Heat Exposure and Heat Acclimation: Effects on Iron Regulation and Running Durability in Female Athletes

by

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DISSERTATION ABSTRACT

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Doctor of Philosophy in Human Physiology

Title: Heat Exposure and Heat Acclimation: Effects on Iron Regulation and Running Durability in Female Athletes

Heat exposure is paradoxical in nature as it can function as both an asset and an adverse influence on exercise performance and physiological well-being. Acute heat exposure during exercise results in decrements in performance, yet chronic heat exposure has proven its utility in producing meaningful adaptations to negate the impact of ambient heat stress and extend to improved performance. The project explores heat exposure in both contexts via acute and chronic heating protocols and how the narrative changes as physiological adaptation occurs. With warming global temperatures and increasing participation of female athletes in sport, there is a need to explore female performance physiology implicated by heat stress.

Using heat as an acute stressor, we explored how heat exposure during exercise may impact iron regulatory hormones post exercise in female runners. Our results indicate an exacerbated release of inflammatory cytokines (Interleukin-6) and a key iron regulating hormone (hepcidin) after exercising in ambient heat compared to a cool condition. Together, these findings suggest potential blocked iron absorption post exercise, providing insights into the implications of environmental stress on iron regulation. Drawing upon these findings, we sought to investigate if chronic heating could negate the deleterious effects of acute heat exposure on iron regulation to restore normal iron regulation.

Chronic heating, or heat acclimation, is popular due to advantageous adaptations for performance benefits, but what remains unclear is the extent of the *other* benefits of heat

acclimation that can extend to improved physiological well-being for a female athlete population. We wanted to explore both performance and health, by creating a project addressing running durability and iron regulation after heat acclimation. Our results indicate that our novel heat acclimation protocol was effective in evoking adaptations that indicate heat preparedness for female athletes and may extend to improved running durability, but the benefits of our chronic heating study did not translate to improved iron regulation.

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DEDICATION

For my dad. You taught me to ask questions, to never settle for easy answers, and to always seek the deeper understanding of the world around me. Your encouragement and support gave me the strength to pursue this journey, even when the path was difficult. This dissertation is a testament to the values you instilled in me. Thank you for being my greatest champion.

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CHAPTER I

DISSERTATION PROPOSAL

PROJECT SUMMARY

The goal of the proposed research is to determine if a heat acclimated phenotype improves performance resiliency in endurance trained female athletes. A secondary aim of this work is to determine if heat acclimation provides an individual with physiological resiliency, evoking enhanced resistance to stressors commonly experienced during exercise. Performance resiliency is a new, dynamic metric of exercise performance that assesses the durability of the athlete and adds consideration for fatigue over time. Performance resiliency may be more predictive of race day outcomes than the static, traditional performance metrics. Well characterized enhancements in cardiovascular, thermoregulatory, and metabolic capacity that arise from heat acclimation improve traditional performance measures. There is compelling evidence to suggest these adaptations will translate to improve performance resiliency. In addition to performance resiliency, this research will focus on endurance trained female athletes and implement an intervention to address key exercise induced issues that female athletes are more susceptible to than their male counterparts. Specifically, female athletes have a higher prevalence of iron deficiency and gut permeability issues than males. No studies to date have addressed these issues simultaneously, nor explored if there is a relationship between their occurrences. Despite the known disparity of iron deficiency in female athletes compared to males, current literature on iron regulation does not focus on female specific recommendations and interventions for improving iron status. A heat acclimation intervention could not only improve sport performance but also combat known issues specific to the female athlete. There is

a need for focused research efforts and approaches to better understand iron regulation in endurance trained female athletes.

PROJECT NARRATIVE

Heat acclimation has proven benefit for traditional performance markers in athletic populations. However, these results need to be contextualized to the modern era where sustainable heat acclimation protocols, new performance metrics, and female focused approaches are emphasized. We plan to heat acclimate female athletes to explore performance resiliency, an emerging performance marker. Additionally, we will explore the idea of physiological resiliency, where a heat acclimated phenotype provides durability against negative conditions and results in positive adaptations catered towards the female athlete.

SPECIFIC AIMS

Heat acclimation (HA) protocols increase traditional performance metrics (Lorenzo et al., 2010) by evoking physiological adaptations that result in improved cardiac output and thermoregulatory capacity. However, no studies have examined whether HA can improve physiological resiliency. Resiliency represents an individual's ability to resist fatigue and maintain performance (Jones, 2024) which may be a more predictive and compelling estimate for race-day performance than traditional metrics. In addition to improving performance-based resiliency, we believe HA offers a broader physiological resiliency which may protect athletes from the insults of intense exercise. In particular, we believe the issues surrounding iron regulation in female athletes may be combated with a heat acclimated phenotype. There is a need for unique approaches to better understand iron regulation in athletes with high training demands

(Sim et al., 2014) and develop novel strategies to improve iron status in females (Peeling, 2022). Female athletes experience iron deficiencies more than males and there are currently no female specific strategies to promote iron status. We believe a HA phenotype may be protective of iron status in addition to improving traditional and non-traditional performance metrics. We propose to evaluate resiliency in female athletes after HA and explore if HA is protective of iron status by targeting inflammatory signaling, the interplay of iron regulatory hormones, and endotoxin gut permeability.

Iron regulation is highly modulated by inflammatory responses induced from physiological stressors, such as exercise. Hepcidin is a key hormone regulating iron status, which is produced in the presence of inflammatory cytokines (Ganz, 2006) and functions to reduce iron availability in circulation (Nemeth & Ganz, 2009). Previous work shows high intensity (Peeling, 2010) and long duration (Peeling et al., 2017) exercise leads to elevated hepcidin post exercise. Exercise is known to produce an inflammatory response due to mechanical and metabolic stress. Another potential source of inflammation during exercise contributing to elevated hepcidin levels is gut permeability. When core body temperature is critically elevated, endotoxins from the lumen of the gut can permeate through the protective barriers of the intestines and enter the circulation during exercise naturally and cause additional, excessive inflammation. Repetitive, high intensity exercise typical in competitive athletes may result in an inflammatory profile that exhibits characteristics of chronic inflammation, potentially leading to elevated hepcidin levels for prolonged periods. Prolonged, elevated hepcidin could be detrimental to iron status and performance of athletes.

Heat acclimation may mitigate elevated iron regulatory and inflammatory responses with cellular and systemic adaptations. Heat acclimation studies show reduced inflammatory profiles

post-acclimation (M. Kuennen et al., 2011) and reduced gut permeability (M. R. Kuennen et al., 2010). Additionally, heat exposure can illicit hypoxic tissue responses that can inhibit hepcidin production and promote erythropoiesis. This may be one process by which prolonged heat acclimation results in an increase in red blood cell mass and performance (Lundby et al., 2021). Our objective is to explore HA as an intervention to modulate the inflammatory load placed on athletes, protect iron status, and enhance oxygen delivery with the goal of improving performance.

AIM 1. Characterize the acute inflammatory and iron regulatory response to an intense exercise bout in female athletes. Determine if endotoxin gut permeability is associated with inflammation and increased hepcidin. We hypothesize that trained athletes will experience increased presence of endotoxin in circulation during intense exercise, resulting in a heightened inflammatory profile and increased serum hepcidin.

AIM 2. Measure performance metrics following a heat acclimation protocol to explore the impacts of heat acclimation on performance resiliency. We hypothesize that short term heat acclimation will result in improved resiliency.

AIM 3. Leverage a heat acclimation protocol to reduce inflammatory profiles, mitigate endotoxin gut permeability and hepcidin. We hypothesize that short term heat acclimation will result in a lower inflammatory profile during exercise, in part, due to improved gut barrier function. A lower inflammatory profile will lend to better iron status, and ultimately improved performance during intense exercise.

RESEARCH STRATEGY

Significance

Heat acclimation is a compelling strategy to provide both competitive advantage and protective physiological adaptations in female athletes. The concept of heat acclimation is over a century old, but its translation into sport performance has grown interest over the past few decades. Heat acclimation is the process by which repeated environmental heat exposures evoke physiological adaptations that improve an individual's ability to accommodate heat load. These natural adaptations to heat can be leveraged to improve sport performance (Figure 1.1). Adaptations to heat center around cardiovascular, thermoregulatory and metabolic changes (J. D.

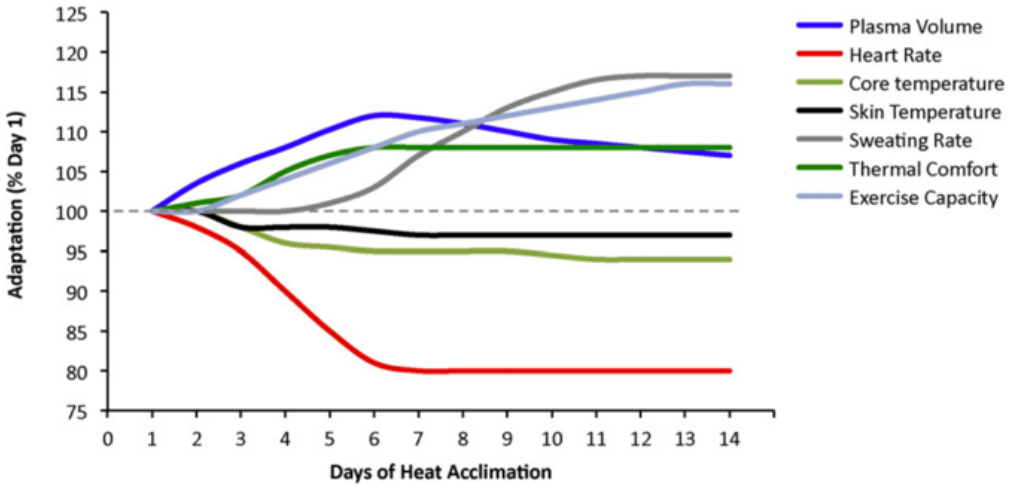


Figure 1.1. Time course and induction of the physiological adaptations to heat acclimation. Notably, a majority of adaptation to heat acclimation takes place after seven days of consecutive heat exposure. Cardio-stability and core temperature adapt quicker (<7 days), while sudomotor function takes longer (>7 days). (Périard et al., 2021)

Périard et al., 2021). Repeated heat exposures evoke an expansion of plasma volume which provides stability to the cardiovascular system as well as maintenance of blood flow to working muscles and non-working tissues (J. D. Périard et al., 2015, 2016, 2021). Thermoregulatory adaptations manifest as faster onset and greater sweat rates, enhanced skin blood flow responses,

and lower basal core temperature. Together, these factors improve heat dissipation capacity, enabling more work to be done before reaching a critical temperature. Further, whole-body (Sawka et al., 2011) and skeletal muscle metabolism (Febbraio et al., 1994; Young et al., 1985) adaptation enhances glycogen sparing, providing additional performance benefits. These key adaptations to heat are the fundamental basis for improvements in performance metrics that compel many athletes to heat acclimate. Traditional performance metrics such as maximal oxygen uptake (VO_2max) (James et al., 2017; Sawka et al., 1985) and lactate threshold (Chalmers et al., 2014) improve after heat acclimation. Further, heat acclimation is an effective strategy for improving traditional performance metrics in both hot and cool conditions (Lorenzo et al., 2010) having implications for race day performances in diverse environments (Figure 1.2). While there is established and growing literature to support improvements in traditional

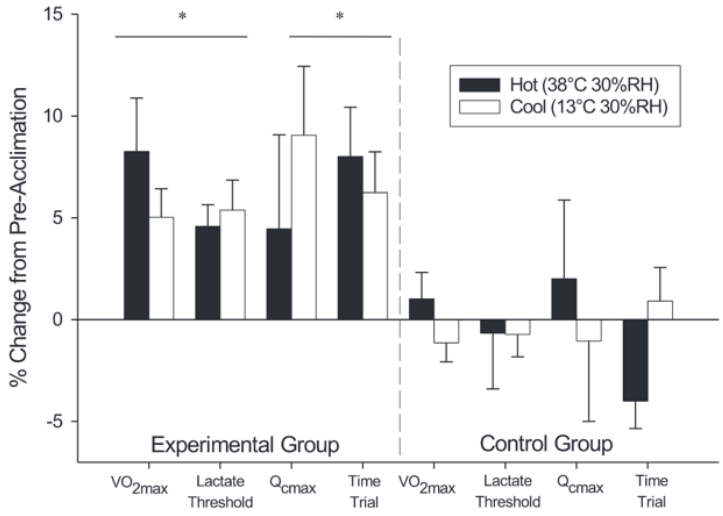


Figure 1.2. Improvements in traditional performance metrics in both hot and cool ambient conditions after heat acclimation as compared to a control group. Improvements of maximal oxygen uptake (VO_{2max}), power at lactate threshold, maximal cardiac output (Q_{cmax}) and time trial performance occurred in the heat acclimation group. These data were the first to indicate that heat acclimation can be leveraged to improve performance in cool conditions. (Lorenzo et al., 2010)

performance markers with heat acclimation, no studies to date have investigated how heat acclimation may impact performance resiliency.

Performance resiliency is defined as the ability of an individual to withstand functional decline following acute and/or chronic stressors (Jones, 2024) and may be a more indicative performance measure for endurance performance than the static, traditional performance metrics. Assessing how performance decrements over time provides powerful understanding into race day outcomes. To date, no studies have characterized how a heat acclimated phenotype responds to resiliency testing. Potential interventions that enhance resiliency are of great interest to athletes, coaches, and sport scientists. A heat acclimated phenotype has many characteristics that would suggest it will improve resiliency. Heat acclimation adaptations centering around cardiovascular, thermoregulatory and metabolic systems should prolong an athlete's ability to maintain a critical workload for longer. While this has been demonstrated in the literature with traditional performance metrics, **there is a gap in the literature surrounding performance resiliency changes after heat acclimation.**

We believe heat acclimation will improve performance resiliency for competition as well as a broader physiological resiliency which combats common issues experienced by female athletes. We define physiological resiliency as the ability to successfully overcome negative conditions and adapt to being more durable to chronic stressors. Despite growing attention and concern for female athletes, only 4-13% of all sports science and sports medicine research was conducted on female only cohorts (Kelly et al., 2023) and during 2010-2019, only 25% of thermoregulatory exercise studies were conducted on mixed-sex cohorts (Hutchins et al., 2021). **A large under-representation of female athlete research is still present** (Costello et al., 2014; Devries & Jakobi, 2021). Female athletes face unique physiological problems that will require

tailored solutions (Sim et al., 2019). Beyond competitive advantage, a heat acclimated phenotype may be a viable intervention to combat common issues faced by female athletes.

Specifically, we believe **a heat acclimated phenotype may offer physiological resilience against issues of iron regulation by augmenting the post-exercise inflammatory response and improve gut barrier function**. Female athletes experience a higher prevalence of iron deficiencies compared to men. There is a need for specific research focused on exercise responses and iron regulation of female athletes. Iron regulation is mediated by inflammation, where increased inflammation negatively impacts iron status (P. Lee et al., 2005). Inflammation is an important stimulus post exercise, responsible for driving exercise induced adaptations. However, too much inflammation can make it hard for athletes to recover properly (L. Smith, 2000). Additionally, inflammation post exercise can be exacerbated by poor gut barrier function (Sheahen et al., 2018). Increased gut permeability enables potentially harmful microorganisms to translocate from the lumen of the intestines into systemic circulation where an inflammatory response can be evoked (Lambert, 2009). Previous studies have shown that gut permeability is increased after long exercise bouts (Brock-Utne et al., 1988; S. K. Gill et al., 2015), exercise evoking high core temperatures (Pires et al., 2017), and high impact exercise (Dokladny et al., 2016). Therefore, compounding stimuli of exercise and poor gut barrier function may result in an exacerbated inflammatory response, which could be harmful to iron status.

Heat acclimation has other benefits beyond performance, and we believe these adaptations can be leveraged to combat issues specific to the female athlete. Heat acclimation has been shown to reduced gut permeability (Lambert, 2004) and cause functional changes to the epithelial linings of the intestinal tract resulting in a more robust gut barrier (Lim et al., 2009). Additionally, a characteristic of a heat acclimated phenotype is reductions in inflammatory

markers (Amorim et al., 2015). Together, these factors may lead to improvements in iron status by decreasing the inflammatory response after exercise.

