.30 mph) on a motorized treadmill (T150; COSMED, Traunstein, Germany). In each testing session, HR, $T_{rec}$, rating of perceived exertion (RPE), thermal sensation (TS), and fatigue were recorded every five minutes. HR was measured with a chest strap (H10®, Polar Electro™, Kempele, Finland) and participants were instructed to insert a rectal probe 10cm passed the anal sphincter and for internal body temperature to be recorded (MP160; BIOPAC Systems Inc., Goleta, CA, USA). Mean skin temperature ($T_{sk}$) was calculated by continuously collecting measurements from the thigh, calf, chest, and upper arm throughout the test (iButton; iButton Link LLC., Whitewater, WI, USA). SR was assessed by taking a nude body mass measurement prior to and immediately post exercise. Sweat electrolyte concentrations (sodium [$Na^+$], potassium [$K^+$], and chloride [$Cl^-$]) were also assessed via the whole-body wash-down technique. Euhydration was ensured prior to each test with the examination of urine specific gravity (USG) and urine color (M±SD, USG, 1.010±0.009; urine color, 1±1) and no fluid was provided during the test.
All test descriptions can be seen in Table 1. The first test (Test\textsuperscript{1}) was performed in an un-acclimatized physiological state (May-June in New England, USA). The second test (Test\textsuperscript{2}) was performed following HAz that involved self-directed summer training (August-September in New England, USA). The days between the Test\textsuperscript{1} and Test\textsuperscript{2} were recorded (M±SD, 109±9 days). The third test (Test\textsuperscript{3}) occurred following five days of HA, which involved exercise to achieve hyperthermia (38.50-39.75°C) (hyperthermic zone heat acclimation [HZHA] for sixty minutes in the heat (M±SD; \(T_{\text{amb}}\), 39.13±1.37°C; %RH, 51.04±8.42%; WBGT 33.16±1.95°C; wind speed, 0±0 mph). The combined HAz and HA that occurred prior to Test\textsuperscript{3} is referred to as “dual heat acc” (DHA). The total number of days of HA (M±SD, 6 ±1 days), the number of days between HA sessions (M±SD, 2±1 days), the number of days between Test\textsuperscript{2} and the first day of HA (M±SD, 4±2 days) and between the last day of HA and Test\textsuperscript{3} were recorded (M±SD, 3±1 days).

Following Test\textsuperscript{3}, participants were randomly assigned to three HT groups: 1) control group (HT\textsubscript{CON}) that received no additional heat training following HA (n=7), 2) minimal heat training (HT\textsubscript{MIN}) group that completed eight heat training sessions in eight weeks (n=8), and 3) maximal heat training (HT\textsubscript{MAX}) group that completed sixteen heat training sessions in eight weeks (n=9). A timeline of the entire study design can be seen in Figure 1.

Self-directed training outside of the laboratory was also recorded throughout the duration of the study utilizing the participant’s own training devices. (Garmin, n=21 [Forerunner® Fenix®, Vivoactive® Garmin™ Ltd., Olathe, Kansas, USA]; Polar H10 and Polar Beat application, n=4 [H10®, Polar Electro™, Kempele, Finland]).\textsuperscript{35} In addition to these devices, three participants also utilized cycling computers to track their cycling training (Wahoo ELEMNT Bolt, n=1 [ELEMNT Bolt, Wahoo Fitness®, Atlanta, GA, USA], Garmin Edge, n=1 [Edge®, Garmin Ltd.,
Olathe, Kansas, USA], Bryton Rider 15 [Rider 15®, Bryton™ Inc., Taipei City, Taiwan]). No training instruction was given. The location of training was determined by the GPS device and the latitude/longitude of that training session location was utilized to determine the closest weather station. Meteorological data from training sessions that were performed outside (with the exception of swimming) were extracted from the nearest available automated surface observing station (ASOS), with a mean distance of 16±11 km. The location of training was determined by the GPS device and the latitude/longitude of that training session location was utilized to determine the closest weather station. Daytime WBGTs (7 a.m. - 7 p.m.) were modeled using Heat Stress Advisor software package (version 2005; Zunæis Foundation, Tulsa, OK; Coyle 2000), which is designed to work with weather station data; nighttime WBGTs were computed using the Liljegren model with solar radiation set to zero. Total distance, average HR, session duration, T\textsubscript{amb}, %RH, and WBGT were reported. Trends in training between group were examined previously and it was determined that trends in training amongst the three groups were similar throughout the study protocol.

**Statistical Analysis:** Repeated measures ANOVA (pooled for Test\textsuperscript{#1} - Test\textsuperscript{#3}) were utilized to determine changes in physiological and perceptual changes between tests throughout DHA. Following random assignment, a repeated measures ANOVA was utilized to ensure that there were no differences in demographic information (VO\textsubscript{2max}, body mass, and age). Two participants were unable to complete Test\textsuperscript{#1} (n=1, HT\textsubscript{MIN}; n=1 HT\textsubscript{MAX}) and one participant was unable to complete Test\textsuperscript{#4} (HT\textsubscript{CON}). Test\textsuperscript{#1} data were replaced with Test\textsuperscript{#2} data, since these values were considered baseline values in this study design. Test\textsuperscript{#4} data were replaced with the average of Test\textsuperscript{#3} and Test\textsuperscript{#5}. All data were checked for normality with the Shapiro-Wilk test and in the
presence of a significant Mauchly’s Test of Sphericity, Greenhouse-Geisser correction was used. Pairwise differences between groups and within groups at various time points were assessed post-hoc using paired (within group) and independent (between groups) t-tests. Cohen’s d (within group) and Hedge’s g (between group) effect sizes (ES) were calculated to quantify the magnitude of pairwise differences. ES was interpreted according to the following thresholds: < 0.2 = trivial, 0.2–0.6 = small, 0.7–1.1 = moderate, 1.2–2.0 = large, and > 2.0 = very large.39 Statistical significance was set at p<0.05, a priori. Data are reported as M±SD, mean differences (MD) with 95% confidence intervals (CI) and ES. All statistical analyses were completed using SPSS Statistics for Mac, version 25 (IBM Corp., Armonk N.Y., USA).

**Results:** The days between HT sessions for HT\textsubscript{MIN} and HT\textsubscript{MAX} were recorded (M±SD, HA\textsubscript{MIN}, 7±2 days; HA\textsubscript{MAX}, 4±2 days). All participants completed a test approximately four weeks following HA (Test\textsuperscript{#4}) and eight weeks following HA (Test\textsuperscript{#5}). The days between the most recent HT session and Test\textsuperscript{#4} and Test\textsuperscript{#5} were recorded for HT\textsubscript{MIN} and HT\textsubscript{MAX} (M±SD, HT\textsubscript{MIN} Test\textsuperscript{#4}, 7±2 days; HT\textsubscript{MAX} Test\textsuperscript{#4}, 3±1 days; HT\textsubscript{MIN} Test\textsuperscript{#5}, 8±4 days; HA\textsubscript{MAX} Test\textsuperscript{#5}, 3±1 days). Additionally, the days between Test\textsuperscript{#3} and Test\textsuperscript{#4} and Test\textsuperscript{#4} and Test\textsuperscript{#5} were recorded for HT\textsubscript{CON} (M±SD, Test\textsuperscript{#3} and Test\textsuperscript{#4}, 29±2 days; Test\textsuperscript{#4} and Test\textsuperscript{#5}, 25±4 days). The testing sessions were considered HT for both HT\textsubscript{MIN} and HT\textsubscript{MAX} at week four and week eight. To account for possible changes in aerobic fitness, VO\textsubscript{2max} (VO\textsubscript{2max}\textsuperscript{#1-5}) was assessed five times throughout this protocol. There were no changes in VO\textsubscript{2max} at any time point throughout this protocol (M±SD, VO\textsubscript{2max}\textsuperscript{#1}, 57.48±7.03 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; VO\textsubscript{2max}\textsuperscript{#2}, 59.66±7.92 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; VO\textsubscript{2max}\textsuperscript{#3}, 58.96±7.92 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; VO\textsubscript{2max}\textsuperscript{#4}, 59.99±7.85 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; VO\textsubscript{2max}\textsuperscript{#5}, 59.23±8.80 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}, p>0.05). There were no differences between the HT\textsubscript{CON}, HT\textsubscript{MIN}, and HT\textsubscript{MAX} in VO\textsubscript{2max} (M±SD HT\textsubscript{CON} 58.56±4.69
ml·kg⁻¹·min⁻¹; HT_MIN 58.13±9.66 ml·kg⁻¹·min⁻¹; HT_MAX 56.49±5.55 ml·kg⁻¹·min⁻¹; p=0.800), age (M±SD HT_CON 33±8 yrs; HT_MIN 34±13 yrs; HT_MAX 37±14 yrs; p=0.706), and body mass (M±SD HT_CON 72.01±9.79 kg; HT_MIN 72.35±7.22 kg; HT_MAX 73.18±10.13 kg; p=0.961) following DHA.

**Physiological Measures from Dual Heat Acc:** There were significant mean differences in maximal HR (p<0.001), average HR (p<0.001), ending T_rec (p<0.001), average T_rec (p=0.001), delta T_rec (p=0.026), SR (p=0.033), and T_sk (p<0.001) between Test #1, Test #2, and Test #3 (Table 2).