The proposed research will provide a fresh perspective on heat acclimation as **we explore how a heat acclimated phenotype will affect performance resiliency, as well as physiological resiliency of gut permeability and iron status in female athletes.** We hypothesize that *performance resiliency* will improve after heat acclimation due to the established benefits and literature supporting improvement in traditional performance metrics. Additionally, we hypothesize positive changes in *physiological resilience* manifested as improved gut barrier function and protected iron status. The scientific rationale for these hypotheses is further developed under Approach.

Innovation

Performance resiliency is an emerging exercise science metric gaining attention due to its practicality and applicability to race day predictions. With gaining attention surrounding the determination of factors that influence performance resiliency, there is also interest in discovering interventions that improve resiliency. This study will be the first to explore if heat acclimation results in a more resilient athlete.

To date, no studies have explored if heat acclimation has protective effects on iron status. This will be the first study to explore iron regulation changes in a heat acclimated phenotype. In addition to changes in the inflammatory profile, the erythropoietic state evoked by heat exposure has promising implications for iron maintenance. Further, we speculate that gut permeability issues in females may contribute to their higher prevalence of iron deficiency. No studies have

specifically explored the interactions between female athletes, gut permeability, and iron regulation.

We propose to use an intermittent heat acclimation protocol which offers practicality within the demands of high performing athletes. Many traditional heat acclimation protocols require athletes to have a daily heat exposure. This method of heat acclimation may be impractical to implement for competitive athletes due to travel, training, and competition schedules. Rather, incorporating intermittent heat exposures into a scheduled training regime over a longer period may be equally as effective, less burden on the athlete, and produce a more sustainable heat acclimation protocol with higher compliance. Previous research comparing daily versus intermittent heat exposures (every 3 days) concluded similar adaptations in resting core temperature and heart rate reductions after the same number of active heating sessions (Fein et al., 1975). Additionally, research on intermittent sauna exposure in endurance trained female athletes for three weeks (3x per week) concluded intermittent heat exposure is a pragmatic tool to improve exercise performance (N. V. Kirby et al., 2021). Current research suggests short term heat acclimation (<4 days) may be insufficient to evoke adaptation and that female athletes may require more heat exposures to respond (9 days) (N. V. Kirby et al., 2019; Mee et al., 2015). Therefore, the focus of these research efforts will be on trained endurance females, capable of running during active heat acclimation, with a moderate-term acclimation protocol (9 days). The goal is to produce a heat acclimation protocol that is effective, female specific, and representative of endurance trained female athletes.

Approach

Background Information and Relevant Literature:

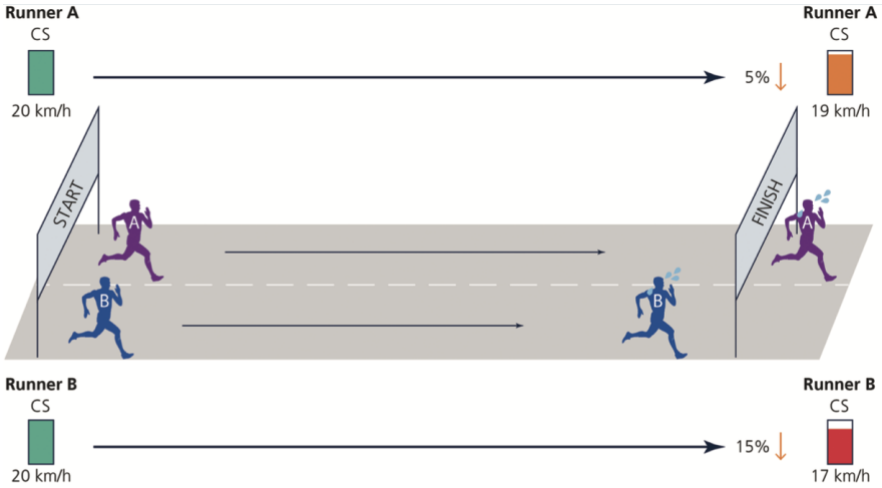


Figure 1.3. Decrements in critical speed are a better determinate of competitive performance than static metrics. Pre-race, both athletes have the same critical speed (20 km/h) and thus would be considered to have similar athletic prowess. However, athlete A is more resilient and fatigues less over the race, resulting in improve performance compared to the less resilient, athlete B. Quantifying fatigue and durability of performance provides more accurate estimates of race-day performance. (Jones et al., 2024)

The performance metric of interest for this study is resiliency. Performance resiliency is defined as the ability of an individual to withstand functional decline following acute and/or chronic stressors (Jones, 2024). Performance resiliency is a compelling assessment of performance because it captures how an athlete manages fatigue and maintains workloads over the time course of an event. While traditional performance metrics are static, performance resiliency captures the dynamic nature of competition and incorporates an assessment of the decrement in workload overtime due to fatigue. To assess performance resiliency, critical speed will be determined. Critical speed represents the workload at the highest sustainable steady state and is a key demarcation between the heavy and severe exercise domains. Critical speed can be maintained for 20-30 minutes (Jones et al., 2019), but exercising for longer than this results in a

decline in workload or task failure as fatigue accumulates. A more resilient athlete will be able to maintain their critical speed longer, with less decrement than a less resilient athlete. Decrement in critical speed over the course of a race varies between athletes and may be a primary factor that sets some athletes apart from the rest (Figure 1.3). There is great interest in determining the factors which influence resiliency, as well as finding interventions that may improve it. There is speculation surrounding the factors that may influence performance resiliency, but current research suggests central fatigue and glycogen depletion are key determinants (Clark et al., 2018, 2019). Many aspects of a heat acclimated phenotype suggest enhanced durability and longevity by mitigating increases in metabolites, sparing energy stores and combating hyperthermia better than non-acclimated athletes. Glycogen utilization is thought to be a key factor in maintaining critical speed, where depletion is associated with fatigue or inability to maintain the critical workload. Improved glycogen sparing and sustained running economy are potential avenues that could result in enhanced energy conservation and glycogen maintenance in the working muscles (King et al., 1985). Enhanced cardiovascular and thermoregulatory capacity should enable athletes to work at critical speed for a longer time or with less decrement as compared to a non-heat acclimated athlete. Slower accumulation of metabolites, a mitigated rise in core temperature during exercise and reduced perceptual effort post heat acclimation may translate to slower onset of central fatigue. **There is compelling evidence that suggests heat acclimation will improve performance resiliency in female runners**.

Heat acclimation may provide physiological resiliency to female athletes that is advantageous for general health and durability against the stressors of chronic training. Specifically, we believe issues of iron status and gut permeability can be targeted with heat acclimation to promote more robust physiological resiliency. Hepcidin is a hormone at the center of iron regulation and may be manipulated with heat exposures to protect iron status. Hepcidin is thought to be the key point of systemic iron regulation that causes many athletes to struggle with iron balance and iron status (Kong et al., 2014). Humans are unable to excrete iron, therefore iron absorption is tightly controlled. Hepcidin functions by blocking and degrading ferroportin channels, the only known iron exporting channels, resulting in iron sequestration intracellularly and removal of free iron from circulation (Figure 1.4). When hepcidin is produced, less dietary iron is absorbed, and more iron is stored intracellularly (Galy et al., 2024; Nemeth, Tuttle, et al.,

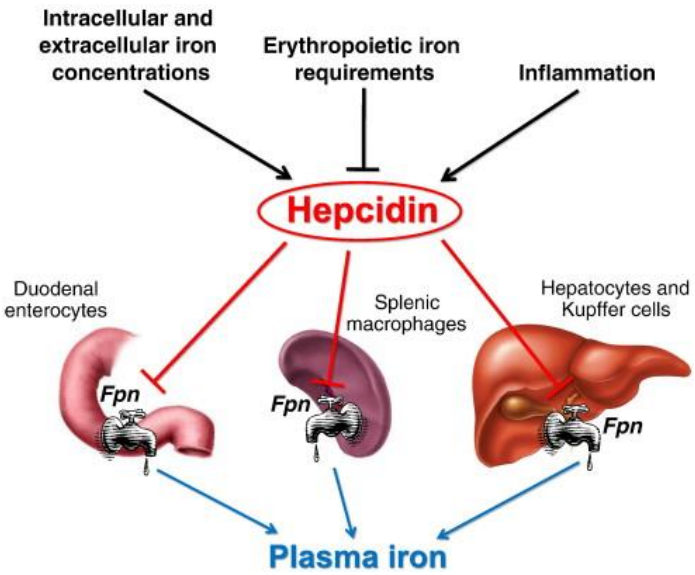


Figure 1.4. Hepcidin, the iron regulatory hormone and the factors that control its production. Hepcidin functions to sequester iron from circulation by blocking and degrading ferroportin, the only known iron exporting channel in the body. Hepcidin results in reduced dietary iron absorption at the duodenal enterocytes of the small intestine, reduced iron recycling at the macrophages of the spleen, and inhibits release of stored iron from the hepatocytes/Kupffer cells of the liver.[56] (Nemeth and Ganz, 2012)

2004). These actions can have consequences in iron balance and homeostasis if hepcidin is chronically elevated. Hepcidin is produced in response to inflammation, specifically the cytokine interleukin-6 (IL-6) promotes its production and release (Nemeth, Rivera, et al., 2004). Exercise of varying intensity and modality that produces IL-6, resultantly increases hepcidin (Peeling et al., 2017). Inflammation is tightly associated with iron regulation, and thus athletes experiencing frequent exercise may also be promoting unwanted changes in iron regulation. Exercise induced hepcidin release can be exacerbated if exercise sessions are frequent and of high intensity as seen in competitive athletes.

Interestingly, hepcidin is also a part of the innate immune response, acting as an anti-microbial peptide (Nemeth & Ganz, 2021). Hepcidin mediated removal of iron from circulation can be an orchestrated protective maneuver if bacteria are present in circulation. Bacteria require iron to replicate, therefore, less iron available in circulation is an immune system response. Bacteria entering the blood stream may occur not only during infection, but also during exercise. Increased gut permeability enables potentially harmful microorganisms to translocate from the lumen of the intestines into systemic circulation where an inflammatory response can be evoked (Neu et al., 2021). Endotoxins from the gut are an additional source of inflammation, evoking an immune response that may be detrimental in post-exercise. Augmented gut permeability post-exercise is common in athletes and may contribute to unwanted inflammatory responses. Female athletes experience a greater than two-fold increase in incidence of exercise induced intestinal dysfunction as compared to males (Wilson, 2017). Hepcidin may have compounding release and response to exercise with endotoxin leakage as both exercise and gut permeability function to increase hepcidin release via inflammatory cytokines (Vargas & Marino, 2016). Together, these factors may compound resulting in an exacerbated inflammatory response which could result in

chronically elevated hepcidin. **Despite females experiencing a higher prevalence of iron deficiency and gut permeability dysfunction, the relationship between gut permeability and hepcidin have never been explored.**

Finding interventions that mitigate gut permeability and reduce unwanted inflammation, may help protect iron status. Current investigations surrounding iron status have focused on mitigating hepcidin production by suppressing inflammatory responses post-exercise or stimulating erythropoietic activity. Badenhorst and colleagues provided athletes with a high carbohydrate diet, resulting in lower IL-6 concentration post exercise (Badenhorst et al., 2015). Subsequently, hepcidin production was lower as compared to a low carbohydrate diet group. These data indicate that changes in cytokine release is an effective strategy for suppressing hepcidin production. In a different study, athletes were provided with a hypoxic stimulus post-exercise to evoke an erythropoietic state, known to inhibit hepcidin release (Badenhorst et al., 2014). Hypoxic stimuli after exercise lead to a reduced hepcidin response. Reductions in hepcidin enables more dietary iron to be absorbed and increase the amount of free iron in circulation for red cell development. Both strategies were acutely successful in mitigating hepcidin release; however, it is unknown if these findings may translate to a chronic intervention. Additionally, to date no studies have used heat acclimation as a possible intervention for reducing the post-exercise hepcidin response, therefore protecting iron status.

Heat acclimation shows promise as an intervention that can protect iron status and mitigate hepcidin release via similar mechanisms as previous research by reducing cytokine production and promoting an erythropoietic state. A heat acclimated phenotype exhibits a lower inflammatory profile after exercise as compared to before acclimation (Barberio et al., 2014). This may be due in part to known metabolic changes in glycogen sparing with heat acclimation

which promotes lower IL-6 levels post-exercise. Additionally, heat acclimation has been shown to improve the integrity of the gut barrier lining, making it harder for endotoxins to translocate into systemic circulation (Kemna et al., 2005). Improved gut barrier function could translate to reductions in post exercise inflammation. Alternatively, heat training can evoke an erythropoietic or hypoxic response which may also function to lower hepcidin production. Hepcidin production is inhibited by the release of erythroferrone, a hormone produced by mature erythrocytes exposed to erythropoietin (Pagani et al., 2019). Previous research has shown an increase in hemoglobin mass after prolonged heat acclimation, indicating erythropoietic effects of heat acclimation and improvements in iron absorption and utilization (Cubel et al., 2024; Rønnestad et al., 2021). Literature indicates that there is potential for heat acclimation to drive reductions in post exercise cytokines, promote an erythropoietic state and improve gut barrier function – all of which may improve physiological resiliency and protect iron status. **There is a need to investigate heat acclimation as a modulator of iron regulation and to tailor this investigation to endurance trained female athletes.**

Research Design and Rigor

We will test our hypotheses on a cohort of young (18-35 years old) endurance trained female runners. We will study highly active individuals training for athletic competition. Subject screening will consist of the completion of a medical history questionnaire and physical activity/heat therapy questionnaire. After screening, participants will give written informed consent to participate in the study. All participants will record their contraceptive uses and menstrual cycle at the beginning of the study. Participants will be encouraged to maintain their

normal training during the study and incorporate heat acclimation periods into their training plans. The first visit consists of a graded exercise test to determine maximal oxygen uptake (VO_2max), gas exchange threshold (GET), fitness level, and workloads for the subsequent visits. Subjects will be assigned into either the heat acclimation or time control group based on block randomization of the graded exercise test results to ensure equal fitness in both groups. For the heat acclimation group, a total of 12 experimental visits will occur. The control group will complete a total of 3 visits. For both groups, visit #2 will consist of the resiliency test. Afterwards, the heat acclimation group will perform a heat acclimation protocol spanning 3 weeks, with 3 heating sessions per week (a total of 9 heat exposures). The final visit of the study will be identical to visit #2. The control group will complete the resiliency testing in visit #2, then enter a time control period for 3 weeks, where participants will be asked to maintain their normal training behaviors. After three weeks, the control group will come back to lab and repeat the resiliency test of visit #2.

Heat Acclimation. Participants will complete 3 visits per week for 3 weeks. The first and last day of heat acclimation will include experimental measures of thermal perception, rating of perceived exertion, heart rate, water loss, core body temperature, gut permeability testing, and blood sampling. Heat acclimation visits will take place in an environmental chamber set to 38°C and 40% relative humidity. Participants will run for 30 minutes at a workload that represents 70% VO_2max. The first and last day of heat acclimation will contain additional testing to compare iron regulation/status, gut permeability, sweat rates and acclimation status within subject pre- and post- acclimation. Blood samples will be collected prior, immediately after, and once per hour for 3 hours after exercise (Figure 1.5).

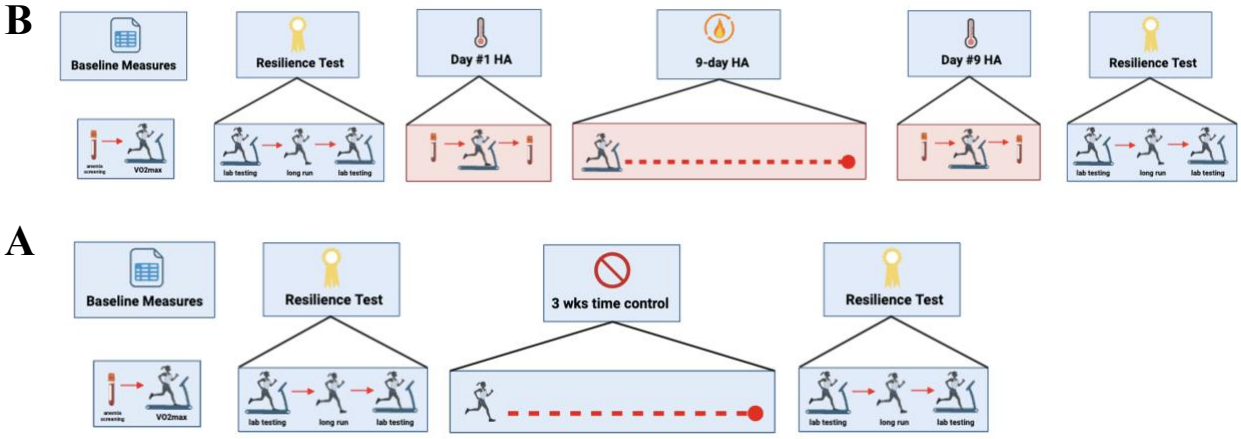


Figure 1.5. Protocol Schematic. For the heat acclimation group (A), a total of 12 visits will span a 4-week period, resulting in ~25 hours of in-lab participation per subject. For the control group (B), a total of 3 visits will span a 4-week period, resulting in ~8 hour of in-lab participation per subject.

Control Group. The individuals not completing heat acclimation will undergo the same testing periods, but do not complete the heat acclimation visits and act as a time control. These participants will be asked to maintain their regular training during this time. They will complete the baseline measurements, and two resiliency tests separated by 3 weeks to compare changes in resiliency across groups.

Performance Resiliency Testing. The resiliency protocol will consist of a critical speed determination followed by intense, fatiguing exercise and ending with a second critical speed determination. The decrement in workload at critical speed between pre- and post- fatiguing exercise will represent how resilient an individual is. Muscle oxygenation sensors will be used to make determinations of critical speed utilizing the zero-slope protocol. When oxygen delivery and oxygen utilization are matched, this represents an athlete's maximal exercising capacity

achievable of a steady state. This can be determined using a graded protocol where exercise encompasses both compensable and incompensable workloads while wearing muscle oxygenation sensors (MOXY sensors). Muscle oxygenation sensors provide information about the oxygen supply and usage at the level of the active muscle. Muscle oxygenation sensors use near-infrared spectroscopy to determine the kinetics of oxygen and blood flow during exercise, where the slope provides information about the exercise intensity. A stable, matched oxygen delivery to oxygen consumption rate (a slope of zero) indicates a critical workload that represents an athlete's maximal sustainable exercise intensity, or critical speed. Workloads greater than this (negative slope) are not sustainable as oxygen consumption is greater than delivery and will ultimately result in fatigue or a lower exercise intensity. Workloads less than this are sustainable (positive slope), but do not represent the maximal steady state aerobic workload an athlete is capable of. Determining critical speed before and after a fatiguing bout of exercise will provide information about how critical speed changes with fatigue. Additionally, implementing a zero-slope protocol using MOXY sensors diminishes the influence of motivation from the experiment as this test is predominately submaximal exercise. The resiliency test will be administered before and after the heat acclimation period or before and after a 3-week time control.

Blood Sampling. Blood sampling will take place on the first and last day of the heat acclimation (visits #3 and #11). Blood samples will be used to characterize iron status (total iron, transferrin, and ferritin), hepcidin, inflammation, and gut barrier markers. Samples will be taken before, immediately after, 1, 2, and 3 hours after exercise to capture both the peak hepcidin concentration and peak cytokine response.

Gut Barrier Function. Gut barrier function will be determined via Double Sugar Absorbance Test (DSAT), the gold standard for determinations of gut permeability. Thirty minutes prior to the end of exercise a mixture of lactulose and rhamnose will be consumed. These sugars are not digestible and should minimally pass through the alimentary system under conditions of good gut barrier function. However, during exercise these sugars can permeate through the epithelium layers in higher concentrations. Lactulose and rhamnose sugars are different in size, and the differential concentration and presence in circulation enables us to functionally determine whether the damage to the gut is due to permeability changes via the transcellular or parvocellular route. Subjects will consume a sugar-water mixture prior to the end of exercise. After exercise, blood samples will be taken. The presence, ratio, and concentration of these sugars in the blood will provide a quantitative understanding of the severity and type of gut barrier disfunction which occurred. Participants will undergo this procedure on the first and last day of heat exposure.

Data Analysis and Study Timeline

Primary outcome variables include performance resiliency, iron status, iron regulatory hormone, and gut barrier function pre- and post-heat acclimation (Table 1.1). Performance resiliency will also be compared to a time-control non-heat acclimated group. A sample size calculation for two equally sized groups, with an effect size of 1.2 and power of 0.90 was determined to be 20 subjects, 10 in each experimental group (G*Power). Further, previous similar research has been powered to reach significance with similar sample size (Lorenzo et al., 2010)**.** We will use t-tests to detect differences between the heat acclimated and control groups

for performance resiliency. Mixed models will be used to assess iron regulation and gut permeability changes before and after heat acclimation and across time.

Table 1.1. Primary and secondary outcome measures to be collected

Outcomes	
Primary	Performance Resilience Iron status & Iron Regulation Gut Barrier Function
Secondary	VO_2max, core temperature, water loss, heart rate, thermal perception, inflammatory markers, gut inflammation markers.

Table 1.2. Study timeline for proposed research and subject enrollment goals for Heat Acclimation Group (HA) and Thermoneutral Control Group (TN) per quarter (Q). Q3 of Year 1 will not contain data collection during the summer months. Q3 of Year 2 will be dedicated to manuscript and dissertation preparation.

	Year 1 (01/2025-01/2026)				**Year 2 (01/2026-09/2026)**		
	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Cumulative Enrollment	4	8	~0	12	16	20	~0
Heat Acclimation	2 HA	2 HA	~0	2HA	2HA	2HA	~0
Thermoneutral Control	2 TN	2TN	~0	2TN	2TN	2TN	~0

Potential Problems and Alternative Strategies. Potential problems center around new methodologies needed to perform the DSAT gut permeability test. We plan to collaborate with researchers at the University of Loughborough to analyze our samples and prepare methodology. If we are unable to successfully set up this collaborative effort, there are alternative testing strategies in place that can be done to quantify adjacent outcomes, that our lab is currently capable of analyzing. Instead, we would use 2 markers which will assess gut inflammation (Lipocalin-2/NGAL) and intestinal fatty acid binding protein (I-FABP) to characterize the inflammation and damage to the gut. An additional potential problem will be recruiting women

from the Eugene-Springfield area to participate in this study. This study requires many hours (~25 in-lab hours) of time and dedication to the experiment and may be hard to find individuals willing to participate. A reasonable timeline and subject enrollment expectation has been put forth (Table 1.2) and can be reassessed in Q4 of year 1 if progress does not match the enrollment expectation. Changes to the protocol can be made to reduce participant burden and make data collection more easily attainable.

CHAPTER II

NEW INSIGHTS

OVERVIEW

"There is nothing new under the sun, but there are new suns" - Octavia Butler

Participation of female athletes has exploded over the past 50 years with the 2024 Paris Olympics being the first to include equal representation of male and female athletes. Simultaneously, global temperatures continue to rise, resulting in specific concern over athlete heat preparedness and the fate of outdoor athletic competitions. Research efforts need to adapt to address the growing demands for understanding female physiology and environmental stressors. While the focus of research efforts has changed to meet modern demands, at their core these concepts are rooted in scientific investigations that began over a century ago.

RESILIENCE

In 1925, A.V. Hill was the first to correlate athletic race performance times and distances (Hill, 1925), later leading to the establishment of the power duration relationship (Monod & Scherrer, 1965). Over 100 years later, the physiological phenomena outlined by A.V. Hill are still relevant today. The principles of fatigue and deterioration of performance have been studied across species as a highly conserved phenomenon (Poole et al., 2016). The mechanisms of fatigue and endurance associated with the power duration relationship continue to intrigue scientists and are of great debate and interest today. Further, exploring fatigue over the course of a prolonged exercise bout has led to an emergence of new interest. As defined by Jones,

resilience is the ability to resist functional decline following acute and/or chronic stressors (Jones, 2024).

Resilience, or durability, is an overarching concept that can be applied to a host of physiological performance metrics by quantifying their deterioration before ("fresh" state) and after ("fatigued" state) an endurance-based exercise task. Typically, performance metrics are quantified in an optimized, rested state. However, resilience is a new way to quantify performance by showing the dynamic nature of fatigue, challenging the fundamental notion that these performance metrics are static. While there are many performance metrics where deterioration over time can be assessed, there is concentrated efforts to determine changes in critical power/speed (cycling/running). Critical speed delineates the heavy and severe exercise domains, meaning it is an important marker for the transition from sustainable to unsustainable exercise (Muniz-Pumares et al., 2024). Critical speed critically delineates distinct muscle metabolic (Black et al., 2017; Vanhatalo et al., 2016), respiratory (Poole 1988), cardiovascular (Copp et al., 2010), and blood metabolite phenomena (Vanhatalo et al., 2016). Critical speed is a physiological boundary representing the highest sustainable effort an athlete can maintain (Black et al., 2014). The amount of deterioration provides integral insight into race day predictions and overall performance (Hunter & Muniz-Pumares, 2025). Characterizing the deterioration of critical speed and critical power are of great interest to sport scientists, athletes and coaches.

The initial resilience studies were conducted by Clark and colleagues, being the first to exhibit reductions in critical power after fatiguing endurance exercise (Clark et al., 2018). Using 3-minute all out cycling tests to capture critical power before and after fatiguing exercise provided insight into the decline of performance after 2 hours of heavy intensity cycling exercise. The results indicate an -8% reduction in critical power demonstrating the deteriorating

nature of critical power after a fatiguing cycling bout (Figure 2.1). There is continued and growing interest in the factors that influence resilience and interventions that may improve resilience.

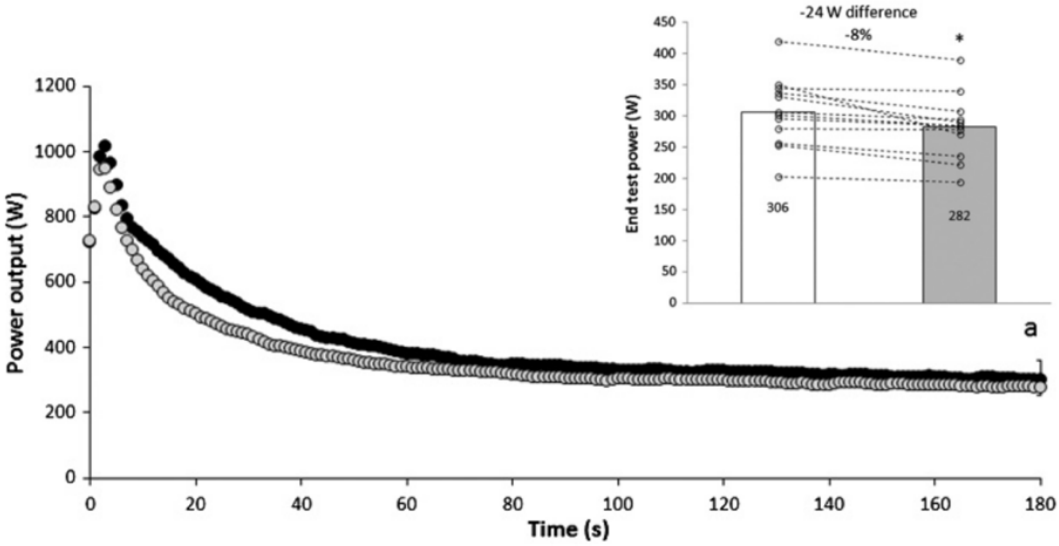


Figure 2.1. Three minute all out critical power tests before (black fill) and after (grey fill) fatiguing exercise. End test power or critical power was reduced on average 24 Watts after two hours of fatiguing exercise. Adapted from (Clark et al., 2018)

Currently, there is limited research on the factors or mechanisms that may influence resilience. Improvements in resilience are likely a combination of physiologically driven (fueling, resistance to muscle damage, muscle fiber density), biomechanically driven (energetic return, tendon elasticity, maintenance of running economy), and psychologically driven factors. Resilience itself may be hard to experimentally isolate as the characteristics that make up an athlete's unique "resilience profile" may vary, and the limited literature suggests high interpersonal variability (Clark et al., 2018). Despite a lack of mechanistic understanding of resilience, a small base of research exploring interventions to improve resilience exists. Strength training programs in runners and duathletes (10 - 11 weeks) concurrent with regular training

improved running economy in male runners (Zanini, Folland, Wu, et al., 2025) and improved 5 minute all out trials in female duathletes (Vikmoen et al., 2017) after a prolonged exercise effort. Both strength training studies speculate that their findings of enhanced resilience were via mechanisms of improved energetic return during running or muscular fiber type adaptations. Similarly, studies have demonstrated running with "super shoes" improve the energetic return during running (Senefeld et al., 2021), thus leading to improvements in running economy that extend to improved economy over prolonged exercising efforts.

Other studies highlight the potential benefits of physiological adaptations in energetics that are favorable for maintaining critical racing intensities. Previous studies have highlighted that more experienced cyclists are more fatigue resistant than novice athletes (Gallo et al., 2022). Similarly, greater training volume in runners is correlated with improved resilience, likely via muscle fiber adaptations (Zanini, Folland, & Blagrove, 2025a). Years of large training volumes and habitual training can lead to fiber type switching towards myosin heavy chain I fibers (Hoff et al., 2013; Kwon et al., 2024; Trappe et al., 2006). Type I muscle fibers have a heavy reliance on fat oxidation, resulting in improved carbohydrate sparing. During prolonged exercise, as muscle recruitment patterns shift towards type II muscle fibers, there is a heavier reliance on carbohydrate metabolism to support the exercise task. Substrate fueling during prolonged exercise also presents an opportunity for improved resilience, as a previous study by Clark and colleagues exhibited partial restoration of the decline in performance after 90 minutes of heavy domain cycling via carbohydrate ingestion (Clark et al., 2019). While it is well established that carbohydrate ingestion improves endurance performance, this is the first time it was seen in the context of resilience. Other potential interventions for improving resilience have been purported (altitude acclimatization, heat acclimation, fueling management) but no studies to date have

characterized them (Jones & Kirby, 2025). The nature of resilience is multifactorial, dependent on the culmination of physiological, biomechanical and psychological traits.

The newly garnered attention towards resilience research has led to commentary by the scientific community about how to define it and how to research it. Maunder and colleagues were the first to use the term "durability" in 2021 (Maunder et al., 2021), follow by Jones' commentary (Jones, 2024). Due to the ambiguity of the metric, others are trying to define and isolate different aspects of fatigue – classifying the metrics of fatigue onset and resilience as distinct definitions based on intensity, duration, physiological boundaries, etc. (Meixner et al., 2025). We highlighted a need for the consideration of sustainability or maintainability—shorter endurance-based tasks were not included in the standing definitions and will have distinct physiological implications on fatigue (Lucernoni et al., 2025). Resilience is not clearly defined nor agreed upon, and discussion about defining resilience is ongoing.

In addition to defining resilience, there are no standard protocols yet developed to assess resilience (Hunter et al., 2025). There is great interest in assessing decrements in critical speed specifically. Clark and colleagues were the first to do it in cyclist (Clark et al., 2018), and there has been one case study on an ultramarathoner (Mougin et al., 2026), but no other research efforts. To date, no studies have explored deteriorations in critical speed in running; this may be due in part to methodological constraints. Current assessments of critical speed would require multiple day trials or a three-minute all out test which would require a self-propelling treadmill or need to be done on an outdoor track with GPS and relies heavily on participant motivation. However, methodological developments of the use of near-infrared spectroscopy (NIRs) to estimate muscle oxygenation for estimating critical speed enable individuals to quantify critical speed in running without reliance on effort-based maximal exercise. The zero-slope protocol

enables researchers to use varied intensity, submaximal exercise bouts that span the exercise domains to quantity critical speed or power. Previous research highlights the accuracy of the zero-slope protocol estimations at delineating sustainable from unsustainable exercise (Matthews et al., 2023). This new methodological development opens the opportunity to characterize deteriorations in critical speed in a single day.