**Sweat Electrolytes:** Differences between Test #1, Test #2, and Test #3 were observed in [Na⁺] (p<0.001), [Cl⁻] (p<0.001). Post-hoc testing revealed no differences in [Na⁺] and [Cl⁻] in Test #2 compared to Test #1 (M±SD, Test #1 [Na⁺], 45.26±16.32 mEqu·L⁻¹; Test #2 [Na⁺], 45.708±18.47 mEqu·L⁻¹; [Na⁺] 95% CI [-5.95, 5.05], ES=0.03, p=0.867; Test #1 [Cl⁻], 43.81±3.43 mEqu·L⁻¹; Test #2 [Cl⁻], 42.88±17.67 mEqu·L⁻¹ [Cl⁻] 95% CI [-2.09, 6.78], ES=0.07, p=0.746). [Na⁺] and [Cl⁻] was lower in Test #3 (M±SD, Test #3 [Na⁺], 34.54±9.54 mEqu·L⁻¹; Test #3 [Cl⁻], 33.51±10.00 mEqu·L⁻¹) compared to Test #2 ([Na⁺] 95% CI, [5.25, 17.08], ES=0.76, p=0.001; [Cl⁻] 95%CI [3.90, 14.83], ES=0.51, p=0.002) and compared to Test #1 ([Na⁺] 95% CI, [5.88, 15.24], ES=0.80, p<0.001; [Cl⁻] 95%CI [5.84, 14.75], ES=1.11, p<0.001). There were no differences in [K⁺] (p>0.05) between Test #1 through Test #3 (p>0.05). RPE was lower in Test #3 (M±SD, 9±2) compared to Test #2 (M±SD, 11±2) and Test #1 (M±SD, 10±2) (Test #1 vs Test #3 95%CI [0, 2], ES=0.50, p=0.004; Test #2 vs Test #3 95%CI [1, 2], ES=1.00, p<0.001).
**Differences Between HT Programs:** To examine the effectiveness of the HT programs, within-group comparisons from Test\(^3\), Test\(^4\), and Test\(^5\) can be seen in Table 3 and between-group comparisons from Test\(^3\), Test\(^4\), and Test\(^5\) can be seen in Table 4.

**HR:** At Test\(^5\), the highest trial HR was significantly higher in HT\(_{\text{CON}}\) compared to HT\(_{\text{MAX}}\) (M±SD, HT\(_{\text{CON}}\), 173.88±22.22 bpm; HT\(_{\text{MAX}}\), 151.00±16.52 bpm, p<0.05), but was not different than HT\(_{\text{MIN}}\) (M±SD, 159.33 bpm) (Figure 2). Within group comparisons demonstrated that HT\(_{\text{CON}}\) had significantly greater maximal HR at Test\(^4\) (M±SD, 164.25±16.33 bpm) and Test\(^5\) (M±SD, 173.88±22.22 bpm) compared to Test\(^3\) (M±SD, 152.13±15.72 bpm), while HT\(_{\text{MIN}}\) and HT\(_{\text{MAX}}\) did not demonstrate differences between Test\(^3\), Test\(^4\) and Test\(^5\) (M±SD, HT\(_{\text{MIN}}\) Test\(^3\) 151.89±13.9 bpm, Test\(^4\) 151.67±16.68 bpm, Test\(^5\) 159.33±12.58 bpm; HT\(_{\text{MAX}}\) Test\(^3\) 149.10±17.42, Test\(^4\) 151.10±19.35, Test\(^5\) 151.00±16.52 bpm).

**T\(_{\text{rec}}\):** While there were no between group difference in T\(_{\text{rec}}\) at Test\(^3\), Test\(^4\), and Test\(^5\) (p>0.05), within group analysis revealed that the HT\(_{\text{CON}}\) demonstrated significantly higher ending T\(_{\text{rec}}\) at Test\(^5\) (M±SD, 39.2±0.62°C compared to Test\(^3\) (M±SD, 38.51±0.35°C, p<0.05). There were no within group differences in ending T\(_{\text{rec}}\) across tests in the HT\(_{\text{MIN}}\) group (M±SD, Test\(^3\) 38.77±0.62°C; Test\(^4\) 38.63±0.58°C; Test\(^5\) 38.97±0.48°C, p>0.05) or in the HT\(_{\text{MAX}}\) group (M±SD Test\(^3\) 38.92±0.44°C; Test\(^4\) 38.81±0.51°C; Test\(^5\) 39.00±0.52°C, p>0.05). To account for the variability that was observed in ending T\(_{\text{rec}}\) responses, percent change from Test\(^3\) to Test\(^4\) and Test\(^5\) was calculated (Figure 3).
HT\textsubscript{CON} Test\textsuperscript{#4} percent change was not different from Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#3} 0.00±0.00%; Test\textsuperscript{#4} 0.64±1.33%; [95%CI] -1.75, 0.48%; ES=0.68; p=0.218), however, Test\textsuperscript{#5} was significantly higher than Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#3} 1.81±1.43%; [95%CI] 0.62, 3.01%; ES=1.79; p=0.009).

HT\textsubscript{MIN} Test\textsuperscript{#4} percent change was not different from Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#3} 0.00±0.00%; Test\textsuperscript{#4} -0.35±1.71%; [95%CI] -0.96, 1.67%; ES=0.29; p=0.553) and Test\textsuperscript{#5} was not different from Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#5} 0.55±1.52%; [95%CI] -1.71, 0.62%; ES=0.51; p=0.314).

Similarly, HT\textsubscript{MAX} Test\textsuperscript{#4} percent change was not different from Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#3} 0.00±0.00%; Test\textsuperscript{#5} -0.26±1.01%; [95%CI] -0.46, 0.98%; ES=0.36; p=0.432) and Test\textsuperscript{#5} was not different than Test\textsuperscript{#3} (M±SD, Test\textsuperscript{#5} 0.23±0.78%; [95%CI] -0.79, 0.33%; ES=0.41; p=0.368).

Between group differences revealed statistical differences between HT\textsubscript{CON} and HT\textsubscript{MAX} (HT\textsubscript{CON} vs HT\textsubscript{MAX}, [95%CI] 0.46, 2.7%; ES=1.37; p=0.009), but not between HT\textsubscript{MIN} (HT\textsubscript{CON} vs HT\textsubscript{MIN}, [95%CI] -0.26, 2.8%; ES=0.85; p=0.098) at Test\textsuperscript{#5}. There were no between group differences at Test\textsuperscript{#4} (HT\textsubscript{CON} vs HT\textsubscript{MIN}, [95% CI] -0.61, 2.59%; ES=0.65; p=0.207; HT\textsubscript{CON} vs HT\textsubscript{MAX}, [95% CI] -0.27, 2.07%; ES=0.76; p=0.122).

**Sweat Rate and Tsk:** SR was not different between groups for Test\textsuperscript{#3}, Test\textsuperscript{#4}, Test\textsuperscript{#5} (M±SD, HT\textsubscript{CON}, Test\textsuperscript{#3} 1.85±0.44 L·hr\textsuperscript{-1}, Test\textsuperscript{#4} 1.59±0.24 L·hr\textsuperscript{-1}, Test\textsuperscript{#5} 1.55±0.29 L·hr\textsuperscript{-1}; HT\textsubscript{MIN}, Test\textsuperscript{#3} 1.98±0.50 L·hr\textsuperscript{-1}, Test\textsuperscript{#4} 1.80±0.23 L·hr\textsuperscript{-1}, Test\textsuperscript{#5} 1.77±0.37 L·hr\textsuperscript{-1}; HT\textsubscript{MAX}, Test\textsuperscript{#3} 1.86±0.52 L·hr\textsuperscript{-1}, Test\textsuperscript{#4} 1.91±0.37 L·hr\textsuperscript{-1}, Test\textsuperscript{#5} 1.92±0.46 L·hr\textsuperscript{-1}; p=0.103). T\textsubscript{sk} was not different between groups for Test\textsuperscript{#3}, Test\textsuperscript{#4}, Test\textsuperscript{#5} (M±SD, HT\textsubscript{CON}, Test\textsuperscript{#3} 35.62±0.43°C, Test\textsuperscript{#4} 35.43±0.52°C, Test\textsuperscript{#5} 35.88±0.54°C; HT\textsubscript{MIN}, Test\textsuperscript{#3} 35.29±0.52°C, Test\textsuperscript{#4} 35.77±0.70°C, Test\textsuperscript{#5} 35.48±0.47°C; HT\textsubscript{MAX}, Test\textsuperscript{#3} 35.46±0.60°C, Test\textsuperscript{#4} 35.39±0.66°C, Test\textsuperscript{#5} 35.35±0.57°C, p=0.057).
**Sweat Electrolyte Concentration:** In terms of sweat electrolyte concentration from Test\(^3\), Test\(^4\), and Test\(^5\), there were differences in [Na\(^+\)] (p=0.049) but not [Cl\(^-\)] (p=0.085) or [K\(^+\)] (p=0.126). Between-group post-hoc analysis demonstrated that HT\(_{CON}\) had significantly higher [Na\(^+\)] than HT\(_{MAX}\) at Test\(^5\) (M±SD, HT\(_{CON}\) 1309.57±399.50 mEq·L\(^{-1}\); HT\(_{MAX}\), 901.28±310.25 mEq·L\(^{-1}\); 95% CI [-761.85, -53.74], ES=1.16, p=0.027). Within-group post-hoc analysis showed that [Na\(^+\)] was significantly higher at Test\(^4\) compared to Test\(^3\) in HT\(_{CON}\) (M±SD, Test\(^3\) 832.45±257.16 mEq·L\(^{-1}\); Test\(^4\) 1286.76±439.93 mEq·L\(^{-1}\); 95% CI [218.40, 690.22] mEq·L\(^{-1}\); ES=1.26, p=0.003) and at Test\(^5\) compared to Test\(^3\) (M±SD, Test\(^3\) 1309.57±399.5 mEq·L\(^{-1}\); 95% CI [203.85, 750.39] mEq·L\(^{-1}\); ES=1.42, p=0.004). [Na\(^+\)] was significantly higher at Test\(^4\) compared to Test\(^3\) in HT\(_{MIN}\) (M±SD, Test\(^3\) 766.34±225.25 mEq·L\(^{-1}\); Test\(^4\) 1024.51±254.78 mEq·L\(^{-1}\); 95% CI [69.81, 446.54] mEq·L\(^{-1}\); ES=1.07, p=0.013) and at Test\(^5\) compared to Test\(^3\) (M±SD, Test\(^5\) 1309.75±286.89 mEq·L\(^{-1}\); 95% CI [75.35, 471.48] mEq·L\(^{-1}\); ES=1.06, p=0.013). [Na\(^+\)] was significantly higher at Test\(^4\) compared to Test\(^3\) in HT\(_{MAX}\) (M±SD, Test\(^3\) 789.49±201.19 mEq·L\(^{-1}\); Test\(^4\) 964.32±251.23 mEq·L\(^{-1}\); 95% CI [53.95, 295.71] mEq·L\(^{-1}\); ES=0.77, p=0.010), however, there was no difference between Test\(^3\) and Test\(^5\) (M±SD, Test\(^5\) 901.28±310.25 mEq·L\(^{-1}\); 95% CI [-68.12, 292.70] mEq·L\(^{-1}\); ES=0.43, p=0.193).