HEAT ACCLIMATION AND IRON REGULATION

Heat acclimation, or repeated heat exposures to induce favorable adaptations has been explored for 100 years due to its wide utility for occupational heat preparedness, military preparations, potential as non-pharmacological alternative, and popularity for athletic performance (J. D. Périard et al., 2015). The first-ever attempts to heat acclimate individuals comes from a unique historical context and serves as the foundation for decades of research. Between the 1890s and 1940s gold was discovered in South Africa that posed extreme thermoregulatory strain on the miners due to mining techniques, mine depth and geological position (Schneider, 2016). There were many reported cases of heat stroke and heat casualty leading to reduced productivity of the mines. Dr. Aldo Dreosti was the first to conduct a series of experiments to determine heat preparedness of recruits for the mines and subsequent heat acclimation protocol (Dreosti, 1935). Heat acclimation protocols spanned from 4 days up to 14 consecutive days of heat exposure depending on the initial individual response of a heat tolerance test. The 7–14 day heat acclimation period being the most common and frequently referred to as the foundation for future efforts of heat acclimation in the coming decades.

Throughout the rest of the 20th century, interest in the utility of heat acclimation was predominately occupational or military focused. Today, there is increased interest in leveraging

heat acclimation protocols to improve heat tolerance and performance for athletes. Despite this growing interest, many researchers continue to use “traditional” consecutive day heat exposures as initially done by Dr. Dreosti, with most protocols using 5 to 10 days of continuous heat exposure. The prominence of continuous heating protocols can be seen even today as a recent meta-analysis highlights the average heat protocol of 8 ± 4 heat exposures over long durations of low intensity (90 ± 36 minutes/exposure) with minimal consideration for intermittent heating protocols (McDonald et al., 2025). While the use of continuous heat acclimation is highly effect at inducing hallmark adaptations of heat acclimation, it is not tailored to the needs of a training and competing athlete. Consecutive day heating protocols may impair athlete’s sleep, recovery, and food intake post heating (Ihsan et al., 2021). Further, there is risk for overreaching or overtraining the athlete if they add heat acclimation to their current training or must disrupt training to perform a traditional continuous heat acclimation protocol (Casadio et al., 2017). Intermittent heating protocols or mixed method heating protocols may offer an alternative way to heat acclimate, reducing overall stress on the athlete. Studies have been successful in evoking heat adaptation using intermittent or mixed methods heating protocols for athletic populations while preserving normal training. Kirby and colleagues reported heat adaptation and improved performance metrics using sauna post exercise (30 minutes, 101-108°C) three times per week for three weeks in endurance trained runners (N. V. Kirby et al., 2021). Other studies have successfully implemented mixed method heat acclimation protocols (active and passive heating bouts) concurrently with normal training in elite athletes (Fenemor et al., 2022; James et al., 2024). These studies demonstrate successful heat acclimation results while taking a departure from the traditional heat acclimation methods with an emphasis on maintaining normal training.

Continuous heat acclimation protocols for athletes are likely too demanding and require changes to training or careful planning leading up to competition to implement. However, when heat acclimation protocols are designed to minimize physiological stress and integrate seamlessly with regular training, athletes may be able to heat acclimate throughout a training block. Maintaining a heat acclimated phenotype throughout the competitive season and not just leading up to a key competition may be beneficial (Sekiguchi et al., 2022). Additionally, there are benefits beyond performance metrics that may lead to improved physiological well-being and resistance to repeated physical stress.

One such benefit of heat acclimation may be improved iron regulation. Iron is an essential mineral that supports critical life functions. Many enzymatic processes rely on iron-containing enzymes such as ATP production, DNA synthesis, and oxygen transport. Due to iron's ability to transition between Fe (II) and Fe (III) valence states, iron can accept or donate electrons readily, making its functional use broad across many different cell types. Iron electron donation also makes it dangerous as its reactivity can lead to cell damage and iron toxicity. Therefore, while iron is essential, tight regulation and management of iron in different body compartments is mandatory. While a majority of iron is stored or used, the small daily fluctuations in dietary iron absorption and iron loss are of most interest. These small fluctuations over time can lead to iron deficiency or anemia if iron loss and gain is not balanced. Iron is lost via hemolysis, hematuria, sweat, menstrual bleeding and gastrointestinal bleeding (Peeling et al., 2008). Iron is gained solely through dietary intake. In humans, there is no direct iron excretory pathway, therefore dietary intake of iron is tightly controlled (Ganz & Nemeth, 2012). One key hormone responsible for regulating systemic iron intake is hepcidin. Hepcidin is a peptide hormone that functions to block and degrade the only known iron exporting channel, ferroportin

(Nemeth, Tuttle, et al., 2004). Ferroportin channels are ubiquitously expressed on cell types, but are highly expressed on macrophages, enterocytes of the small intestine, and the liver. When hepcidin concentrations are high, iron export and dietary iron intake are greatly diminished and circulating “free” iron is stored intracellularly, reducing the overall labile iron pool (Galy et al., 2024). These actions can have consequences in iron balance and homeostasis if hepcidin is chronically elevated.

Athletes are susceptible to iron deficiency, with female athletes having an even larger proportion of reported indices of iron deficiency (Cowell et al., 2003; Fallon, 2008). Exercise can lead to increased hepcidin concentrations, resulting in reduced dietary iron absorption and a mismatch of iron loss and iron gain. Post exercise, muscle derived interleukin-6 and other cytokines result in an acute phase response signaling hepcidin production and release from the liver. Hepcidin concentration peaks ~3 hours after exercise in a dose dependent manner based on exercise intensity in both running and cycling (Sim et al., 2013). Elevated hepcidin concentrations after prolonged running (~90 minutes) can result in 36% reduction in dietary iron absorption (Barney et al., 2022). Further, hepcidin can stay elevated above baseline for up to 24 hours (Ishibashi et al., 2022), meaning athletes who regularly exercise once or twice a day may experience chronic elevations in hepcidin which can pose an issue over time (Ishibashi et al., 2017). Chronic elevations in hepcidin are thought to be the reason athletes struggle with iron deficiency (Peeling, 2022) and therefore the emphasis of investigations is to mitigate post exercise hepcidin production.

Heat acclimation may be able to mitigate hepcidin release via adaptions in hematopoietic stimulation or reductions in circulating cytokines. Recent heat acclimation studies have demonstrated that prolonged heat acclimation (5 weeks) increases hemoglobin mass (Cubel et

al., 2024; Oberholzer et al., 2019; Rønnestad et al., 2021). While the specific mechanisms behind the hemoglobin mass expansion are unknown, it is purported that the hypoxic effects of heat exposure, plasma volume expansion (Jenkins et al., 2025), or the presence of elevated heat shock proteins (Ely et al., 2014) incurred from heat acclimation results in the kidneys to produce an erythropoietic response. Hypoxic and erythropoietic responses to heat acclimation likely impacts iron regulation. Previous research has shown that erythropoietin and its precursor erythroferrone inhibit hepcidin production (Peyssonnaux et al., 2008). Prolonged heating interventions may be beneficial for athletes for increased oxygen carrying capacity, but also dietary iron uptake. A previous investigation was successful in mitigating post exercise hepcidin release via hypoxic stimulus (Badenhorst et al., 2014). Participants were exposed to normobaric hypoxia (~2,900 m above sea level) during a three-hour post-exercise recovery period. Exercise consisted of 48 minutes of interval running (2x; 8 x 3 min) at 85% VO_2max. Blood samples taken during the recovery period demonstrated a mitigated hepcidin release compared to a control condition. The authors hypothesize that this response is due to the stabilization of hypoxia inducible factor alpha or accumulation of erythroid proteins (Badenhorst et al., 2014) that both have modulating effects on hepcidin transcription (Galy et al., 2024).

Additionally, heat acclimation has demonstrated reduced post exercise cytokine production (M. R. Kuennen et al., 2010; B. J. Lee & Thake, 2017). This may be due to the upregulation of heat shock proteins which are protective against cellular damage and may mitigate distress signals from exercising tissue (M. Kuennen et al., 2011). If heat acclimation results in reduced production and release of IL-6 post exercise, it may reduce hepcidin production as well. A previous investigation manipulated post exercise IL-6 production via carbohydrate ingestion to explore its effects on post exercise hepcidin (Badenhorst et al., 2015).

Carbohydrate intake is known to influence IL-6 cytokine signaling, as IL-6 act as an "energy sensing" cytokine released by muscle during glycogen depletion in the muscle (Pedersen et al., 2001). A high carbohydrate diet reduced IL-6 concentration post exercise resulting in lower baseline and trending lower peak hepcidin concentration post exercise compared to a low carbohydrate group. Reductions in cytokine production after heat acclimation may translate to lower basal hepcidin and lower peak post exercise hepcidin concentration, posing long term positive implications on iron status in athlete. A heat acclimated phenotype may extend beyond heat preparedness or performance benefit, and result in more robust physiology via improved iron regulation.

CHAPTER III

PROPOSAL REVISIONS

OVERVIEW

Several key decisions were made regarding the study design during the candidacy exam meeting and after initial project data collection. These changes strengthened the overall study design and rigor of the project.

OVERALL STUDY DESIGN CHANGES

The study design was modified to become a near-parallel study design between the control and heat groups. The control and heat groups were asked to maintain consistent training throughout the duration of the study and to add the intervention visits as additional training load per week. The heat acclimation group and control group were asked to report to lab to complete active training bouts the same number of times and same relative intensity, but in different environmental conditions. With this modification to the protocol, both groups completed the same amount of additional training load per week in a monitored lab setting. This change strengthens the study overall as we ensured that both groups completed a similar additional training load during the intervention phase, mitigating concern of changes in training volume as a confounding factor.

Additionally, the order of research visits was changed. The heat tolerance testing occurred prior to the resilience test in both groups, pre and post intervention to remove any confounding effect that one session of heat acclimation may have on the performance testing. A revised protocol schematic is provided (Figure 3.1).

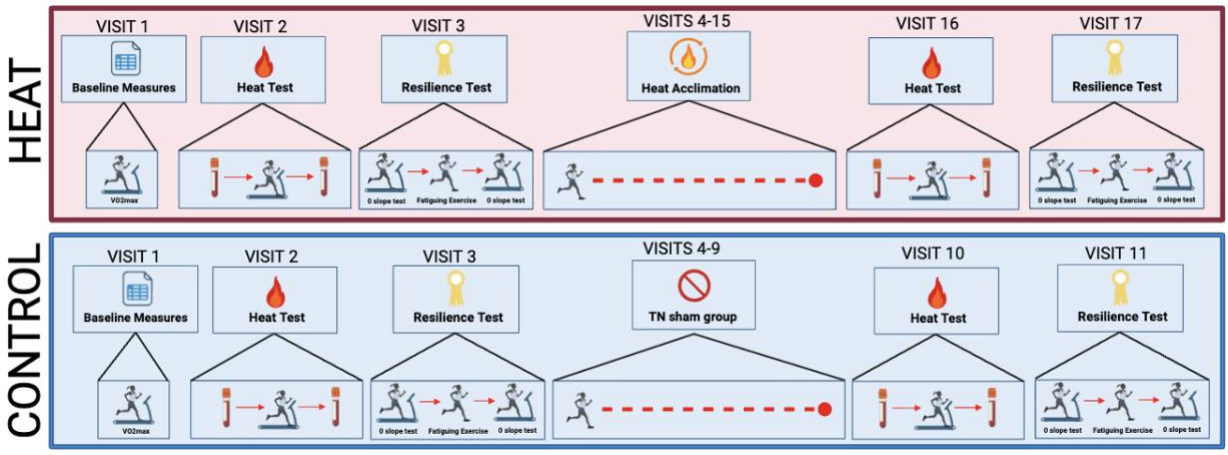


Figure 3.1. Revised Protocol Schematic with near-parallel design between the control and heat groups. The heat group reported to the lab for 17 visits, totaling 23 hours in-lab hours spanning, 5 weeks. The control group reported to the lab for 11 visits, totaling 17 hours in-lab, spanning 5 weeks.

Dual Sugar Absorbance Test (DSAT)

During the comprehensive exam, the Dual Sugar Absorbance Test was removed from the protocol. The decision was based partially off the results from the first research study (Aim #1 & Chapter IV) where we did not observe changes in the presence of lipopolysaccharide in female athletes after an acute bout of running in the hot environmental conditions. One key conclusion from this study was that a trained population may be adapted to withstand the conditions of the heat test with no leaky gut. Due to our uncertainty in the possible changes in gut permeability in this population, we decided to remove it from the protocol. Additionally, this methodology was not previously established in our laboratory, needing further refining and validation prior to using it in a research study.

Heat Acclimation Protocol

The heat acclimation protocol was changed from what was proposed to a three-week intervention period including two passive heating visits (hot water immersion) and two active

heating visits (running in an environmental chamber) per week (Figure 3.2). The order of these visits each week was determined at discretion of the participant to incorporate the additional training volume into their current training plans. The active heating sessions were performed in an environmental chamber where participants were asked to run at a speed that reflects 70% VO_2max for 30 minutes. The temperature of the environmental chamber started at 38°C (40% relative humidity) and progress weekly by 2°C, ending with 42°C (40% relative humidity) to maintain a novel stimulus throughout the intervention. The passive heating visits were performed via hot water immersion in 41°C water for 30 minutes. Passive heating sessions were not progressively increase in water temperature or time.

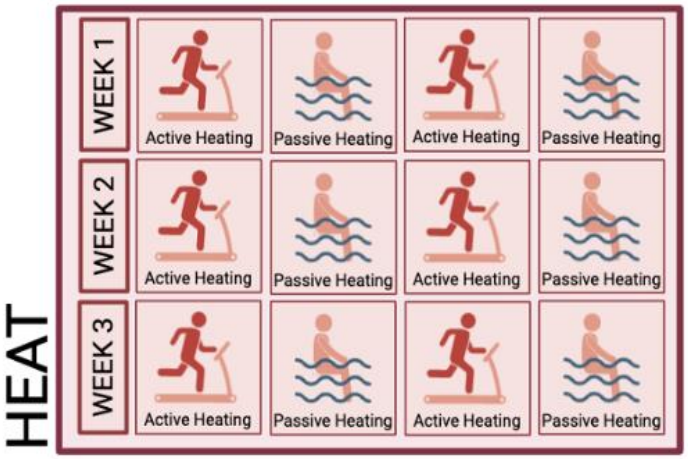


Figure 3.2. Heat group intervention.

This heat acclimation protocol is novel. While previous investigations have suggested using mixed-method intermittent heat acclimation protocols for athletes (Pryor et al., 2019) to our knowledge, no studies have implemented them in this manner. Intermittent heat acclimation protocols provide more practicality for athletes with training plans and is thought to be less disruptive than traditional, continuous heat acclimation protocols. Due to the novelty of this

intermittent heat acclimation protocol, the first participants (n = 3) in the heat acclimation group served as a pilot to ensure the protocol was successful in producing a heat acclimated phenotype. We sought verification heat acclimation via reductions in peak body core temperature, elevated sweat rate and reduced heart rate when comparing pre- and post-intervention heat test visits.

Control Group Protocol

The control group performed training sessions to match the increase in training volume of the active heating sessions for the heat group (Figure 3.4). Running sessions occurred twice per week for 3 weeks at the same relative intensity (70% VO_2max) for the same amount of time (30 minutes) but in thermoneutral conditions (~22°C and 40% relative humidity). The control group did not replicate the water immersion sessions completed by the heat acclimation group.

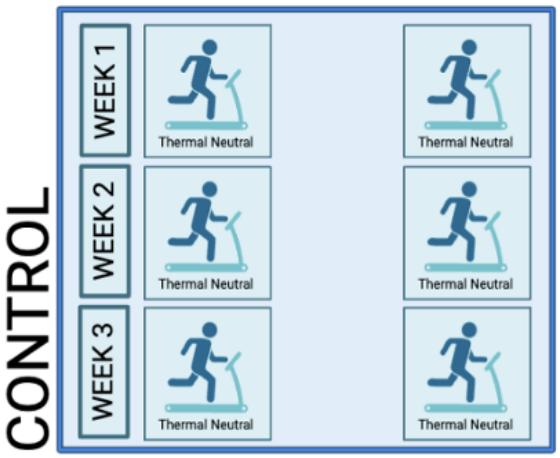


Figure 3.4. Control group intervention.

SAMPLE SIZE CALCULATIONS

The proposed sample size calculations were modified as follows based on the expected variation in hepcidin concentration and based on the findings of the study complete in Chapter IV. The hypothetical expected meaningful change in hepcidin concentration is 2nM which has been studied in our research as well as others (Badenhorst et al., 2014, 2015). A 2nM reduction in hepcidin would be associated with an approximate 25% reduction in iron absorption (Barney et al., 2022). Using this anticipated meaningful change in mean concentration, reported standard deviation of hepcidin from Badenhorst and colleagues (SD = 0.82 pg/mL), an α of 0.05 and β of 0.15 (power = 0.85) we calculated the necessary sample size to be 24 participants in a continuous endpoint, two independent sample groups study (Badenhorst et al., 2014).

Upon establishing the necessary participant count for our biomarker analysis, we did secondary sensitivity measure to assess the amount of change we will be able to detect during the resiliency testing with our chosen sample size. Using the reported standard deviation in critical power from Clark et al., (SD = 2.26%) with 12 participants in each group, an α of 0.05 and β of 0.15 (power = 0.85) – we can assess down to a 2.8% difference between groups with the sample size calculated (Clark et al., 2018).

PERFOMRANCE RESILIENCE TESTING

The established protocol is provided below (Figure 3.5). Initially, we planned to characterize the speed for the time to exhaustion trial once during the initial resilience test and repeat the same workload in the post-resilience testing as well. With this protocol, we would be able to assess changes in critical speed with heat acclimation and assess a time to exhaustion trial before and after heat acclimation. This decision was made thinking that 5% above critical speed

would still represent a heavy workload that would result in task failure within 20-40 minutes after the intervention period. As long as the individual went to exhaustion both times (regardless of if it was longer for the second visit) we would still be able to assess the reduction in critical speed as resilience.

However, the test does not accurately capture "resilience" as the trial will likely be easier during the post-testing and no longer be representative of a heavy-to-severe domain exercise trial. We saw this response with the first participant who completed the heat acclimation protocol. The increase in critical speed they had from the pre to post heat acclimation was large enough where the TTE in the resiliency test was now well below their critical speed, and the exercise no longer had characteristics of heavy-to-severe domain exercise (no slow component, exercised for >40 minutes, no negative slope in muscle oxygenation). While this finding was encouraging for the idea that heat acclimation may result in improved critical speed, it was clear that the protocol needed to be revised to better capture performance resilience.

We revised the protocol to where we characterize the TTE during the first resilience test for the TTE, then during the second resiliency test post intervention we re-characterize critical speed and set a new workload for the TTE. While this makes it impossible for us to use the TTE data as a direct comparison within individuals, it strengthens the resilience test, as now both testing periods are the same relative intensity. This makes our understanding of the decrement in critical speed stronger, knowing that the trials will be an equal level of relative difficulty. Additionally, this accounts for any training effect that may be present as both groups are asked to increase training volume throughout the intervention.

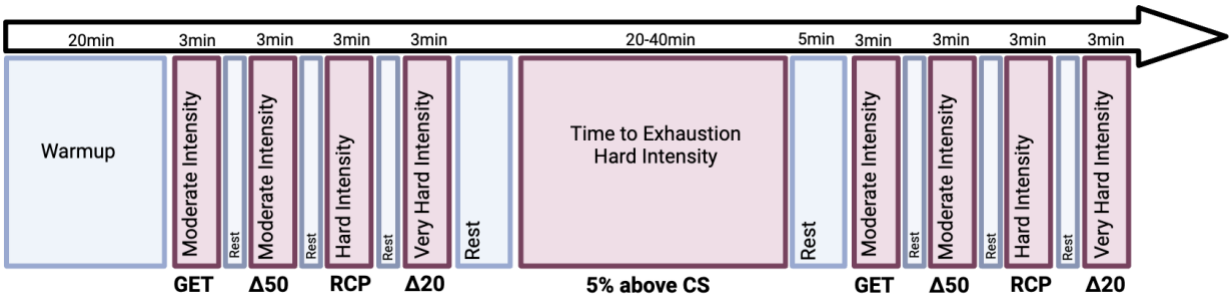


Figure 3.5. Resilience testing timeline. Each stage is 3 minutes in length at increasing intensity spanning both compensable and incompensable workloads calculated based off the results of a VO_2max test. Speed at Gas Exchange Threshold (GET), Respiratory Compensation Point (RCP), halfway between them (Δ50), and 20% above RCP (Δ20; RCP + 0.2*(MAX-RCP)) are the chosen workloads. After the first set of intervals, critical speed is determined using muscle oxygenation sensors and the zero-slope protocol. Participants were instructed to exercise at 5% above critical speed until failure. Following a short break (5 minutes) the interval stages were repeated for a recharacterization of critical speed.

REVISED AIMS

AIM 2. Assess endurance performance durability following combined active and passive heat acclimation to explore the impacts of heat acclimation on athlete resilience. We hypothesize that heat acclimation will result in improved resilience.

AIM 3. Leverage a combined active and passive heat acclimation protocol to protect iron status by mitigating hepcidin release and reduce inflammatory profiles. We hypothesize that heat acclimation will result in a lower inflammatory profile during exercise lending to less hepcidin production post-exercise and ultimately improved iron status.

CHAPTER IV

EFFECTS OF EXERCISE IN ACUTE HEAT ON BIOMARKERS OF GUT PERMEABILITY, SYSTEMIC INFLAMMATION, AND IRON REGULATION IN FEMALE ATHLETES

INTRODUCTION

Iron is an essential mineral, critical for many physiological processes and athletic performance. The athletic population is particularly susceptible to poor iron status with female athletes having a significantly higher prevalence of iron deficiency compared to males (35%, 10%; respectively) (Fallon, 2008; Sim et al., 2019). Further, 60% of elite collegiate female athletes experienced anemia during their career (Cowell et al., 2003). If iron consumption and absorption from the diet is not adequate and/or chronic stressors occur, iron stores can be depleted. Iron can be lost during exercise via hemolysis, hematuria, sweat, menstrual bleeding and gastrointestinal bleeding (Babić et al., 2001; DeRuisseau et al., 2002; Mclnnis et al., 1998; Zoller & Vogel, 2004). The liable iron pool can be increased by dietary iron absorption. However, there are obstacles that can make iron absorption from the diet difficult. Iron absorption is stringently regulated as humans have no excretory pathway to remove excess iron, relying on blocking absorption of ingested iron from the intestines or sequestering iron within cells. Hepcidin is the key iron regulating hormone. Hepcidin functions by blocking the only known cellular iron exporting channel (ferroportin) leading to reduced iron availability in circulation and reduced absorption of dietary iron (Ganz & Nemeth, 2012). If hepcidin is high, then the ability to absorb iron to increase iron stores is limited (Barney et al., 2022). Exercise is a large stimulus for release of hepcidin due to the post exercise inflammatory release of cytokines.

Interluekin-6 (IL-6) has been identified as an important inflammatory and energy sensing cytokine that regulates production of hepcidin (P. Lee et al., 2005; Nemeth, Rivera, et al., 2004; Nemeth & Ganz, 2009). Previous research has established an increase in hepcidin hormone concentration after exercise across different modalities and intensities (Sim et al., 2013). Hepcidin concentration peaks ~3 hours after an acute exercise session (Peeling et al., 2009b) and can remain elevated for >12 hours after exercise. Due to the nature of competitive athlete training, this indicates that hepcidin can be chronically elevated, making it difficult to absorb dietary iron (Ishibashi et al., 2022; Peeling, 2010). Hepcidin has been implicated as a main mechanism that leads to iron deficiency in athletes (Peeling et al., 2009a). However, much of the preliminary work on exercise and hepcidin was conducted on male athletes with limited focus on female athletes (Ishibashi et al., 2017, 2022), despite females being more susceptible to iron deficiency. Further, there is limited consideration for how environmental stimuli may impact this response. Seasonal changes in ambient training temperature and competitions align within warmer months (Barney et al., 2022), meaning athletes may be more susceptible to issues with iron regulation during this time. Exercise in hot environmental conditions can result in higher IL-6 production due to changes in relative exercise intensity (McKay et al., 2021) and previous work in a field-based investigation showed a positive correlation between ambient temperature and hepcidin concentration (McKay et al., 2020). Only one controlled study of iron regulation post exercise in the heat has been done on male athletes resulting in greater elevations in IL-6, but not hepcidin in the hot ambient conditions (McKay et al., 2021). However, females may have a different response due to baseline ferritin (Peeling et al., 2017). Hepcidin concentration is reliant on serum ferritin which tends to be lower in the female population and therefore may drive a different response. There is a gap in the literature examining female athletes and issues

pertinent to them (Costello et al., 2014; Hutchins et al., 2021). Therefore, the primary aim on this research was to observe changes in hepcidin and IL-6 concentration after exercise in the heat in female athletes.

A secondary aim of the project was to explore gut permeability as a potential mediator of this response. No studies to date have examined the relationship between exercise-induced increases in gut permeability and iron regulation in athletes. A majority of the cytokines post exercise are muscle-derived; however, other sources of inflammation may exacerbate or prolong elevated inflammation post exercise. Hepcidin release in response to inflammation is a part of the innate immunity to pathogens (Ganz & Nemeth, 2012). Bacteria require iron to replicate, thus hepcidin functions to sequester iron from circulation to mitigate bacterial replication (Ganz, 2019; Vargas & Marino, 2016). Exercise can cause cell wall material from gram-negative bacteria (endotoxins) to translocate from the lumen of the intestine into systemic circulation, often referred to as "leaky gut" (Chantler et al., 2021), which may enhance the release of inflammatory cytokines.

Previous studies have shown that gut permeability is increased after long exercise bouts, exercise evoking high body core temperatures, or activities involving repeated ground impact exercise (such as running) (Brock-Utne et al., 1988; S. K. Gill et al., 2015; Lambert, 2004). Changes in gut permeability have been attributed to the redistribution blood flow away from the splanchnic region during exercise and thermoregulatory challenge (Rowell et al., 1968) which can lead to cell damage and increased gut permeability (Van Wijck et al., 2012). Exercise or environmental elevations in body core temperature can compromise the integrity of the protective lining in the intestine (Dokladny et al., 2016). Previous studies have indicated that body core temperatures exceeding 39°C will illicit augmented permeability in exercising

individuals (Pires et al., 2017). If an exercising individual attains high body core temperatures, they may be susceptible to larger increases in inflammatory cytokines due to the compounding stimuli of exercise and endotoxins, which may exacerbate hepcidin production, but this is currently unknown.

Only one previous study has explored the connection between endotoxin injection and hepcidin response in humans. Kemna and colleagues demonstrated that injection of lipopolysaccharide (a cell wall component of gram-negative bacteria, also called endotoxin) resulted in a mounted inflammatory response and ultimately an increased release of hepcidin with a dramatic decline in circulating iron (Kemna et al., 2005). While these data confirm the relationship between endotoxin in circulation and hepcidin release, these results need to be contextualized to exercising athletes. It is unknown if athletes experience high enough concentrations of circulating endotoxin to evoke a similar hepcidin response, or if there are protective adaptations that take place with training which may prevent leaky gut.

The purpose of this study was to examine the time course and magnitude of gut permeability, inflammatory biomarkers and hepcidin responses evoked by exercise under cool and hot environmental conditions in female athletes. We hypothesized that exercise in a hot environment would increase interleukin-6 and hepcidin concentrations post-exercise compared to a cool exercise condition. We further hypothesized that these elevations are due to the presence of gut endotoxins in circulation.

METHODS

Subjects. Before participating in the study, volunteers provided written informed consent. The study conformed to the principles of the Declaration of Helsinki, except for registration in a

database. The research protocol was approved by the Institutional Review Board at the University of Oregon. Thirteen endurance trained female runners (Table 4.1) completed three exercise visits. Participants were limited to Tier 2 or Tier 3 athletes as defined by McKay and colleagues (McKay et al., 2022). Seven participants were using contraceptives consisting of: five intrauterine devices (one copper, four hormonal), and two oral contraceptive users. Additionally, ten participants self-reported regular (~28 day) menstrual cycles. Upon completion of the informed consent, a blood sample was collected to assess iron status (serum iron, transferrin, and ferritin). Participants were excluded from the study if they exhibited iron status values outside of the normative ranges (transferrin: 188-341 mg/dL; iron, total: 40-190 mcg/dL; ferritin: 16-154 ng/mL).

Table 4.1. Participant anthropometrics, exercise speed, and iron status. Values are means ± SD.

Variable	
Age (y)	26.1 ± 5.9
Height (cm)	164.5 ± 6.5
Weight (kg)	62.0 ± 6.7
Body mass index (kg m^{-2})	23.0 ± 2.3
VO_{2max} (ml kg^{-1} min^{-1})	57.7 ± 7.3
Exercise Speed (km·h^{-1})	10.5 ± 1.3
Exercise Grade (%)	1.0 ± 0
Serum Iron (mcg/dL)	106.2 ± 53.5
Serum Transferrin (mg/dL)	267.4 ± 28.6
Serum Ferritin (ng/mL)	32.2 ± 16.5
n	13

Study Design.

Visit 2: Participants completed a graded exercise test to assess VO_2max on a treadmill in at room temperature (21 - 22°C) with a fan on for convective cooling. Participants were asked to abstain from food (3 hours), vigorous exercise (24 hours), caffeine (6 hours), over the counter

medications or supplements (12 hours) and alcohol (12 hours) prior to their graded exercise test. Upon arrival to the lab, height and weight were recorded, and participants were instrumented with a heart rate monitor (Polar H10, Polar Electro). Participants provided a urine sample to test for hydration status (Atago; Master-URC/NM, Tokyo, Japan) and pregnancy status (if applicable, hCG-Urine Test, Sekisui Diagnostics, San Diego, CA). If urine specific gravity >1.020, participants were asked to drink 5 mL of water per kilogram of body weight. For the graded exercise test, participants were given a standard 3 minute warm up (9.66 km/h and 1% grade). Treadmill speed for the test was self-selected by participants to reflect a 10-kilometer race pace. After warm-up, treadmill speed was increased to the testing pace. Treadmill incline was increased 1% per minute until volitional exhaustion. During the test, rating of perceived exertion was collected every minute (Borg Scale) (Borg, 1982). Upon completion of the test, participants were given a 10-minute break before a supra-maximal VO_2max verification. Supra-max validation consisted of a maximal running test set to 110% of the final workload achieved in the graded exercise test (Poole & Jones, 2017). Participants were instructed to run as long as possible, and the supra-max verification test ended at volitional exhaustion. During exercise, participants wore a mouthpiece, and expired gases were collected and analyzed (Parvo, Parvometrics, Sunvalley, UT). Criteria for a valid VO_2max test were: a respiratory exchange ratio of ≥ 1.1, rating of perceived exertion ≥ 17, a plateau in heart rate/VO_2 observed despite further increases in workload, and VO_2 value within 5% of supra-max validation.

Visits 3 & 4: Prior to arriving at the lab on experimental study days, participants consumed a body core temperature pill at least 4 hours prior to exercise (e-Celsius performance pill, BodyCap, Caen, France or HQ Inc., Palmetto, FL) and maintained a 24-hour food log that was

repeated for the subsequent experimental session. Additionally, participants were asked to follow the same abstentions as described for Visit #2. Exercise sessions were conducted at least a week apart and occurred in the morning and fasted to mitigate any potential circadian influence. Menstrual cycle was not controlled for during the experimental sessions. Previous research has examined similar hepcidin responses to exercise across the phases of the menstrual cycle and during oral contraceptive use (Alfaro-Magallanes et al., 2021). Upon arriving to the lab for the experimental visit days, participants provided a urine sample to test for hydration status (Atago; Master-URC/NM, Tokyo, Japan) and pregnancy status (if applicable, hCG-Urine Test, Sekisui Diagnostics, San Diego, CA). If urine specific gravity <1.020, participants were asked to drink 5 mL of water per kilogram of body weight. Subjects provided a nude body weight before and after exercise to calculate water loss estimates. Visits 3 and 4 were experimental exercise sessions that took place in an environmental chamber. These visits were identical, except for the ambient temperature in the room. One visit was in hot conditions (35°C and 40% relative humidity) while the other was in cool conditions (11°C and 40% relative humidity). The order of the visit conditions was randomized. The exercise duration was 45 minutes and participants ran at 70% of their VO_2max. During exercise participants were asked to rate their thermal perception and rating of perceived exertion every 5 minutes. Body core temperature and heart rate were monitored continuously during exercise (Table 4.2). If a body core temperature of 39.5°C was reached during exercise, the exercise bout was ended.