*Ratings of Perceived Exertion, Thermal Sensation and Fatigue:* There were no differences in RPE ((M±SD, HT\(_{CON}\), Test\(^3\) 10±2, Test\(^4\) 10±2, Test\(^5\) 11±3; HT\(_{MIN}\), Test\(^3\) 9±1, Test\(^4\) 9±2, Test\(^5\) 10±2; HT\(_{MAX}\), Test\(^3\) 9±2, Test\(^4\) 9±1, Test\(^5\) 9±1; p=0.225), TS (M±SD, HT\(_{CON}\), Test\(^3\) 5±0.6, Test\(^4\) 5.5±1.2, Test\(^5\) 5.3±0.8; HT\(_{MIN}\), Test\(^3\) 5±0.6, Test\(^4\) 6±1.8, Test\(^5\) 5.3±0.5; HT\(_{MAX}\), Test\(^3\) 5.3±0.7, Test\(^4\) 6.5±2.6, Test\(^5\) 5.5±0.8; p=0.676), or fatigue (M±SD, HT\(_{CON}\), Test\(^3\) 3±1,
Test#4 3±2, Test#5 4±2; HTMIN, Test#3 2±1, Test#4 2±1, Test#5 2±1; HTMAX, Test#3 2±1, Test#4 2±1, Test#5 2±1; p=0.191) throughout HT.

Discussion: Findings from this study point to the effectiveness of a twice weekly (HTMAX), and possibly a once weekly (HTMIN), HT program to maintain the many physiological benefits of HA (Figure 4). These results may be useful for individuals who perform physical activity in cooler climates and are in need of an effective strategy that can be incorporated with other training regimens. One notable finding from this research was that HTMAX had a ~22 bpm lower maximum HR and ~16 bpm lower average HR than HTCON at Test#5. Participants in the HTMAX group did not report significant losses from DHA in most physiological variables, including average HR, maximum HR, average Trec, ending Trec, and Tsk at Test#5. Participants in the HTMIN group demonstrated some physiological decrements from DHA, specifically in terms of sweat [Na+], however, the majority of physiological adaptations (including average HR, maximum HR, average Trec, ending Trec, and delta Trec) were not different from Test#3. By Test#5, HTCON saw declines in several physiological adaptations that occurred with DHA, including average HR, highest HR, ending Trec and delta Trec, and sweat [Na+]. One unexpected finding from this study was that HTMAX did not demonstrate any changes in SR, following DHA, although [Na+] decreased indicating greater sweating efficiency. This made further analysis into HT difficult to analyze and interpret. The specific method of HA used to induce physiological adaptations, the HT method and frequency, and the participant’s aerobic fitness levels most likely contributed to the findings of this HT program.

Little research has investigated the decay of physiological responses following DHA. Daanen et al. examined the impacts of one method of DHA that involved nine consecutive days
of moderate environmental stress followed by three days of severe environmental stress and determined that the optimal physiological responses were observed three and seven days following DHA. These findings bring two distinctive points to light. First, similarly, to any fitness training program, individuals need time to recover following a HA protocol for the full benefits to be observed. This is evident from previous research and from the improvements observed in several physiological outcomes at Test #4 in the present study. The HT protocols for the four weeks following DHA, seemed to allow the athletes to recover while continuing to gain physiological benefits. Second, decay does not happen as rapidly as previously assumed (days), especially in a fit population that undergoes a strenuous HA protocol, such as the present DHA and HZHA.

Findings from this study support the first notion due to the improved HR, Trec, sweat [Na+], and SR observed ~3 days following the cessation of DHA. Support from the second point is also evident from this data, as it appears that in this particular highly aerobically trained population, even the HTCON group observed minimal physiological decay in highest HR (~7 bpm) and sweat [Na+] (~454 mEq·L⁻¹) four weeks after DHA. All other variables demonstrated nonsignificant changes at Test #4 in HTCON. The maintenance of these physiological adaptations, although somewhat similar to previous literature, are most likely related to multiple components of the study design, including the thermal load of this HZHA protocol, the high aerobic fitness of these participants, and the moderate environmental conditions experienced during free-living training throughout this period (M±SD, WBGT, 15.85±5.31°C). At Test #5, HTCON exhibited ~15 bpm higher average HR, ~22 bpm greater maximum HR, ~0.7 °C higher ending Trec, ~0.5°C higher delta Trec, and ~1310 mEq·L⁻¹ higher [Na+] compared to Test #3,
providing clear evidence of decay. This was expected, as the environmental conditions experienced during free-living training in this phase was low (M±SD, WBGT, 9.72±5.89°C).

While decay in HT<sub>CON</sub> is important to examine and establish, the main purpose of this study was to determine the minimal effective dose of HT in aerobically trained individuals. Even though it has been postulated that re-induction of HA following a brief bout of decay would be much shorter than HA, few studies have investigated this point.<sup>41,42</sup> From these two investigations, it appears that fitness plays a large role in the time it takes to re-establish HA. In a study investigating individuals with average aerobic fitness (VO<sub>2max</sub> 34 ml·kg<sup>-1</sup>·min<sup>-1</sup>), the investigators reported that it took about six days to re-acclimate participants following HA. However, in a study examining aerobically fit individuals, it took only two days to re-establish HA twelve days after the cessation of HA and four days to re-establish twenty-six days after HA.<sup>41</sup> The contrast in fitness levels and outcomes between these studies points to one of the main possible reasons for effectiveness of twice weekly HT in the current study, as these participants all had relatively high aerobic fitness levels and therefore, needed less continued HT to maintain HA benefits.

When comparing the HT<sub>CON</sub>, HT<sub>MIN</sub> and HT<sub>MAX</sub>, a variety of individual responses must be considered (Supplemental Figures 1-3). This variety of responses is expected and has been previously discussed in terms of difficulty in assessing and controlling heat tolerance measures.<sup>43</sup> HT<sub>MAX</sub> and HT<sub>CON</sub> seemed to produce more consistent outcomes, with statistically significant differences and moderate (average HR) and large (maximum HR, %change ending T<sub>rec</sub>, and [Na+]) effects between the groups. This finding is novel and is not consistent with previous literature that demonstrated no statistical differences between a control group and once every fifth day HT session 25+ days following HA.<sup>29</sup> Alternatively, HT<sub>MIN</sub> did not demonstrate any
statistically significant differences from HT\textsubscript{CON} in any variables, although moderate effects were observed in highest reached HR and % change in ending $T_{rec}$. It appears that there were certain individuals in this group who responded to HT once weekly well and others who did not.

Future investigations are needed to determine the rationale behind the variety of responses observed in HT\textsubscript{MIN}. One limitation to the current study is the absence of control of free-living training throughout the HT program. While training was monitored throughout this period, future research should aim to more closely control training performed outside of the HT sessions. Although this distinction was needed to answer the current research question, one limitation to the current study design is the difference in protocols between testing and HT. A similar study design using the same protocol for testing and HT could be investigated to gain a more detailed description of the changes that are occurring over the four and eight-week period following DHA.

**Conclusion:** To summarize, HT\textsubscript{MAX} (twice weekly heat training) provides clear evidence for the ability to maintain and possibly improve physiological adaptations following DHA. HT\textsubscript{MIN} (once weekly heat training) may be sufficient for some individuals to maintain gains made from DHA, however, future research is needed to differentiate the responders and non-responders to this program. Following a unique DHA and HT protocol, participants in this aerobically trained sample did not demonstrate signs of decay in all variables following four weeks without heat exposure. Not only is this method of HA and HT unique and effective, it allows for optimal training-stress balance. This protocol should be considered by elite athletes who aim to reach peak performance and safety during competition in the heat.
References


Figure 1. Study design overview.

Table 1. Complete description of trials throughout the study

<table>
<thead>
<tr>
<th>Test Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test #1</td>
<td>Baseline trial in an un-acclimated state; pre-summer</td>
</tr>
<tr>
<td>Test #2</td>
<td>Trial following heat acclimatization through free-living summer training; post summer; pre-heat acclimation</td>
</tr>
<tr>
<td>Test #3</td>
<td>Trial following 5-day short term heat acclimation; post dual heat acc</td>
</tr>
<tr>
<td>Test #4</td>
<td>Trial 4 weeks after dual heat acc</td>
</tr>
<tr>
<td>Test #5</td>
<td>Trial 8 weeks after dual heat acc</td>
</tr>
</tbody>
</table>
Table 2. Changes in physiological variables following heat acclimatization and heat acclimation.

<table>
<thead>
<tr>
<th></th>
<th>1 vs 2</th>
<th>2 vs 3</th>
<th>1 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MD±SE</td>
<td>95%CI</td>
<td>ES</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>-4.41±1.30</td>
<td>L: -7.09</td>
<td>U: -1.73</td>
</tr>
<tr>
<td>Max</td>
<td>-11.15±2.08</td>
<td>L: -11.42</td>
<td>U: -2.88</td>
</tr>
<tr>
<td>Internal Temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>-0.04±0.05</td>
<td>L: -0.14</td>
<td>U: 0.07</td>
</tr>
<tr>
<td>Ending</td>
<td>-0.13±0.07</td>
<td>L: -0.28</td>
<td>U: 0.01</td>
</tr>
<tr>
<td>Min</td>
<td>0.05±0.06</td>
<td>L: -0.08</td>
<td>U: 0.17</td>
</tr>
<tr>
<td>Delta</td>
<td>-0.15±0.09</td>
<td>L: -0.34</td>
<td>U: 0.03</td>
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<tr>
<td>Skin Temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>-0.39±0.09</td>
<td>L: -0.57</td>
<td>U: -0.22</td>
</tr>
<tr>
<td>Sweat Rate (L.h⁻¹)</td>
<td>-0.02±0.05</td>
<td>L: -0.07</td>
<td>U: 0.11</td>
</tr>
</tbody>
</table>

Test*¹: Baseline-Unacclimated; Test*²: Post Heat Acclimatization; Test*³: Post Dual Heat Acc

Negative values indicate later test is lower than earlier test. Positive values indicate later test is higher than earlier test.