Blood Sampling. Immediately prior to exercise, subjects provided a blood sample via straight needle draw. Immediately post exercise, subjects were removed from the environmental chamber, and an intravenous catheter was placed for a post exercise blood sample and further

sampling. Hourly, blood samples were collected for 3 hours post exercise. One, 8 mL serum separator tube was collected at each time point. The blood samples were allowed to clot at room temperature for 30 minutes before being centrifuged, aliquoted into 1 mL cryovials, and frozen at -80°C for biomarker analysis.

Blood Analyses. Iron status (serum iron, transferrin, ferritin) blood analyses were conducted by an external clinical laboratory. Lipopolysaccharide (LPS) was used as the biomarker for leaky gut as this surface protein is expressed on the outside of gram-negative bacteria. The presence and change in LPS in serum samples was used to indicate changes in gut permeability. Lipopolysaccharide concentration was determined via LAL Chromogenic Endpoint Assay (Hycult Biotech (HIT302)). Interluekin-6, Tumor Necrosis Factor alpha (TNFα), and Hepcidin were analyzed using enzyme linked immunosorbent assays (Quantikine HS ELISA Human TNFα (HSTA00E), Quantikine HS ELISA Human IL-6 (SS600C), Quantikine HS ELISA Human Hepcidin (DHP250); respectively). Blood analyses were conducted in duplicate for all subjects at all time points, except one subject missing immediately post exercise and 3 hours post exercise timepoints in the cool condition. Out of the 130 sample timepoints for each biomarker that were measured, two are missing due to not getting a blood sample. For specific markers, some samples were excluded as follows: IL-6; 4 samples over the limit of detection. Hepcidin; 4 samples removed due to high coefficients of variations between tested duplicates. LPS; 10 samples removed due to high coefficients of variation between tested duplicates (7) or samples under the limit of detection (3).

Statistical Analyses. Values are expressed as means (95% Confidence Intervals) unless stated otherwise. Alpha was set to 0.05 for all statistical inferences including familywise error rates. Fixed effects were condition (hot or cool condition) and timepoint (baseline, 0h, 1h, 2h, and 3h post exercise). Inferences were drawn from two-way mixed-effects models for measurements across time and between conditions. (Prism 10, GraphPad, Boston, MA, USA). Inferences regarding changes over time within each condition were drawn from Dunnett's multiple comparison test versus baseline, with reported p-values and confidence intervals adjusted for multiplicity. Inferences regarding the difference between groups were drawn from Šidák's multiple comparisons test, restricted to comparisons at the same timepoint between group.

Limitations. The primary limitation of this study is that we did not track and match menstrual cycles for the exercise sessions. Since the primary outcome of these research efforts was to observe inflammatory and iron regulatory responses in the heat and was not menstrual cycle specific, we did not match cycle phase. Controlling for menstrual phase would limit the findings to one specific temporal occurrence (i.e. only applicable in the follicular phase) and would limit the ecological validity of the results. We recorded contraceptive type and use across participants, and we did not find any systematic impacts on our data or findings. We were not adequately powered to address hormonal contraceptive changes on the primary outcome variables. It is important to note that the menstrual cycle influences resting body core temperature (Baker et al., 2020), however, the starting body core temperatures between the hot and cool exercise bouts were similar (Table 4.2). Menstrual cycle may also influence gut permeability, however, there is currently a lack of evidence to support whether this exists in female athletes (Pugh et al., 2022). Additionally, the use of LPS as a biomarker of gut permeability may present challenges. We

followed the manufacturers specifications for performing the assay including using endotoxin free water. However, the appearance of LPS in circulation may be difficult to capture due to the liver and antibodies that neutralized LPS in vivo.

RESULTS

Exercise. Participants completed 45 minutes of exercise in the cool condition, and average exercising time in the hot condition was ~42 minutes as five of the exercise sessions were stopped early (four due to body core temperature exceeding 39.5°C and one asked to stop due to lightheadedness). Body core temperature was elevated in both hot and cool conditions after the first 5 minutes of exercise that persisted through the end of the exercise session. The rise in body core temperature was greater in the hot condition from minute 25 ($p = 0.03$) until the end of exercise ($p < 0.01$). Peak body core temperature during the hot condition was greater than the cool condition (Δ2.1°C (1.8, 2.3), Δ1.0 °C (0.8, 1.3), respectively; $p < 0.01$; Figure 4.1). Peak heart rate and sweat rate were greater in the hot condition compared to the cool condition ($p < 0.01$, $p < 0.01$; Table 4.2).

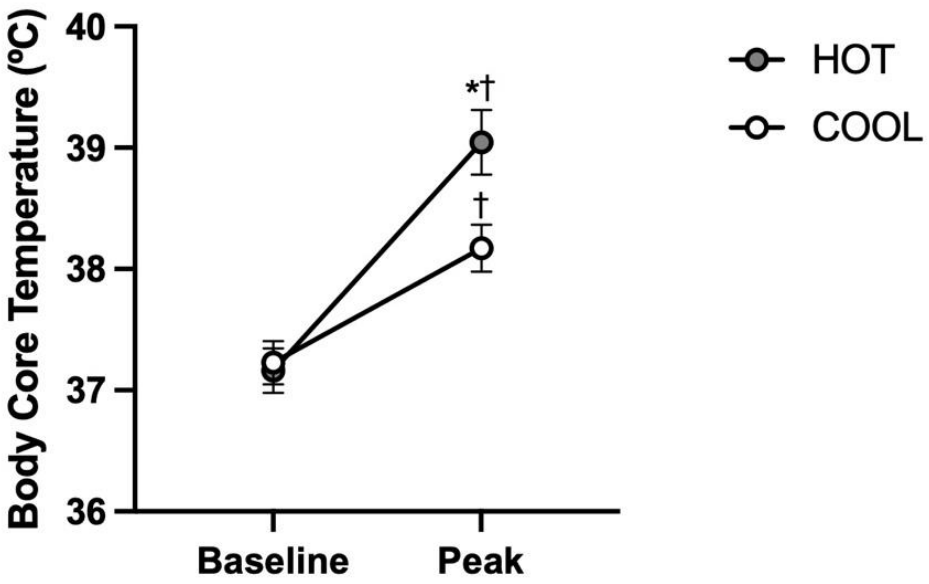


Figure 4.1. Mean body core temperature (95% CI) during exercise in hot and cool conditions. * Denotes significance between conditions. † Denotes a difference from pre-exercise within condition.

Table 4.2. Measures associated with the exercise bouts. Values are means (95% confidence intervals). * Denotes a difference compared to the cool condition at same timepoint. † Denotes a difference from the pre-exercise time point within condition.

Variable and timepoint	Cool Condition	Hot Condition
Heart Rate (BPM)		
Start Exercise	143 (132, 153)	146 (137, 156)
End Exercise	154 (142, 165) †	167 (148, 187) †*
Sweat Loss (L)		
Post Exercise	0.50 (0.41, 0.59)	0.85 (0.73, 0.97) *
Body Core Temperature		
Start Exercise	37.3 (37.1, 37.5)	37.2 (37.0, 37.4)
End Exercise	38.2 (38.0, 38.4) †	39.2 (38.9, 39.5) †*

Lipopolysaccharide. Lipopolysaccharide remained at a similar level through the duration of all timepoints and both exercise trials (Figure 4.2).

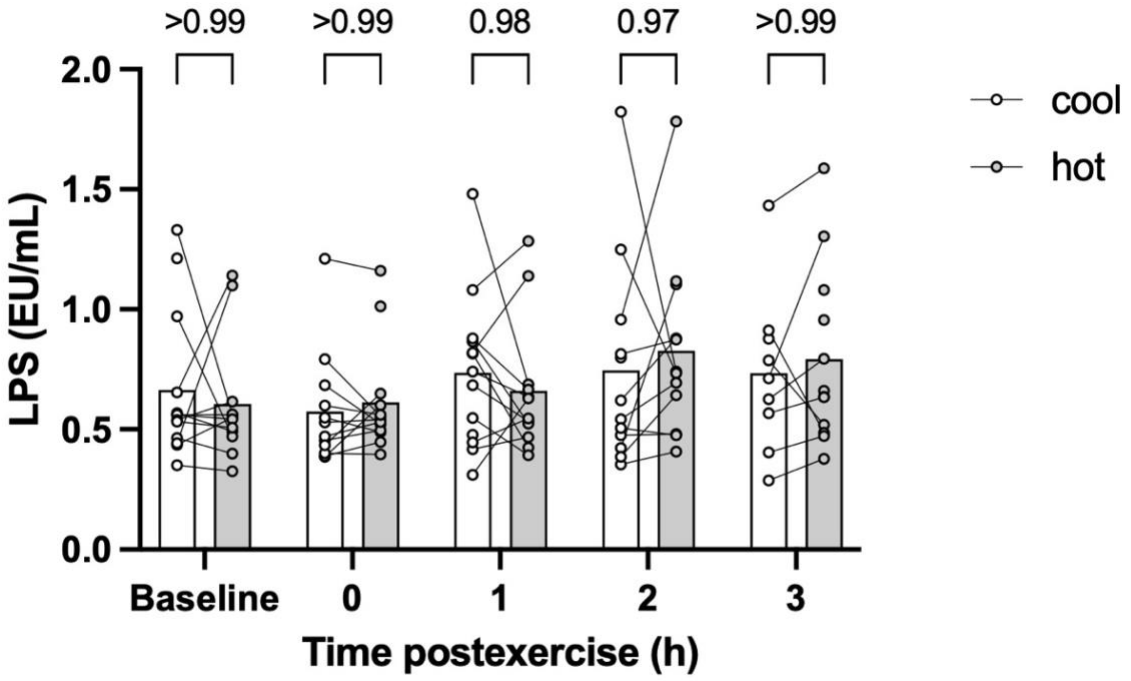


Figure 4.2. Lipopolysaccharide concentration from pre-exercise in the hot and cool conditions immediately, 1h, 2h, 3h post exercise.

Tumor Necrosis Factor Alpha. TNFα remained at a similar level through the duration of all timepoints and both exercise trials (Figure 4.3).

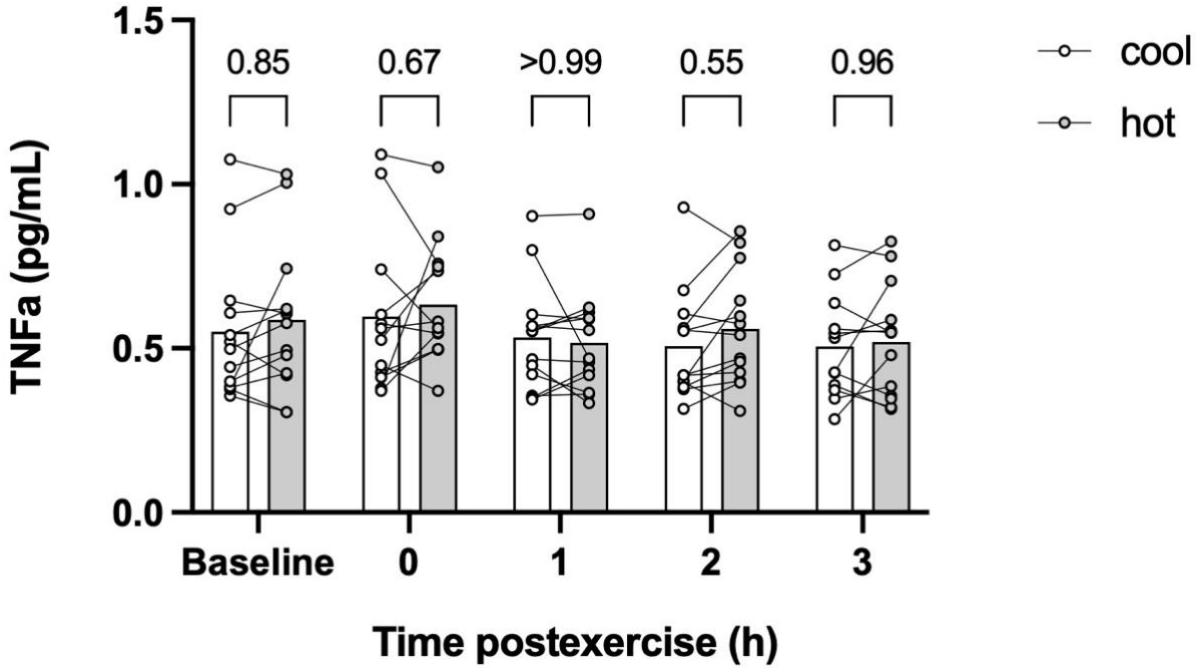


Figure 4.3. Tumor necrosis factor alpha concentration from pre-exercise in the hot and cool conditions immediately, 1h, 2h, 3h post exercise.

Interleukin-6. IL-6 was elevated post exercise in hot and cool conditions immediately post exercise compared to pre-exercise baseline (Δ1.2 pg/mL (0.9, 1.4), $p < 0.01$; Δ0.7pg/mL (0.5, 0.9), $p < 0.01$; respectively; Figure 4.4). One hour post exercise, IL-6 concentration was greater in the hot condition (1.6 pg/mL (1.0, 2.2) versus the cool condition (1.0 pg/mL (0.8, 1.3); $p = 0.04$). Elevations in the ΔIL-6 compared to baseline were greater in the hot condition compared to the cool condition at 1h (Δ1.1 pg/mL (0.7, 1.5), Δ0.4 pg/mL (0.1, 0.7), $p < 0.01$; respectively) and 2h post exercise (Δ1.0pg/mL (0.5, 1.4), Δ0.3 pg/mL (0.01, 0.7), $p = 0.01$; respectively).

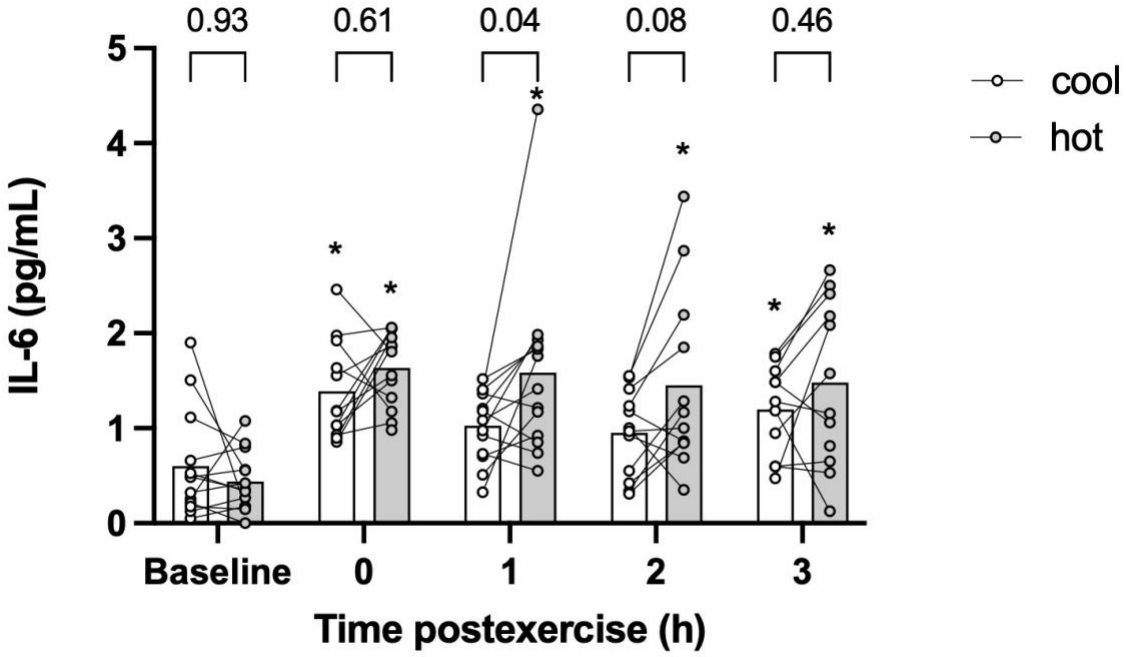


Figure 4.4. Interluekin-6 concentration from pre-exercise in the hot and cool conditions immediately, 1h, 2h, 3h post exercise. * Denotes a difference from pre-exercise within condition ($p < 0.05$).