*Indicates statistical significance, p<0.05
Table 3. Group comparisons of physiological outcomes of no heat training (HT\textsubscript{CON}), once per week heat training (HT\textsubscript{MIN}), and twice per week heat training (HT\textsubscript{MAX}) following dual heat acc.

<table>
<thead>
<tr>
<th></th>
<th>Test\textsuperscript{#3} vs Test\textsuperscript{#4}</th>
<th>Test\textsuperscript{#3} vs Test\textsuperscript{#5}</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MD±SE</td>
<td>95%CI</td>
</tr>
<tr>
<td><strong>Heart Rate (bpm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT\textsubscript{CON}</td>
<td>5.76±3.52</td>
<td>-2.32, 13.84</td>
</tr>
<tr>
<td>HT\textsubscript{MIN}</td>
<td>-1.54±2.64</td>
<td>-7.62, 4.54</td>
</tr>
<tr>
<td>HT\textsubscript{MAX}</td>
<td>-0.25±2.74</td>
<td>-6.44, 5.95</td>
</tr>
<tr>
<td>Highest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT\textsubscript{CON}</td>
<td>12.13±12.11</td>
<td>7.14, 17.11</td>
</tr>
<tr>
<td>HT\textsubscript{MIN}</td>
<td>-0.22±3.81</td>
<td>-9.01, 8.57</td>
</tr>
<tr>
<td>HT\textsubscript{MAX}</td>
<td>2.00±4.29</td>
<td>-7.71, 11.71</td>
</tr>
<tr>
<td><strong>Internal Temperature (°C)</strong></td>
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<tr>
<td>Trial Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT\textsubscript{CON}</td>
<td>-0.15±0.17</td>
<td>-0.24, 0.54</td>
</tr>
<tr>
<td>HT\textsubscript{MIN}</td>
<td>-0.09±0.16</td>
<td>-0.45, 0.27</td>
</tr>
<tr>
<td>HT\textsubscript{MAX}</td>
<td>-0.07±0.09</td>
<td>-0.28, 0.14</td>
</tr>
<tr>
<td>Delta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT\textsubscript{CON}</td>
<td>0.24±0.18</td>
<td>-0.18, 0.67</td>
</tr>
<tr>
<td>HT\textsubscript{MIN}</td>
<td>-0.14±0.22</td>
<td>-0.65, 0.37</td>
</tr>
<tr>
<td>HT\textsubscript{MAX}</td>
<td>-0.10±0.12</td>
<td>-0.39, 0.18</td>
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<tr>
<td><strong>Skin Temperature (°C)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT\textsubscript{CON}</td>
<td>-0.19±0.20</td>
<td>-0.67, 0.29</td>
</tr>
<tr>
<td>HT\textsubscript{MIN}</td>
<td>0.47±0.16</td>
<td>0.11, 0.84</td>
</tr>
<tr>
<td>HT\textsubscript{MAX}</td>
<td>-0.07±0.17</td>
<td>-0.46, 0.31</td>
</tr>
</tbody>
</table>

Test\textsuperscript{#3}: Post dual heat acc; Test\textsuperscript{#4}: Four weeks following dual heat acc; Test\textsuperscript{#5}: Eight weeks following dual heat acc

Negative values indicate later test is lower than earlier test. Positive values indicate later test is higher than earlier test.

*Indicates statistical significance, p<0.05
Table 4. Within-group comparisons of physiological outcomes of no heat training (HTCON), once per week heat training (HTMIN), and twice weekly heat training (HTMAX).

<table>
<thead>
<tr>
<th></th>
<th>HTCON vs HTMIN</th>
<th>95% CI</th>
<th>P-value</th>
<th>HTCON vs HTMAX</th>
<th>95% CI</th>
<th>P-value</th>
<th>HTMIN vs HTMAX</th>
<th>95% CI</th>
<th>P-value</th>
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<td>MD±SE</td>
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<td>Average HR</td>
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<tr>
<td>Test^3</td>
<td>0.11±5.62</td>
<td>0.01</td>
<td>0.985</td>
<td>-3.33±5.52</td>
<td>0.29</td>
<td>0.555</td>
<td>-3.44±5.33</td>
<td>0.30</td>
<td>0.528</td>
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<tr>
<td>Test^4</td>
<td>0.19±7.30</td>
<td>0.48</td>
<td>0.340</td>
<td>-9.34±6.74</td>
<td>0.66</td>
<td>0.185</td>
<td>-2.14±5.49</td>
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<td>0.745</td>
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<tr>
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<td>-9.94±7.32</td>
<td>0.66</td>
<td>0.195</td>
<td>-16.59±7.38</td>
<td>1.07</td>
<td>0.039</td>
<td>-6.65±5.93</td>
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<tr>
<td>Highest HR</td>
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<tr>
<td>Test^3</td>
<td>-0.24±7.18</td>
<td>0.25</td>
<td>0.974</td>
<td>-3.03±7.92</td>
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<td>0.708</td>
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<td>0.43</td>
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<td>Test^4</td>
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<td>0.138</td>
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<td>0.73</td>
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<tr>
<td>Test^5</td>
<td>-14.54±8.62</td>
<td>0.82</td>
<td>0.112</td>
<td>-22.88±9.12</td>
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<td>0.023</td>
<td>-8.33±6.80</td>
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<td>Average T_rec</td>
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<tr>
<td>Test^3</td>
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<td>0.41</td>
<td>0.382</td>
<td>0.32±0.17</td>
<td>0.85</td>
<td>0.082</td>
<td>0.14±0.18</td>
<td>0.34</td>
<td>0.458</td>
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<tr>
<td>Test^4</td>
<td>-0.06±0.19</td>
<td>0.16</td>
<td>0.754</td>
<td>0.79±0.16</td>
<td>0.29</td>
<td>0.573</td>
<td>0.15±0.19</td>
<td>0.39</td>
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<td>Test^5</td>
<td>0.01±0.14</td>
<td>0.03</td>
<td>0.944</td>
<td>-0.05±0.15</td>
<td>0.16</td>
<td>0.734</td>
<td>-0.06±0.17</td>
<td>0.17</td>
<td>0.718</td>
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<tr>
<td>Ending T_rec</td>
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<tr>
<td>Test^3</td>
<td>0.26±0.25</td>
<td>0.51</td>
<td>0.305</td>
<td>0.41±0.19</td>
<td>1.02</td>
<td>0.048</td>
<td>0.15±0.24</td>
<td>0.28</td>
<td>0.554</td>
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<tr>
<td>Test^4</td>
<td>-0.12±0.24</td>
<td>0.32</td>
<td>0.618</td>
<td>0.07±0.22</td>
<td>0.13</td>
<td>0.769</td>
<td>0.19±0.25</td>
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<tr>
<td>Test^5</td>
<td>-0.23±0.21</td>
<td>0.53</td>
<td>0.304</td>
<td>-0.19±0.22</td>
<td>0.43</td>
<td>0.395</td>
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<tr>
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<td>0.355</td>
<td>0.28±0.18</td>
<td>0.72</td>
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<td>0.13</td>
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<td>-0.09±0.24</td>
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<td>0.702</td>
<td>0.28±0.22</td>
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<td>0.215</td>
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</table>

Test^3: Post dual heat acc; Test^4: Four weeks post Test^3; Test^5: Eight weeks post Test^3

Negative values indicate later test is lower that earlier test.

*Indicates statistical significance, p<0.05
Figure 2. Change in maximum heart rate from post-dual heat acc (Test#3) to four weeks following dual heat acc (Test#4) and eight weeks following dual heat acc (Test#5).

HT<sub>CON</sub>: Control group who did not complete any heat training following dual heat acc; HT<sub>MIN</sub>: Group who completed one heat training session per week following dual heat acc; HT<sub>MAX</sub>: Group who completed two heat training sessions per week following dual heat acc.

* Indicates between group differences between HT<sub>CON</sub> and HT<sub>MAX</sub>

^ Indicates within group differences from Test#3 (HT<sub>CON</sub>)

# Indicates within group differences from Test#3 (HT<sub>MIN</sub>)

+ Indicates within group differences from Test#3 (HT<sub>MAX</sub>)

Statistical significance set at p<0.05
Figure 3. Percent change in ending internal body temperature from post-dual heat acc (Test\textsuperscript{#3}) to four weeks following dual heat acc (Test\textsuperscript{#4}) and eight weeks following dual heat acc (Test\textsuperscript{#5}).

HT\textsubscript{CON}: Control group who did not complete any heat training following dual heat acc; HT\textsubscript{MIN}: Group who completed one heat training session per week following dual heat acc; HT\textsubscript{MAX}: Group who completed two heat training sessions per week following dual heat acc

*Indicates statistical significance, $p<0.05$, from Test\textsuperscript{#3}.
Figure 4a-c. Percent change (%) of physiological adaptations from a baseline post-heat acclimation state in a control (HT\textsubscript{CON}) group (a), once per week heat training (HT\textsubscript{MIN}) group (b), and twice per week heat training (HT\textsubscript{MAX}) group (c).

a.

b.

c.
Supplemental Figure 1 a (HT\textsubscript{CON}), b, (HT\textsubscript{MIN}) and c (HT\textsubscript{MAX}). Individual responses of highest reached heart rate from Test\#1 (baseline-un-acclimated), Test\#2 (post-heat acclimatization), Test\#3 (post heat acclimation), Test\#4 (four weeks following dual heat acc), and Test\#5 (eight weeks following dual heat acc). Gray lines represent individual participants and the black line represents the group mean.
Supplemental Figure 2 a (HT_{CON}), b, (HT_{MIN}) and c (HT_{MAX}). Individual responses of max internal body temperature from Test^1 (baseline-un-acclimated), Test^2 (post-heat acclimatization), Test^3 (post heat acclimation), Test^4 (four weeks following dual heat acc), and Test^5 (eight weeks following dual heat acc). Gray lines represent individual participants and the black line represents the group mean.
Supplemental Figure 3 a (HT\textsubscript{CON}), b, (HT\textsubscript{MIN}) and c (HT\textsubscript{MAX}). Individual responses of sweat rate from Test\textsuperscript{#1} (baseline-un-acclimated), Test\textsuperscript{#2} (post-heat acclimatization), Test\textsuperscript{#3} (post heat acclimation), Test\textsuperscript{#4} (four weeks following dual heat acc), and Test\textsuperscript{#5} (eight weeks following dual heat acc). Gray lines represent individual participants and the black line represents the group mean.
Ch. 6 Distinguishing Factors of Individual Changes in Internal Body Temperature, Heart Rate, and Sweat Rate Following Heat Acclimatization and Heat Acclimation

**Background:** There is a need to understand the individual variability in physiological improvements when undergoing HAz or HA, and others do not. **Purpose:** the purpose of this study was to examine the individual nature of HAz and HA and factors that may contribute to the varied responses. **Methods:** 28 endurance athletes (mean[M]±standard deviation[SD]; age, 35±12 years; height, 178.81±6.39 cm; body mass [BM], 72.95±8.90 kg; body fat percent [BF%], 10.96±5.31%; height, 178.59±6.24 cm; VO$_{2max}$ 58.80±8.27 ml·kg$^{-1}$·min$^{-1}$). completed testing trials. These trials involved 60 minutes of exercise (59.31±1.73% vVO2max) in an artificial environmental laboratory (M±SD; ambient temperature [T$_{amb}$], 35.11±0.62°C; relative humidity [%RH], 47.61±0.38%; Wet Bulb Globe Temperature [WBGT] 29.53±0.63°C; wind speed, 4.02±0.12 mph) at three time points: 1) baseline (Test$^1$), 2) post-HAz (Test$^2$), 3) post-Dual Heat Acc (DHA) (Test$^3$). Throughout the tests, internal body temperature (T$_{rec}$), heart rate (HR), and sweat rate (SR) were collected. HAz involved free-living summer training in the summer and HA utilized a novel hyperthermic zone (HZ) approach for five days. HZHA involved internal temperature between 38.50 and 39.75°C for sixty minutes. Based on positive vs negative absolute change, we distributed participants into two groups for each physiological response (T$_{rec}$, HR, and SR). Independent t-test were performed for a variety of factors between groups for each response. Statistical significance was set at 0.05, a priori. **Results:** There were differences in T$_{rec}$ between groups at Test$^2$ (M±SD, improved T$_{rec}$ 39.19±0.41°C; not improved T$_{rec}$ 38.68±0.65°C; [95%CI], [0.92, 0.10]°C; ES=0.29; p=0.017). There were no differences in HR between groups at Test$^2$ (M±SD, improved HR 161±17 bpm; not improved HR 149±15
bpm; [95%CI], [-2, 26] bpm; ES=0.73; p=0.079), although the groups were approaching statistical differences. **Conclusions:** If the ultimate goal is a lower $T_{rec}$ or HR, this data does not support that every individual, especially those who are not stressed in a given environment and intensity, would benefit from HA. Individual and team needs analyses should be established prior to the start of a HAz or HA program and should guide the practitioner in designing and implementing a program.

**Introduction:** Heat acclimatization (HAz), which occurs in a natural environment, and heat acclimation (HA), which occurs in an artificial environment are effective methods for the physically active to optimize safety and performance in hot environments.\(^1\) Classic markers of successful HAz or HA include a lower internal body temperature and heart rate and a higher sweat rate.\(^2\) All of these outcomes are known to positively improve several aspects of performance\(^3\) and safety.\(^4\) Although this concept has been extensively studied for many years\(^5\) a recent meta-analysis concluded that the optimal methodological approach to this type of heat mitigation strategy is still unknown and that several factors influence individual outcomes greatly.\(^1\)

The impact of several participant characteristics, including age, aerobic fitness, and body composition, and some training factors on HAz and HA have been examined independently and the results are mixed.\(^6\)–\(^9\) In 2014, a study was released that assessed young and older cyclist pre and post HA.\(^10\) These highly trained participants were matched and the authors concluded that the older well-trained individuals achieved the same cardiovascular adaptations as their younger counterparts, however, internal body temperature and sweat loss was not impacted by age.\(^10\) Additionally, it is well-established that aerobic fitness contributes to thermal tolerance and this
concept remains true in terms of HA. In 1977, Pandolf et al. demonstrated that aerobic fitness and time for rectal temperature to plateau throughout HA were significantly related ($r = -0.68$). Some investigations have also thoroughly examined the interactions of training and HA by controlling training outside of the lab and have concluded that full adjustments to specific environments can take months or years to occur. Others have speculated that physical training could have been a co-founding factor in study designs. Specifically, middle-age men seem to have a greater thermal tolerance prior to HA than younger individuals and the author hypothesized that this was most likely due to a higher training volume in the middle-aged men.

While accounting for participant characteristics and training information is influential in the attainment of thermal tolerance, the best method of HA to use, even in a controlled laboratory, is questionable. Previous literature has examined the variability of various HA study designs and concluded that the type of performance test used to assess HA can be strongly influenced by the methods used in the HA program. Many protocol lengths, frequencies, methods, environmental conditions, intensities, and durations have been investigated and all have reported advantages and disadvantages. Additionally, the individual internal body temperature or heart rate may also contribute to the magnitude of physiological improvement observed from HAZ and HA.

Several studies have investigated factors that are known to influence heat tolerance, however, few have assessed factors that could have an impact on HAZ or HA outcomes. Specifically, there is a need to understand why some individuals demonstrate certain physiological improvements when undergoing HAZ or HA, and others do not. Therefore, the purpose of this study was to examine the individual nature of HAZ and HA and factors that may contribute to the varied responses. We hypothesize that individuals with greater adaptations to
HAz and HA will demonstrate greater physiological stress prior to HAz and HA than the individuals who do not adapt.

**Methods:** Twenty-eight endurance athletes were included in this study (mean[M]±standard deviation[SD]; age, 35±12 years; height, 178.81±6.39 cm; body mass [BM], 72.95±8.90 kg; body fat percent [BF%], 10.96±5.31%; height, 178.59±6.24 cm; VO$_{2\max}$ 58.80±8.27 ml·kg$^{-1}$·min$^{-1}$). This study was approved by the institutional review board at <removed for review> and all participants provided written informed consent. A within-participant longitudinal study design was utilized, with the participants completing two VO$_{2\max}$ tests, three treadmill running exercise tests, free-living summer training, and five days of HA. For the VO$_{2\max}$ test, participants were asked to don a heart rate monitor (H10®, Polar Electro™, Kempele, Finland) and compete a self-selected 5-minute warm-up. Following warm-up, participants completed a graded maximal exercise test on a treadmill (T150; COSMED, Traunstein, Germany) at 2% grade to volitional exhaustion (TueOne, ParvoMedica, Sandy UT, USA). The mL of oxygen recorded during the final completed stage will be recorded as the VO$_{2\max}$. BF% was collected using skin-fold calipers and 3-site measurements.$^{19}$

The participants began exercising in the heat by completing sixty minutes of steady-state exercise on a motorized treadmill (T150; COSMED, Traunstein, Germany) (Test #1) in an artificial environmental laboratory when they were un-acclimatized, as they resided in New England, USA. Throughout the test, internal body temperature ($T_{rec}$) was continuously monitored by a rectal probe that was inserted 10cm beyond the anal sphincter by the participant (MP160; BIOPAC Systems Inc., Goleta, CA, USA) and heart rate (HR) was measured with a chest strap (H10®, Polar Electro™, Kempele, Finland). Sweat rate (SR) was calculated by taking the difference in nude BM measurements before and immediately post exercise. Following Test #1
participants were instructed to track their self-prescribed training throughout the summer (~June-August) using their own training devices (Garmin, n=21 [Forerunner® Fenix® Vivoactive® Garmin™ Ltd., Olathe, Kansas, USA]; Polar H10 and Polar Beat application, n=7 [H10®, Polar Electro™, Kempele, Finland]).\textsuperscript{20} Test\textsuperscript{#2}, which mimicked Test\textsuperscript{#1}, was completed in late August or early September. The days between Test\textsuperscript{#1} and Test\textsuperscript{#2} were recorded (M±SD; Test\textsuperscript{#1} and Test\textsuperscript{#2}, 109±10 days).

Following Test\textsuperscript{#2}, participants completed five days of HA within eight days. The HA method utilized in this study (hyperthermic zone [HZ]) requires that the participant maintain an elevated $T_{\text{rec}}$ (38.50 -39.75°C) for sixty minutes while exercising in the heat (M±SD; $T_{\text{amb}}$, 38.67±1.03°C; %RH, 51.34±2.42%; WBGT, 33.82±1.20°C; wind speed, 0±0 mph). The exercise sessions began with a higher intensity exercise (~70% $v\text{VO}_{2\text{max}}$) to increase $T_{\text{rec}}$ quickly to the desired level of 38.5°C and was subsequently adjusted throughout the remaining 60 minutes so as not to depart from the desired HZ. Total AUC in $T_{\text{rec}}$ (AUC) and $T_{\text{rec}}$ above 38.50°C (AUC\textsuperscript{38.5}), average HR, and sweat volume (SV) were recorded for each session. Fluid was consumed ad-libitum and the volume recorded throughout HA.

Following HA, a third steady-state exercise test (Test\textsuperscript{#3}) was completed. A complete description of all tests can be seen in Table 1. For all three tests, participants were instructed to arrive to the laboratory euhydrated. Hydration status was confirmed with urine indices (M±SD; urine specific gravity, 1.010±0.008; color, 2±0).\textsuperscript{21} No fluid was provided throughout the 60-minute exercise at any time point. Environmental conditions for three testing sessions were recorded (M±SD; ambient temperature [$T_{\text{amb}}$], 35.11±0.62°C; relative humidity [%RH], 47.61±0.38%; Wet Bulb Globe Temperature [WBGT] 29.53±0.63°C; wind speed, 4.02±0.12
mph). Twenty-five participants completed all three tests and three participants completed Test$^{#2}$, HA, and Test$^{#3}$.

Participant characteristic information, including VO$_{2\text{max}}$, age, BM, BF%, and max heart rate ($HR_{\text{max}}$) were recorded at the beginning of the study protocol. An additional VO$_{2\text{max}}$ was completed prior to Test$^{#2}$ to account for changes in fitness from summer training. $HR_{\text{max}}$ was recorded from the VO$_{2\text{max}}$ test.