Hepcidin. Hepcidin increased post exercise in both conditions, but the extent of the peak elevation was higher in the hot condition (Figure 4.5). Hepcidin was elevated in the cool condition at 2h ($p = 0.04$) and 3h ($p = 0.03$) post exercise. Hepcidin was elevated at all timepoints in the hot condition compared to pre exercise ($p < 0.02$; all timepoints). Hepcidin elevations were similar between the two conditions immediately, 1h, and 2h post exercise. At the 3h post exercise timepoint, hepcidin was higher in the hot condition (15.1ng/mL (8.8, 21.3)) versus the cool condition (7.9 ng/mL (3.5, 12.2), $p = 0.03$; Figure 4.5).

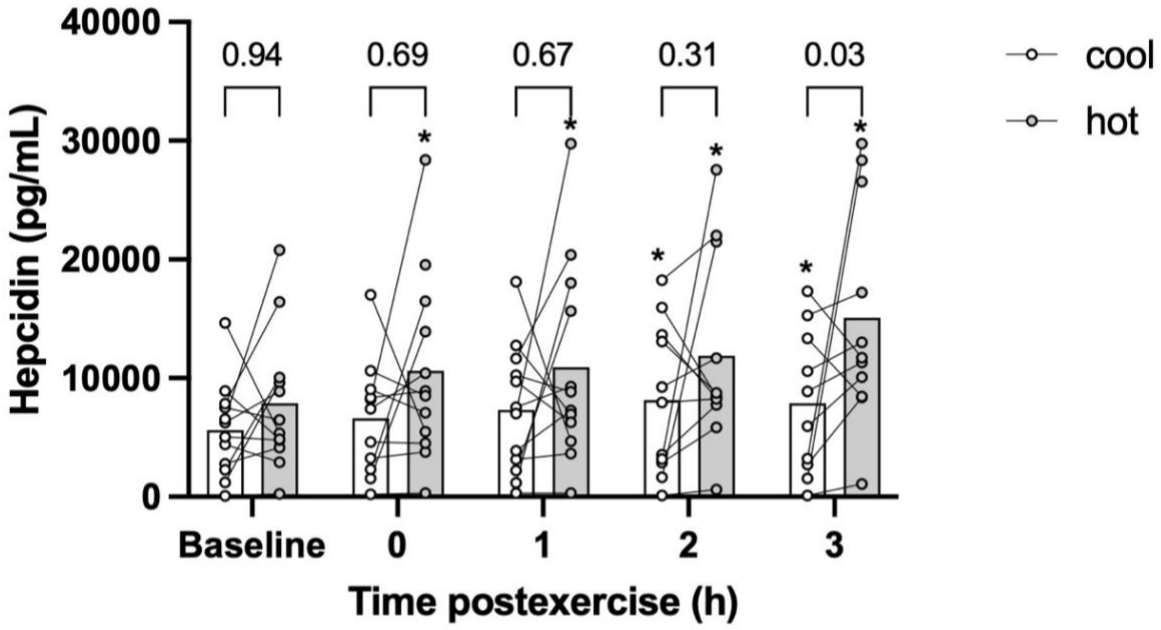


Figure 4.5. Hepcidin concentration from pre-exercise in the hot and cool conditions immediately, 1h, 2h, 3h post exercise. * Denotes a difference from pre-exercise within condition ($p < 0.05$).

DISCUSSION

The purpose of this study was to examine the acute hepcidin, inflammatory and gut permeability biomarker responses elicited by exercise in hot environmental conditions in trained female athletes. We hypothesized that there would be increased gut permeability after exercise that would evoke an exacerbated inflammatory response leading to increased serum hepcidin concentration. The production of inflammatory cytokines such as IL-6 are integral to the signaling and release of hepcidin from the liver. Due to the known relationship between inflammation and hepcidin, we were curious if other sources of inflammation may exacerbate the IL-6 signaling and hepcidin release. Our findings demonstrated that hot environmental conditions resulted in changes to inflammation and hepcidin concentrations post exercise regardless of endotoxin in the circulation. The measured endotoxin (LPS) concentration was not elevated in either condition at any time point. Interluekin-6 was elevated from baseline and sustained in both conditions, but to a greater extent in the hot condition. Additionally, hepcidin was elevated from pre-exercise similarly in both conditions; however, the elevation at 3 hours in the hot condition was greater than the cool condition. These results indicate that exercise in the heat results in a greater hepcidin response.

Gut Permeability during Exercise

Lipopolysaccharide is a component of the outer membrane of gram-negative bacteria used in both the clinical setting and research investigations as a blood biomarker that indicates an increase in gut permeability (Lambert, 2009; Lim et al., 2009). While basal levels of LPS are present, elevations in LPS would indicate increased bacterial translocation from the lumen of the

intestines into circulation. We hypothesized that LPS would be more elevated in athletes after exercising in the heat compared to exercise under cool conditions. However, our results indicate no change in LPS concentration after exercise in either condition. This was not an expected finding, as a previous meta-analysis indicated gut permeability (via measures of LPS, dual sugar absorbance testing, and intestinal fatty acid binding protein) was augmented in all individuals once a body core temperature of 39°C was reached during exercise (Pires et al., 2017). In our study, all but one participant achieved a peak body core temperature >39°C during the hot condition and all participants stayed below 39°C in the cool visit. We hypothesized that the separation in body core temperature between these two conditions would elicit augmented gut permeability in the heat compared to the cool condition. Pires and colleagues' meta-analysis was completed on a wide range of fitness levels and predominately males, therefore may not represent the highly trained endurance female athletes included in this study (Pires et al., 2017). Training can evoke physiological adaptations that are protective against gut permeability issues (Lim et al., 2009) and may have led to adaptations in the female athletes to be more resilient to the hot condition exercise bout. This study is one of few assessing endurance-trained female athletes at a moderate intensity in the heat. These athletes may have adapted to a heating stimulus through their regular training bouts.

Alternatively, an expansion in blood volume due to training can maintain blood profusion to the splanchnic region longer, thus reducing the hypoxic tissue effects (Van Wijck et al., 2012). Additionally, exercise can make the gut barrier more robust as repeated exercise bouts resulting in elevated body core temperature can change the composition of the enterocytes of the gastrointestinal tract, resulting in a less permeable membrane (M. Kuennen et al., 2011). Further, immune changes such as increased production of immunoglobin G (IgG) may function to keep

circulating endotoxins at a minimum. Immunoglobin G functions to bind to foreign pathogen associated molecular patterns, such as LPS, mitigating the subsequent inflammatory response. There is research to suggest that trained athletes have higher concentrations of IgG, potentially to combat chronic endotoxin exposure due to exercise (Barberio et al., 2014).

In our study, the average running speed of the visits was 10.5 km/h (range: 8.4 – 12.6), representing a workload at 70% VO_2max which was prescribed for 45 minutes (average of ~42 minutes in the hot condition). We speculate that the absolute intensity or duration may have been insufficient to cause changes in gut permeability during exercise to evoke an LPS response. While the absolutely running intensity was low, perceived intensity of exercise in the heat was quite high (average rating of perceived exertion was "hard" 16 ± 2 at the end of exercise), and several athletes were removed from the heat early (four due to body core temperature exceeding 39.5°C and one asked to stop due to lightheadedness). That said, previous work has highlighted that the duration of exercise may be more predictive of gut permeability than the ambient temperature (Sheahen et al., 2018). Studies utilizing longer exercise bouts have reported changes in gut permeability and may be more successful in evoking changes in gut permeability than ambient temperature (Brock-Utne et al., 1988).

Immune Response to Endotoxins

Lipopolysaccharide in the systemic circulation can activate TNFα signaling, resulting in an immune-mediated inflammatory response. Since LPS concentration did not change after exercise, we would anticipate no changes in the production and release of TNFα. Tumor necrosis factor alpha is a proinflammatory cytokine that can be activated in response to an acute infection or trauma. During short exercise bouts TNFα is not elevated, however, during long sustained

efforts TNFα can be elevated (Cerqueira et al., 2020; Petersen & Pedersen, 2005). The exercise bout in this study was 45 minutes, therefore, under normal conditions TNFα concentration will not rise. Our results indicate the TNFα did not rise during exercise in either exercise condition suggesting that the elevated IL-6 was not immune-mediated. Our data indicates that the source of the IL-6 release is not from a TNFα activated mechanism, nor LPS induced inflammatory response. The IL-6 response post exercise is likely mediated via primarily muscle derived myokine release in response to exercise.

Sources of Inflammation Post Exercise

Interlukin-6 is an important cytokine released in response to exercise and has direct implications on the production and release of hepcidin. There are many factors that can contribute to elevated IL-6 in the heat. Exercise intensity (Febbraio & Pedersen, 2002), energy sensing (Pedersen et al., 2001), muscle damage (Fortes et al., 2013) and muscle heating in mouse models (Obi et al., 2017; Welc et al., 2012) have all been shown to independently raise IL-6 levels. Interluekin-6 was elevated on both conditions, but to a greater extent in the hot condition. This was the anticipated response as previous work indicates higher intensity exercise elicits a greater rise in IL-6 (Ostrowski et al., 2000). The additional stimulus of heat resulted in an increased relative exercise intensity during the hot condition (Table 4.2), which likely resulted in the increased inflammatory response. Reductions in splanchnic blood flow during exercise are associated with increased relative exercise intensity as well as core temperature and are closely associated with the rise in sympathetic activity. Further, the increase in carbohydrate utilization that accompanies exercise in the heat could promote elevated IL-6 myokine signaling from the working muscle (Febbraio & Pedersen, 2002). Precautions were taken to try to mitigate

variability in this response. Participants were asked to maintain a consistent diet throughout their duration of enrollment in the study, and food logs were kept and matched 24 hours prior to exercise as an attempt to control carbohydrate intake prior to exercise. Therefore, the increase in IL-6 release after the hot condition was most likely due to the ambient heat exposure, and subsequent increase in relative exercise intensity compared to the cool condition.

Iron Regulation and Inflammation

The release of IL-6 during exercise in different ambient conditions may be an important consideration for its effects in iron regulation (Nemeth, Rivera, et al., 2004). Hepcidin is known to block cellular export of iron by blocking and degrading the only known iron exporting channel, ferroportin (Ganz, 2006). This process has important implications for the intake of dietary iron. If hepcidin is present in high concentrations, absorption of dietary iron into circulation is more difficult as ferroportin channels are integral to transporting iron into the circulation (Nemeth & Ganz, 2021). Previous research has shown a marketed reduction in iron absorption once hepcidin is released (Barney et al., 2022). Our results demonstrate an increase in hepcidin from baseline in both conditions, but to a greater extent in the hot condition at the 3h timepoint. This is most likely due to the more greatly sustained elevations in IL-6 during the hot condition. Previous studies examining iron regulation after exercise in hot ambient temperature reported similar increases in IL-6 during exercise in hot conditions (McKay et al., 2021). Despite elevations in IL-6, McKay and colleagues reported no changes in hepcidin production. This study was conducted on a cohort of male subjects exercising at a workload relative to ~55% VO_2max in similar ambient conditions. The current study examined an all-female cohort with a higher relative exercise intensity. These key differences may explain the difference in findings.

An increase in exercise intensity regardless of sex could explain an elevated hepcidin response as previous research has shown hepcidin responds in an exercise intensity dependent manner (Sim et al., 2013). An established sex difference in iron deficiency prevalence and occurrence in athletes would indicate that there may be differences in iron regulation between males and females. There is limited research examining sex differences in the hepcidin response, particularly in highly trained athletes. Work on both male and female cohorts has indicated that 62% of the variability in hepcidin response is due to baseline serum ferritin and serum iron (Peeling et al., 2017). In the current study, individuals outside of the normative range for ferritin, transferrin, and serum iron were excluded. More research is needed to elucidate the sex differences in iron regulation.

CONCLUSION

An acute bout of exercise in the heat elicited a greater IL-6 and hepcidin response compared to that in a cool condition in highly trained female athletes. Peak serum hepcidin (3 hours post exercise) was higher after exercise in a hot environmental condition compared to a cool exercise condition. The greater levels of hepcidin were likely due to a greater increase in IL-6 after exercise in the heat compared to the cool condition. Lipopolysaccharide and TNFα were not different across timepoints or conditions and likely did not contribute to the elevation in hepcidin in the heat. Since LPS concentration did not change during either exercise sessions, it appears that the elevation in IL-6 and hepcidin was independent of gut permeability changes. These results indicate the importance of considering environmental and seasonal impact on iron regulation for training and competing athletes.

CHAPTER V
MIXED METHOD HEAT ACCLIMATION MAY IMPROVE DURABILTY IN FEMALE ATHLETES

INTRODUCTION

Performance durability is an emerging topic that explores exercise metrics in both rested and fatigued states. Accumulation of fatigue will result in the deterioration of performance metrics over time and ultimately influence overall race day outcomes (Hunter & Muniz-Pumares, 2025; Jones, 2024). With limited exiting research on durability, there is a need to explore how different performance metrics fatigue over time and how they may be assessed, trained, and mitigated for competition.

Critical speed is integral to endurance sport performance as it delineates sustainable exercise intensity from non-sustainable intensities (Burnley et al., 2006; Jones & Vanhatalo, 2017; Poole et al., 2016). Critical speed is defined as the highest sustainable oxidative metabolic rate achievable (Jones et al., 2019). In a race environment, critical speed represents an important boundary during endurance-based events with limited, finite capacity above this speed (defined as D^{I}). This metric provides important context into race day pacing as it is highly correlative with endurance exercise performance (Black et al., 2014; Hill, 1925; Kolbe et al., 1995; J. C. Smith et al., 1999). However, over the course of prolonged exercise, critical speed declines as fatigue onsets, or results in task failure (Jones, 2024). Despite having similar traditional performance metrics in an idealized, rested state, some athletes can better sustain their critical speed than others. Initial durability studies have shown high interpersonal variability in response to fatiguing exercise (Clark et al., 2018) highlighting the potential for some athletes to outperform their competition despite similar traditional performance metrics performed in the rested state.

The magnitude and time course of decay provides valuable information into individual performance capacity.

Durability during running can be captured by quantifying the deterioration of critical speed. To our knowledge, quantifying the deterioration in critical speed during running in female runners has not yet been investigated. Recent work on cycling durability can provide insight into the decrements in critical power over time. Initial studies have reported that decrements in critical power after 90 minutes of heavy domain cycling elicited a 10% average drop in critical power (Clark et al., 2018). These results point to the feasibility of utilizing decrements in maximal metabolic steady state (critical speed or critical power) as a viable measure of durability in athletes. Others have reported that decrements in running economy and lactate kinetics over the course of prolonged exercise indicate a measurable deterioration in durability (Zanini, Folland, & Blagrove, 2025b).

Thus, interventions that improve performance durability are of great interest to sport scientists, coaches and athletes (Jones & Kirby, 2025). Currently, only two strength intervention studies have reported improvements in running economy durability after an 11- and 10-week supplementary strength program (Vikmoen et al., 2017; Zanini, Folland, Wu, et al., 2025). While other interventions such as the use of super shoes (Madsen et al., 2025) or carbohydrate ingestion during exercise (Clark et al., 2019) have also proved to be successful at improving durability.

Another potential intervention that may improve durability is heat acclimation. Heat acclimation is the process by which an individual undergoes repeated heat exposures leading to well characterized adaptations (McDonald et al., 2025; J. D. Périard et al., 2015) in cardiovascular stability, electrolyte and fluid balance, and thermoregulation that are ergogenic in both hot and cool exercising conditions (Lorenzo et al., 2010; J. Périard et al., 2024).