When available, meteorological data were extracted from the nearest available automated surface observing station (ASOS), with a mean distance of 16±11 km (number of cases, n=2801). Training location was determined by the GPS device and the latitude/longitude of that training session location was utilized to determine the nearest weather station. Daytime WBGTs (7 a.m. - 7 p.m.) were modeled using Heat Stress Advisor software package (version 2005; Zunis Foundation, Tulsa, OK; Coyle 2000)$^{22}$, which is designed to work with weather station data; nighttime WBGTs were computed using the Liljegren model with solar radiation set to zero.$^{23}$ Total distance, average HR, session duration, $T_{\text{amb}}$, %RH, wind speed, and WBGT were reported. The cases that meteorological data were not available were excluded from the analysis (number of cases, n=651).

Training data were captured from the wearable technology that was used by participants throughout the summer. Several variables were collected from training data and ones that were included in this analysis were: total training time, percent of $HR_{\text{max}}$ (Average HR%), weekly distance, total number of sessions, and running training time. Total training time describes the sum, in minutes, of all training that occurred throughout HAz, including running, cycling, weight training, and other. Average HR% is defined by the percentage of individual’s $HR_{\text{max}}$ (as determined by VO$_{2\text{max}}$ test) that participants trained at throughout the summer in all session
types. *Weekly distance* is the total number of kilometers that participants ran every seven days between Test\(^1\) and Test\(^2\). *Total # of sessions* describes the sum count of all sessions that occurred from Test\(^1\) to Test\(^2\). *Running training time* describes the average number of minutes that were recorded per running session from Test\(^1\) to Test\(^2\).

**Statistical Analysis:** Based on positive vs negative absolute change, we distributed participants into two groups for each physiological response (T\(_{rec}\), HR, and SR). Our approach is unique in that it considers the practical application and evaluates the physiological mechanisms that might distinguish success or failure in response to HAz or HA. The detriment to safety and performance during exercise in the heat with an elevated T\(_{rec}\) has been well-established.\(^{24-26}\) Similarly, an elevated HR typically leads to earlier onset of fatigue and lower performance and this is exacerbated during exercise in the heat.\(^{27,28}\) Finally, one of the primary mechanism to achieve a lower T\(_{rec}\) and HR following HAz or HA is an increase in SR.\(^{29,30}\) An increase in T\(_{rec}\) or HR and a lower SR is not considered successful HAz or HA. Figure 1 demonstrates the distribution of participants who improved and did not improve T\(_{rec}\), HR, and SR from HAz and HA. Figure 2 demonstrates descriptive data of T\(_{rec}\), HR, and SR from improved and not improved groups from HAz and HA.

To ensure physiological differences between the groups, a repeated measures ANOVA with post-hoc comparisons were performed for each variable at each test. To examine differences in those that demonstrated improvement and no improvement of T\(_{rec}\), HR, and SR, independent t-test were performed for each participant characteristic, training metric, and environmental condition from HAz and for AUC, AUC\(^{38.5}\), HA average HR, and HA sweat volume from HA. Cohen’s d effect sizes (ES) were calculated to quantify the magnitude of pairwise differences.
ES was interpreted according to the following thresholds: < 0.2 = trivial, 0.2–0.6 = small, 0.7–1.1 = moderate, 1.2–2.0 = large, and > 2.0 = very large.\textsuperscript{31} Statistical significance was set at p<0.05, a priori. Data are reported as M±SD, 95% confidence intervals (95%CI) and effect size (ES). All statistical analyses were completed using SPSS Statistics for Mac, version 25 (IBM Corp., Armonk N.Y., USA).

**Results:** The days between Test\textsuperscript{#2}, HA sessions, and Test\textsuperscript{#3} were recorded (M±SD; Test\textsuperscript{#2} and HA \#1, 4±2 days; HA \#1 and HA \#2, 1±1 days; HA \#2 and HA \#3, 2±1 days; HA \#3 and HA \#4, 2±1 days; HA \#4 and HA \#5, 1±1 days; total number of HA days, 6±1 days; HA \#5 and Test\textsuperscript{#3}, 3±1). All participants (100%) exhibited improvement in two out of the three variables (HR, T\textsubscript{rec}, and/or SR) by Test\textsuperscript{#3}. There was a significant interaction for T\textsubscript{rec}, HR, and SR for HAz and HA (p<0.05) and, as expected, post-hoc analysis confirmed that the groups responded differently to HAz and HA for each variable (p<0.05) (Table 2).

*Influence of Participant Characteristics on T\textsubscript{rec}, HR, and SR Improvement Following HAz:* There were no significant differences in any subject characteristics between those demonstrating improved T\textsubscript{rec}, HR, or SR following HAz (Table 3). There were no differences in T\textsubscript{rec} between groups at Test\textsuperscript{#1} (M±SD, improved T\textsubscript{rec} 39.23±0.64 °C; not improved T\textsubscript{rec} 38.99±0.47 °C; [95%CI], [-0.73, 0.25] °C; ES=0.41; p=0.316). There were also no differences in T\textsubscript{rec} between groups at Test\textsuperscript{#2} (M±SD, improved T\textsubscript{rec} 38.84±0.54 °C; not improved T\textsubscript{rec} 39.23±0.54 °C; [95%CI], [-0.10, 0.82] °C; ES=0.72; p=0.117). There were no differences in HR between groups at Test\textsuperscript{#1} (M±SD, improved HR 166±15 bpm; not improved HR 155±14 bpm; [95%CI], [-25, 4] bpm; ES=0.74; p=0.148). There were also no differences in HR between groups at Test\textsuperscript{#2}
Influence of Training and Environmental Variables on Trec, HR and SR Following HAz:

Differences in training variables between improved Trec, HR, and SR from HAz are reported in Table 4. No differences were observed amongst any training variables for changes in Trec or SR. Running weekly distance appears to influence HR responses to HAz, as the participants with a lower HR ran more km per week than those with the same or higher HR following HA. Differences in environmental conditions between improved Trec, HR, and SR from HAz are demonstrated in Table 5. No statistical differences were observed amongst groups in terms of environmental conditions.

Influence of Characteristics and Heat Acclimation Variables on Trec, HR and SR Following HA:

Table 6 shows the differences between those who improved and did not improve their Trec from HA. There were no differences between individuals who improved and did not improve HR following HA in age (M±SD, improved HR 36±12 years; not improved HR 34±13 years; [95%CI], [ -12, 9] years; ES=0.16; p=0.773), VO2max (M±SD, improved HR 59.25±8.34 ml·kg⁻¹·min⁻¹; not improved HR 57.87±8.52 ml·kg⁻¹·min⁻¹; [95%CI], [-8.36, 5.60] ml·kg⁻¹·min⁻¹; ES=0.16; p=0.688), BM (M±SD, improved HR 75.06±8.46 kg; not improved HR 68.51±8.57 kg;
[95%CI], [-13.61, 0.52] kg; ES=0.77; p=0.068), or %BF (M±SD, improved HR 11.36±4.93 %; not improved HR 10.09±6.24 %; [95%CI], [-5.74, 3.20] %; ES=0.23; p=0.564), although BM was approaching statistical differences between groups, with the ‘improved’ group having greater BM than ‘not improved’ group. There were no differences between individuals who improved and did not improve HR following HA in AUC (M±SD, improved HR 15447.10±929.70 °C·min; not improved HR 15660.25±942.08 °C·min; [95%CI], [-563.32, 989.64] °C·min; ES=0.23; p=0.577), average HR during HA (M±SD, improved HR 132±12 bpm; not improved HR 136±10 bpm; [95%CI], [-6.35, 12.94] bpm; ES=0.35; p=0.36), or sweat volume (M±SD, improved HR 2.55±0.59 L; not improved HR 2.72±0.45 L; [95%CI], [-0.29, 0.63] L; ES=0.31; p=0.447). There were also no differences in HR between groups at Test#2 (M±SD, improved HR 161±17 bpm; not improved HR 149±15 bpm; [95%CI], [-2, 26] bpm; ES=0.73; p=0.079), although the groups were approaching statistical differences. There were no differences in HR between the groups at Test#3 (M±SD, improved HR 149±16 bpm; not improved HR 154±14 bpm; [95%CI], [-17, 8] bpm; ES=0.32; p=0.477).

There were no differences between individuals who improved and did not improve SR following HA in age (M±SD, improved SR 34±12 years; not improved SR 37±12 years; [95%CI], [-12, 8] years; ES=; p=0.662), VO\textsubscript{2max} (M±SD, improved SR 59.38±8.23 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; not improved SR 57.22±8.74 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; [95%CI], [-4.66, 8.98] ml·kg\textsuperscript{-1}·min\textsuperscript{-1}; ES=; p=0.520), BM (M±SD, improved SR 72.89±10.04 kg; not improved SR 73.06±6.86 kg; [95%CI], [-7.51, 7.19] kg; ES=; p=0.964), or %BF (M±SD, improved SR 10.88±5.51 %; not improved SR 11.09±5.21 %; [95%CI], [-4.66, 8.98] %; ES=; p=0.921). There were no differences between individuals who improved and did not improve SR following HA in AUC.
(M±SD, improved SR 15584.75±746.67 °C·min; not improved SR 15391.16±1211.84 °C·min; [95% CI], [-163.85, 951.01] °C·min; ES=; p=0.604), average HR during HA (M±SD, improved SR 132±11 bpm; not improved SR 136±12 bpm; [95% CI], [-13, 6] bpm; ES; p=0.478), or sweat volume (M±SD, improved SR 2.64±0.49 L; not improved SR 2.53±0.066 L; [95% CI], [-0.34, 0.56] L; ES=; p=614).

**Discussion:** This study aimed to distinguish factors that influence changes in $T_{rec}$, HR, and SR following HAz and HA. The findings from this study point to the need to focus on individual responses to both HAz and HA, which may not follow traditional timelines. There were no factors that differed between individuals who improved and did not improve $T_{rec}$ following HAz. One unexpected finding from this study was the absence of differences in WBGT between $T_{rec}$ groups, which could be explained by the low environmental training stress (~22°C WBGT) throughout this period in the New England area of the United States. The individuals who showed improvements in HR from HAz reported significantly more running distance per week (~35 km) compared to individuals who did not show improvements in HR. Participants who displayed an increase in SR trained in a higher WBGT that approached statistical significance (p=0.08), although the actual mean difference is quite small (~1°C WBGT). Perhaps the most impactful finding from this study was that initial test $T_{rec}$, and possibly HR, determined how individuals would respond to HA. Specifically, individuals who demonstrated improvements in $T_{rec}$ had ~0.50 °C higher $T_{rec}$ prior to HA than those who did not improve. Additionally, individuals who improved HR following HA was approaching statistically significantly lower (~12 bpm) HR than individuals who did not demonstrate improvement.
HAz resulted in improvements in HR (~11 bpm) for 19 individuals, Trec (~0.39 °C) for 15 individuals, and SR (0.23 L·h⁻¹) for 9 individuals. As a group, the patterns of these adaptations (HR first, followed by Trec, and then SR) are in conjunction with previous literature²,³, however, the actual number of days previously thought to achieve full adaptations are not in agreement with previous literature. Ninety-five percent of all adaptations are said to be achieved after fourteen days of heat exposure.²,³ Therefore, the HAz period in the present study of ~109 days should have been long enough to achieve improvements in HR, Trec, and SR in the majority of the participants, however, this was not the case. We hypothesize that these adaptations were not achieved because of the low environmental stress throughout HAz in New England, USA. Even though the expected group patterns were observed, it is critical to point-out the individual participant responses in Figure 2. For example, participant 19 demonstrated improvements in HR and SR but not Trec following HAz, which differs from the previously established timeline.¹ Previous literature that examined heat acclimatization in well-trained cyclist concluded that there may be “fast” and “slow” responders to heat acclimatization.³ The results from the present study supports that conclusion and highlights the need for individualized HAz programs.

No differences were present in subject characteristics information for HAz. While previous research has demonstrated that aerobically trained individuals demonstrate greater thermal tolerance during exercise in the heat, there were no differences in VO₂max in this study and this is most likely due to the low variability in this cohort.¹⁵ Previous research has also questioned the impact of age on during exercise in the heat and findings from this study support what has been previously reported by Padolf et al.⁷ Specifically, our study did not demonstrate
differences in age for any HAz outcome and we hypothesize that this is most likely due to the high training quantity of the participants in this study, regardless of age.

Training, specifically related to running, seems to be impactful for optimal HR HAz outcomes. Following HAz, individuals in the ‘improved HR’ group ran for ~63 minutes compared to those in the ‘not improved HR’ group who ran ~40 minutes, and this difference was approaching statistical differences (p=0.052) with moderate effects (ES=0.96). While little research has investigated this concept in a field setting, previous research in laboratory settings have demonstrated that longer session durations can positively impact performance in the heat.\(^3\) Weekly running distance was also greater with moderate effects in the group that demonstrated improved HR. One previous study reported successful HAz with low training volume\(^34\), however, the environmental conditions in that study (40°C \(T_{amb}\); 12%RH) were substantially greater than the present study and most likely permitted an elevated \(T_{rec}\) with less volume.

HA resulted in more consistent outcomes especially in regards to improved SR, with improvements in HR (~12 bpm) for 19 individuals, \(T_{rec}\) (~0.53 °C) for 19 individuals, and SR (0.31 L·h⁻¹) for 18 individuals. These improvements are most likely linked to the extreme environment and high intensity of this type of HA, which is consistent with previous thought.\(^35\) However, this type of training is not always feasible and may not be available to many athletes.\(^18\) From HA, the ‘improved’ group tended to have individuals with elevated \(T_{rec}\) and HR compared to the ‘not improved’ group prior to the start of the training program.

This finding begs the question: do individuals who are not physiological stressed in a specific environment at a given intensity benefit from, and therefore need, HA? First, as with classic sport performance literature, a needs analysis must be completed.\(^36\) If the ultimate goal is a lower \(T_{rec}\) or HR, this data does not support that every individual, especially those who are not
stressed in a given environment and intensity, would benefit from HA. However, if improved performance is the ultimate goal, HA, even without lower T_rec or HR, may be beneficial. While improved T_rec and HR can result in greater performance, there are several other components, such as improved perception, that can contribute. Therefore, tailored HA programs should be considered by coaches and athletes for the desired outcome.

AUC or AUC_{38.5} was not different between any groups, however, this finding should be taken with caution as this study was part of a larger study, therefore, AUC was not controlled for and resulted in low variability between groups. Future HA studies should investigate the impact of AUC to determine if a more intense protocol, such as HZHA, would prove more beneficial than a traditional HA protocol (such as isothermal, fixed-work rate, or self-selected). Training under these conditions may allow athletes to achieve greater adaptations sooner than with other methods, which is typically thought to be 7-14 days. Specifically, the changes that were seen in SR are not often reported with such a short protocol, as this is one of the last adaptations to occur.

Although one aim of this study was to examine differences in participant characteristics, one limitation to the current design was that there was the little variability in participant characteristics data in this aerobically trained population. Future research should aim to gather individuals of a variety of participant characteristic factors and examine differences in HAz responses. Another limitation to this study is that the environmental conditions throughout HAz were low and may not be applicable to individuals who train in warmer climate. Future research should examine these factors in aerobically trained individuals who train in higher WBGTs.
Conclusion: In summary, there are a variety of individual adaptations to both HAz and HA and the timeline of these adaptations are not always as expected. The exponential growth of the use of wearable technology in the athletic, military, and physically active communities may be a useful tool to examine individual HAz outcomes. This study provides a starting point from which individuals in these cohorts can use wearable technology to understand the adaptations that occur with HAz. While the findings from this study expand upon the current literature, future research and extensive modeling methods are needed to examine ways to quantify variables that are related to successful HAz and HA. Knowing this, future investigations should examine these factors within the ‘improved’ category alone to examine what factors influence the magnitude of change in these individuals. This type of data will provide evidence-based information for coaches, military personnel, and sports medicine professionals that will allow them to make data-driven decisions about their personnel. Additionally, this study provides evidence that a method of HA and a way to quantify internal body temperature during HA (AUC) may be beneficial to optimizing the adaptations that can be made from this heat mitigation strategy. Finally, individual and team needs analyses should be established prior to the start of a HAz or HA program and should guide the practitioner in designing and implementing a program.
References


Table 1. Complete description of trials throughout the study

<table>
<thead>
<tr>
<th>Test Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>Test #1</td>
<td>Baseline trial in an un-acclimated state; pre-summer</td>
</tr>
<tr>
<td>Test #2</td>
<td>Trial following heat acclimatization through free-living summer training; post summer; pre-heat acclimation</td>
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<tr>
<td>Test #3</td>
<td>Trial following 5-day short term heat acclimation; post dual heat acc</td>
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</table>
Figure 1. Distribution of those who improved and did not improve internal body temperature (Trec) heart rate (HR), and sweat rate (SR) from heat acclimatization HA and heat acclimation (HA).
Figure 2. Individual heart rate (HR), internal body temperature (Trec), and sweat rate (SR) responses to heat acclimatization (HAz) and heat acclimation (HA)

<table>
<thead>
<tr>
<th>Participant</th>
<th>Improved Count</th>
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<th>HAz Trec</th>
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</table>

Figure legend

- **Improved**
- **Not Improved**
- **No Data**
Table 2. Descriptive data of internal body temperature ($T_{rec}$), heart rate (HR), and sweat rate (SR) from improved and not improved groups from HAz and HA.

<table>
<thead>
<tr>
<th>Heat Acclimatization</th>
<th>Improved Test#1 (mean±SD)</th>
<th>Not Improved Test#1 (mean±SD)</th>
<th>Improved Test#2 (mean±SD)</th>
<th>Not Improved Test#2 (mean±SD)</th>
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<td>$n=15$</td>
<td>$n=10$</td>
<td>$n=15$</td>
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<tr>
<td>$T_{rec}$ (°C)</td>
<td>39.23±0.64</td>
<td>38.99±0.47</td>
<td>38.84±0.54*</td>
<td>39.20±0.54^</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>$n=19$</td>
<td>166±15</td>
<td>155±14</td>
<td>154±18*</td>
</tr>
<tr>
<td>SR (L·h⁻¹)</td>
<td>$n=9$</td>
<td>1.75±0.24</td>
<td>1.82±0.42</td>
<td>1.98±0.24*</td>
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</table>

<table>
<thead>
<tr>
<th>Heat Acclimation</th>
<th>Improved Test#2 (mean±SD)</th>
<th>Not Improved Test#2 (mean±SD)</th>
<th>Improved Test#3 (mean±SD)</th>
<th>Not Improved Test#3 (mean±SD)</th>
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<tbody>
<tr>
<td>$T_{rec}$ (°C)</td>
<td>$n=19$</td>
<td>39.22±0.42</td>
<td>38.61±0.57</td>
<td>38.69±0.47*</td>
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<tr>
<td>HR (bpm)</td>
<td>$n=19$</td>
<td>161±17</td>
<td>149±15</td>
<td>149±16*</td>
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<tr>
<td>SR (L·h⁻¹)</td>
<td>$n=18$</td>
<td>1.72±0.43</td>
<td>1.84±0.39</td>
<td>2.03±0.43*</td>
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</table>

* Indicates lower $T_{rec}$ and HR and higher SR between Test#1 and Test#2 and Test#2 and Test#3 in the ‘improved’ group

^ Indicates higher $T_{rec}$ and HR and lower SR between Test#1 and Test#2 and Test#2 and Test#3 in the ‘not improved’ group
Table 3. Characteristics of individuals who improved and did not improve internal body temperature ($T_{rec}$), heart rate (HR), or sweat rate (SR) following heat acclimatization.

<table>
<thead>
<tr>
<th></th>
<th>Improved $T_{rec}$ (n=15) mean±SD</th>
<th>Not Improved $T_{rec}$ (n=10) mean±SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36±11</td>
<td>35±13</td>
<td>-9, 11</td>
<td>0.828</td>
<td>0.09</td>
</tr>
<tr>
<td>$\text{VO}_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>56.79±5.98</td>
<td>58.52±8.62</td>
<td>-7.76, 4.29</td>
<td>0.557</td>
<td>0.24</td>
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<tr>
<td>Body Mass (kg)</td>
<td>73.27±10.66</td>
<td>72.69±6.11</td>
<td>-7.16, 8.30</td>
<td>0.879</td>
<td>0.06</td>
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<tr>
<td>Percent Body Fat (%)</td>
<td>10.03±4.57</td>
<td>11.79±5.99</td>
<td>-6.49, 2.97</td>
<td>0.442</td>
<td>0.34</td>
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<tr>
<td>Initial Trial $T_{rec}$ (°C)</td>
<td>39.23±0.64</td>
<td>38.99±0.47</td>
<td>-0.25, 0.73</td>
<td>0.316</td>
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</table>

<table>
<thead>
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<th>Improved HR (n=19) mean±SD</th>
<th>Not Improved HR (n=6) mean±SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
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<td>Age (years)</td>
<td>36±12</td>
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<td>-13, 10</td>
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<td>$\text{VO}_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>57.74±7.98</td>
<td>56.67±4.61</td>
<td>-3.31, 5.46</td>
<td>0.617</td>
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<td>Body Mass (kg)</td>
<td>71.75±8.45</td>
<td>77.11±10.12</td>
<td>-13.92, 3.21</td>
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<td>Percent Body Fat (%)</td>
<td>11.04±5.33</td>
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<td>-3.78, 6.33</td>
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<td>Initial Trial HR (bpm)</td>
<td>166±15</td>
<td>155±14</td>
<td>-4, 25</td>
<td>0.148</td>
<td>0.74</td>
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<table>
<thead>
<tr>
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<th>Improved SR (n=9) mean±SD</th>
<th>Not Improved SR (n=16) mean±SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
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<tr>
<td>Age (years)</td>
<td>39±13</td>
<td>34±11</td>
<td>-6, 15</td>
<td>0.371</td>
<td>0.43</td>
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<tr>
<td>$\text{VO}_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>58.43±7.49</td>
<td>56.95±6.95</td>
<td>-4.68, 7.63</td>
<td>0.625</td>
<td>0.21</td>
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<tr>
<td>Body Mass (kg)</td>
<td>73.10±9.90</td>
<td>73.00±8.74</td>
<td>-7.79, 7.99</td>
<td>0.980</td>
<td>0.01</td>
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<tr>
<td>Percent Body Fat (%)</td>
<td>11.52±5.49</td>
<td>10.29±5.06</td>
<td>-3.27, 5.72</td>
<td>0.578</td>
<td>0.24</td>
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<tr>
<td>Initial Trial SR (L·h$^{-1}$)</td>
<td>1.75±0.24</td>
<td>1.82±0.42</td>
<td>-0.39, 0.25</td>
<td>0.649</td>
<td>0.19</td>
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</table>

*Indicates significant differences between groups, p<0.05
Table 4. Training descriptions of individuals who improved and did not improve internal body temperature ($T_{rec}$), heart rate (HR), or sweat rate (SR) following heat acclimatization.

<table>
<thead>
<tr>
<th></th>
<th>Improved mean±SD</th>
<th>Not Improved mean±SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
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<tr>
<td><strong>Lower $T_{rec}$ (n=15)</strong></td>
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<tr>
<td>Total Training Time (min)</td>
<td>5716.39±2109.68</td>
<td>5878.31±3450.26</td>
<td>-2454.21, 2130.37</td>
<td>0.885</td>
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<tr>
<td>Average Heart Rate (%)</td>
<td>68.48±5.81</td>
<td>66.34±5.28</td>
<td>-2.59, 6.88</td>
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<td>0.38</td>
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<tr>
<td>Running Weekly Distance (km)</td>
<td>377.19±114.90</td>
<td>383.97±207.75</td>
<td>-162.87, 149.33</td>
<td>0.927</td>
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<td>Total # of Sessions</td>
<td>68±26</td>
<td>69±21</td>
<td>-22, 19</td>
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<td>Running Training Time (min)</td>
<td>58.95±27.38</td>
<td>55.78±22.90</td>
<td>-18.55, 24.89</td>
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<td><strong>Lower HR (n=19)</strong></td>
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<tr>
<td>Total Training Time (min)</td>
<td>5625.17±2303.56</td>
<td>6275.10±3799.08</td>
<td>-3265.60, 1965.74</td>
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<td>Average Heart Rate (%)</td>
<td>67.34±5.81</td>
<td>68.51±5.25</td>
<td>-6.68, 4.35</td>
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<tr>
<td>Running Weekly Distance (km)</td>
<td>50.99±34.83</td>
<td>25.16±11.87</td>
<td>6.48, 45.18</td>
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<td>Total # of Sessions</td>
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<td>Running Training Time (min)</td>
<td>63.14±25.56</td>
<td>40.41±15.21</td>
<td>-0.24, 45.68</td>
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<td><strong>Higher SR (n=9)</strong></td>
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<tr>
<td>Total Training Time (min)</td>
<td>5734.92±2273.11</td>
<td>5807.16±2923.92</td>
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<td>Average Heart Rate (%)</td>
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<td>Running Weekly Distance (km)</td>
<td>390.78±125.55</td>
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<td>Total # of Sessions</td>
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<td>Running Training Time (min)</td>
<td>56.69±31.25</td>
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<td>0.06</td>
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*Indicates significant differences between groups, p<0.05
Table 5. Environmental condition descriptions of individuals who improved and did not improve internal body temperature ($T_{rec}$), heart rate (HR), or sweat rate (SR) following heat acclimatization.

<table>
<thead>
<tr>
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<th>Improved mean±SD</th>
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</tr>
<tr>
<td>Ambient Temperature (°C)</td>
<td>23.28±1.49</td>
<td>22.53±2.10</td>
<td>-0.74, 2.22</td>
<td>0.311</td>
<td>0.43</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>63.60±5.04</td>
<td>66.64±9.96</td>
<td>-9.26, 3.18</td>
<td>0.323</td>
<td>0.41</td>
</tr>
<tr>
<td>Heat Index (°C)</td>
<td>30.12±0.98</td>
<td>29.77±0.92</td>
<td>-0.46, 1.16</td>
<td>0.380</td>
<td>0.37</td>
</tr>
<tr>
<td>Wet-Bulb Globe Temperature (°C)</td>
<td>22.88±1.07</td>
<td>22.28±1.62</td>
<td>-0.50, 1.71</td>
<td>0.270</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Lower HR (n=19)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient Temperature (°C)</td>
<td>22.91±2.00</td>
<td>23.21±0.58</td>
<td>-1.37, 0.77</td>
<td>0.565</td>
<td>0.17</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>65.77±8.09</td>
<td>61.77±3.25</td>
<td>-3.09, 11.09</td>
<td>0.255</td>
<td>0.55</td>
</tr>
<tr>
<td>Heat Index (°C)</td>
<td>30.02±1.07</td>
<td>29.85±0.49</td>
<td>-0.76, 1.12</td>
<td>0.698</td>
<td>0.17</td>
</tr>
<tr>
<td>Wet-Bulb Globe Temperature (°C)</td>
<td>22.65±1.49</td>
<td>22.59±0.58</td>
<td>-1.24, 1.37</td>
<td>0.917</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Higher SR (n=9)</strong></td>
<td></td>
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</tr>
<tr>
<td>Ambient Temperature (°C)</td>
<td>23.66±1.67</td>
<td>22.60±1.73</td>
<td>-0.41, 2.54</td>
<td>0.149</td>
<td>0.62</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>62.35±6.07</td>
<td>66.20±7.85</td>
<td>-10.13, 2.42</td>
<td>0.216</td>
<td>0.53</td>
</tr>
<tr>
<td>Heat Index (°C)</td>
<td>30.09±0.81</td>
<td>29.92±1.05</td>
<td>-0.67, 1.00</td>
<td>0.688</td>
<td>0.18</td>
</tr>
<tr>
<td>Wet-Bulb Globe Temperature (°C)</td>
<td>23.25±1.13</td>
<td>22.30±1.32</td>
<td>-0.13, 2.04</td>
<td>0.083</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*Indicates significant differences between groups, p<0.05
Table 6. Differences between individuals who improved (Lower T\text{rec}) and maintained similar or did not improve (Same or Higher T\text{rec}) internal body temperature during testing following heat acclimation.

*Indicates significant differences between groups, p<0.05

<table>
<thead>
<tr>
<th></th>
<th>Improved mean±SD</th>
<th>Not Improved mean±SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lower T\text{rec} (n=19)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area Under the Curve</td>
<td>15412.40±909.62</td>
<td>15733.50±962.58</td>
<td>-1091.52, 449.32</td>
<td>0.399</td>
<td>0.21</td>
</tr>
<tr>
<td>Area Under the Curve &gt;38.5</td>
<td>215.47±37.17</td>
<td>218.59±41.59</td>
<td>-35.21, 28.98</td>
<td>0.843</td>
<td>0.34</td>
</tr>
<tr>
<td>Average Heart Rate (bpm)</td>
<td>133±13</td>
<td>134±9</td>
<td>-11, 9</td>
<td>0.865</td>
<td>0.35</td>
</tr>
<tr>
<td>Sweat Volume (L)</td>
<td>2.58±0.58</td>
<td>2.65±0.49</td>
<td>-0.53, 0.39</td>
<td>0.765</td>
<td>0.20</td>
</tr>
<tr>
<td>VO\text{2max} (ml·kg\text{-1}·min\text{-1})</td>
<td>60.07±7.11</td>
<td>56.94±9.63</td>
<td>-3.50, 9.76</td>
<td>0.341</td>
<td>0.26</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>10.52±4.26</td>
<td>11.87±7.26</td>
<td>-5.82, 3.11</td>
<td>0.538</td>
<td>0.04</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±12</td>
<td>40±10</td>
<td>-17, 2</td>
<td>0.128</td>
<td>0.25</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>73.25±8.82</td>
<td>72.32±9.56</td>
<td>-6.61, 8.46</td>
<td>0.802</td>
<td>0.02</td>
</tr>
<tr>
<td>Test\text{&quot;2 T\text{rec}}</td>
<td>39.19±0.41</td>
<td>38.68±0.65</td>
<td>0.92, 0.10</td>
<td>0.017</td>
<td>0.29</td>
</tr>
<tr>
<td>Test\text{&quot;3 T\text{rec}}</td>
<td>38.71±0.48</td>
<td>38.87±0.54</td>
<td>-0.58, 0.26</td>
<td>0.437</td>
<td>0.91</td>
</tr>
</tbody>
</table>