Cardiovascular and thermoregulatory adaptations during prolonged exercise have been shown to improve exercise capacity and improved performance (Lorenzo et al., 2010; Sekiguchi et al., 2022). Further, adaptations in muscle metabolism and efficiency may translate to better metabolite handling during steady state exercise (Febbraio, 2001; Febbraio et al., 1994). Taken together, the local muscular and systemic changes may lead to improved capacity to maintain race speeds for longer. Yet, there is limited research on the effects of heat acclimation as a tool to sustain submaximal exercise efforts. It is unknown if the established benefits of heat acclimation extend to durability. To date, no studies have explored the use of heat acclimation for improving durability.

Due to the dearth of female athletic performance research, the rapid increase in female distance runners (DeLuca et al., 2023), as well as the increased emphasis on female athletic performance (Osborne et al., 2025) this investigation had two aims: 1) Assess the viability of an intermittent mixed method heat acclimation; 2) Assess if heat acclimation improves running durability in endurance trained female athletes. We hypothesized that heat acclimation protocol would induce thermoregulatory adaptations and would improve durability as defined by improved maintenance of critical speed after fatiguing exercise.

METHODS

Participants

This study was approved by the Institutional Review Board at the University of Oregon and conformed to the principles of the Declaration of Helsinki except for registration in a database. Participants provided written informed consent prior to participation. Twenty-four endurance trained female runners participated in this study (Table 5.1). Endurance trained was

determined by Tier 2 or Tier 3 athletes as defined by McKay and colleagues (McKay et al., 2022). The majority of our participants (n = 19) reported normal, ~28 day menstrual cycles. Thirteen participants reported using hormonal contraceptives (7 oral contraceptives, n = 3 in heat group; 6 Intrauterine devices, n = 4 in heat group). We recorded self-reported cycle phase but did not stager starting times with regard to the menstrual cycle.

Table 5.1. Participant Anthropometrics and intervention running speed.

Variable	Control (*n* = 12)	Heat (n = 12)
Age (y)	29 ± 5	25 ± 5
Height (m)	1.7 ± 0.04	1.7 ± 0.04
Bady mass (kg)	61.9 ± 8.6	65.0 ± 7.4
Body mass index ($kg \cdot m^{-2}$)	22.6 ± 2.7	22.8 ± 2.1
VO_{2max} ($ml\ kg^{-1} \cdot min^{-1}$)	52.9 ± 6.3	52.9 ± 5.6
Running Speed @ 70% max ($km \cdot h^{-1}$)	9.7 ± 2.2	9.5 ± 1.8

Participant characteristics by group. Values are means (± SD).

Following consent, participants were block randomized assigned to either the heat acclimation group (HA) or the control group. Block randomization was based on the results of their VO_2max test to match fitness level of both groups.



Figure 5.1. Protocol Schematic. Participants completed three pre-tests (VO_2max test, heat tolerance test, and durability test), followed by a three-week intervention period, and ending with two post-testing visits that were identical to the pre-testing visits with the exception of the VO_2 max test.

Experimental Design

Participants were asked to fast from food and drink (except for water) at least two hours prior to the experimental visits (Visits 1-5). They were also asked to abstain from vigorous exercise, supplements, alcohol, and recreational drugs for 24 hours prior to each testing visit.

Visit 1: VO_2max Assessment. A graded exercise test was used to assess VO_2max. Grade was kept constant (1% incline) and speed was increased at a rate of 1 $km \cdot h^{-1}$ every three minutes until volitional exhaustion. Expired gases were collected and analyzed via indirect calorimetry (Parvo Medics, True One 2400) for oxygen uptake (VO_2), carbon dioxide production (VCO_2), and Ventilation (VE), Gas Exchange Threshold (GET) and Respiratory Compensation Point (RCP). Following the test, participants were allowed a 10-minute rest before completing a supramaximal verification to ensure maximal oxygen uptake was achieved. The verification trial was performed at a constant speed that was 110% of the final speed achieved during VO2max test (Poole & Jones, 2017).

VO_2max was determined as the highest 30 second average during the ramp protocol. GET and RCP were estimated using the V-slope method (Beaver et al., 1986). To account for the lag in VO_2 during the graded exercise test for determinations of GET and RCP the time spent in each stage was deducted from the total stage time (Lansley et al., 2011).

Visit 2: Heat Tolerance Test. Prior to the heat tolerance test, participants were asked to ingest a core temperature pill (e-Celsius performance pill, BodyCap) at least 4 hours prior to the experimental session and provide at 24 hour dietary recall. When participants arrived at the laboratory, they provided a urine sample to ensure euhydration (urine specific gravity > 1.020;

Atago; Master-URC/NM) and a nude body weight. If urine specific gravity was greater than 1.020, participants were given 5 mL water per kg body weight of water prior to exercise as no fluid intake was allowed during the test. Participants exercised in an environmental chamber set to 38°C and 40% relative humidity for 30 minutes at the speed associated with 70% of VO_2max. Heart rate was measured via chest strap heart rate monitor (Model H10, Polar Electro). If a participant's body core temperature exceeded 39.5°C before the end of the test, then the test was ended (occurred once, at minute 25). After exercise, participants gave a second nude weight to calculate total body water loss.

Visit 3: Durability Test. Running durability was characterized by changes in critical speed before and after fatiguing exercise using the zero-slope method (Matthews et al., 2023) (Figure 5.2).

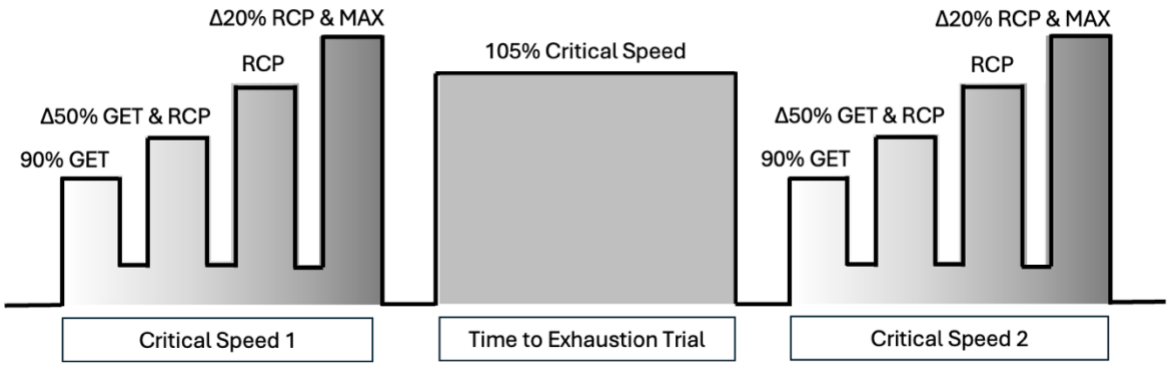


Figure 5.2. Durability Testing Protocol. After a warmup, participants completed four exercise intervals of increasing intensity. To estimate the other intensities, the relative difference (%Δ) between the speed at GET and RCP as well as RCP and MAX were used. Running stages reflect 90% GET (moderate domain), Δ50% GET and RCP (heavy (-) domain; that is 50% of the difference between GET and RCP), RCP (heavy (+) domain), Δ20% RCP and MAX (severe domain). Stages were three minutes, with a two minute active recovery break between each stage. Initial intervals were used to estimate critical speed 1. After completing four stages, participants completed a time to exhaustion trial at 105% critical speed. Upon volitional exhaustion, participants were given a five minute break before repeating the four stages of intervals used to calculate critical speed 2.

When participants arrived at the laboratory, they provided a urine sample to ensure euhydration (urine specific gravity > 1.020; Atago; Master-URC/NM) and a nude body weight. If urine specific gravity was greater than 1.020, participants were given 5 mL water per kg body weight of water prior to exercise. Throughout the duration of the entire test, participants were given a small amount of water (3mL per kg bodyweight) to drink ad libitum during breaks. Participants were instrumented with one Moxy sensor on the rectus femoris of the right and left leg (Moxy sensors; Moxy Monitors) and a heart rate monitor (Model H10, Polar Electro). Placement was approximately halfway between the anterior superior iliac spine and patella.

Participants completed 10 minutes of a self-paced unstructured running warm up followed by 10 minutes of a structured warm up. The 10 minute structured warm up started with a 30 second run at each testing stage followed by 30 seconds of walking (3.2 km/h). The test finished with 5 minutes of running at 90% GET followed by a final minute of walking at 3.2 km/h.

Critical Speed 1. After the warm-up, participants were instrumented with a metabolic mask for measurements of expired gases. Participants started the first critical speed test (Figure 5.2) following instrumentation. This test consisted of running at each stage for three minutes, with a two-minute walking (2 mph) break between. Critical speed was determined using muscle oxygenation sensors and the zero-slope protocol**.** Muscle oxygenation sensors recorded continuously during the running intervals. After the first critical speed test, participants were given a short break (≤ 13.5 minutes, matched within participants) while critical speed was calculated using the zero-slope method.

Exercise speeds were determined based on the results of the VO_2max test and design to span the moderate, heavy and severe exercise domains as previously established (B. S. Kirby et al., 2021; Lansley et al., 2011; Matthews et al., 2023). The speed at GET represents the transition between moderate and heavy domains (Black et al., 2017) while the speed at RCP estimates the transition between heavy and severe domains (Bergstrom et al., 2013). To estimate the other intensities, the relative difference (%Δ) between the speed at GET and RCP as well as RCP and MAX were used. Running stages reflect 90% GET (moderate domain), Δ50% GET and RCP (heavy (-) domain; that is 50% of the difference between GET and RCP), RCP (heavy (+) domain), Δ20% RCP and MAX (severe domain).

Time to Exhaustion. The time to exhaustion (TTE) trial was completed at 5% above their calculated critical speed. Participants were blinded to their time and distance during the TTE and instructed to exercise until volitional exhaustion. Upon voluntary cessation of exercise (n = 22) or upon reaching 40 minutes (n = 2, one in each group) of running, participants were given a 5-minute break before the second critical speed assessment.

Critical Speed 2. The second critical speed test was completed immediately following the TTE trial and was identical to the first critical speed test.

Intervention

Participants were asked to maintain their normal training and add the intervention visits in the lab as added training volume of which they were asked to maintain a training log for the duration of the three-week intervention period (Table 5.2). Additionally, all participants were asked to

avoid training sessions during warmer times of the day to minimize seasonal influence. As we previously mentioned, participants were block randomized to one of two groups: Control or Heat Acclimation.

Control. Those assigned to the control intervention were asked to visit the lab twice a week for three weeks (six total running visits). Control sessions consisted of 30 minutes of running in a thermoneutral room with a fan on at the speed associated with 70% VO_2max. The control group visits were designed to mimic the increase in training volume experienced by the heat acclimation group.

Heat Acclimation. Our heat acclimation protocol consisted of four visits per week for three weeks. Participants were asked to avoid training sessions in the heat of day. Heat acclimation was a mixed method protocol consisting of two active heating sessions (running at 70% VO_2max in an environmental chamber with temperatures of 38°C, 40°C, and 42°C with 40% relative humidity) and two passive heating sessions (water immersion up to the neck in 41°C water for 30 minutes. The ordering and timing of these visits was at the discretion of the participant to incorporate into their current training plans.

Table 5.2. Average weekly running distance outside of study activities.

Variable	Control	Heat
Average Weekly Distance (km)	36.2 ± 22.8	40.8 ± 18.4
Average Weekly Running Time (h)	3.7 ± 2.5	4.2 ± 1.8
Week 1 Distance (km)	41.5 ± 20.4	36.2 ± 27.9
Week 2 Distance (km)	36.4 ± 25.9	38.7 ± 25.8
Week 3 Distance (km)	39.6 ± 18.8	36.9 ± 19.8

Participant characteristics by group. Values are means (±SD).

Visits 4 and 5

After the intervention, the heat tolerance test and durability test were repeated identical to the testing prior to the intervention. After the intervention, a second heat tolerance test was administered at the same exercising speed, environmental conditions and the same time of day to account of circadian variation. Participants were asked to match their dietary consumption before both visits and kept a 24 hour dietary recall. During the durability testing, the zero-slope protocol stages were identical to the first durability test (visit 3) but the time to exhaustion trial speed was recalculated based on critical speed 1 for each independent trial.

Protocols and Equipment

Moxy Sensors. Moxy sensors provide an index of muscle oxygen tissue saturation (SmO_2) via continuous near-infrared spectroscopy (NIRS). The percentage of oxygenation versus non-oxygenated tissue measured by these sensors greatly reflects the microvascular beds of the skeletal muscle underneath the tissue. NIRS sensors have been used effectively to delineate exercise domains, determine maximal metabolic steady state, providing a novel methodology for accurately assessing exercise parameters submaximally (Hudgins et al., 2025).

Data and Statistical Analysis.

Zero-Slope Protocol. For all participants, the intensities of the zero-slope protocol were based on the VO_2max test, spanning the moderate, heavy and severe exercise domains. Moxy sensors recorded at a 2 Hz frequency during the exercise stages on both legs. SmO_2 data was analyzed over time to determine the slope for each stage, omitting the initial nadir accompanied by the onset of exercise and other periods of erratic changes due to signal artifact. At least 90 seconds of each stage were included for the slope determinations for all timepoints and intervals. After completing four stages that spanned all exercise domains, the integral of the SmO_2 slopes were plotted on the y-axis and speed on the x-axis. A linear regression was performed to determine the x-intercept. The x-intercept speed reflects an estimated slope of 0, representing the transition between the heavy and severe domain, or critical speed, as previously determined and validated in previous research studies (Abbotts et al., 2025; Matthews et al., 2023). Stages were omitted from the determination of speed at critical speed if they did not contain reliable data. Zero-slope speed was determined by averaging both legs together. If one leg had a linear regression of R^2 less than 0.85 or contained less than three interval stages, it was omitted from the determination of speed at zero-slope.

Values represent means (95% confidence intervals) unless stated otherwise. Alpha was set to 0.05 for all statistical inferences, including familywise error rates. Two-way mixed effects models were used for comparisons across timepoints and between groups based off pre-planned comparisons (Prism 10, GraphPad, Boston, MA, USA). Inferences over time and before and after intervention were assessed within each group using Šidák's multiple comparisons test (with reported p-values and confidence intervals adjusted for multiplicity). Inferences regarding the

differences between groups were drawn from Šidák's multiple comparisons test, restricted to comparisons at the same timepoint between groups and Pre- and Post- measurements within groups.

Reproducibility & Rigor. None of the participants were using over-the-counter medications at the time of the study, except for oral contraceptives. Women were studied irrespective of menstrual cycle phase and completed a negative pregnancy test prior to all experimental testing days. The menstrual cycle was not controlled, as female athletes typically train and compete across all phases, and prior research suggests minimal influence of menstrual phase on exercise performance (D'Souza et al., 2023; McNulty et al., 2020) nor critical speed estimates made using the zero-slope method (Schoeberlein et al., 2026). We recorded menstrual cycle phase across participants, and we did not find any systematic impacts on our data or findings. Prior to all experimental testing days, participants abstained from alcohol, strenuous exercise, and over-the-counter medications for 24 hours and from food, beverages (except water), and caffeine for two hours before testing. Participants were instructed to maintain a consistent diet prior to each experimental visit, verified through 24-hour dietary recall collected at each session. All durability tests were conducted in a thermoneutral laboratory environment with a fan on the participant. Heat tolerance tests were conducted at the same time of day pre and post intervention to minimize the influence of circadian rhythm.

An a prior assessment of sensitivity of the durability measurement was made using the reported standard deviation in critical power from Clark et al., (SD = 2.26%) with 12 participants in each group, an α of 0.05 and β of 0.15 (power = 0.85) demonstrating we can assess a 2.8% difference in durability between groups with the sample size calculated (Clark et al., 2018). This

is further supported by recent reliability and repeatability work that demonstrated meaningful physiological changes (~3-4%) can be detected using MOXY sensors and zero-slope method derived performance metrics (Hudgins et al., 2025), supporting the sample size of 24 participants.

To date, no study has utilized the zero-slope method to assess durability. This novel approach required the establishment of both within-test and between-test criteria to ensure comparable relative intensity across trials. Importantly, unlike previous durability or intervention-based performance research, we recalculated TTE speeds at each testing session to ensure similar relative intensity for both durability tests. While this decision introduces both strengths and limitations, it was conceptually necessary. If an intervention meaningfully alters physiological capacity, maintaining the same absolute intensity across trials would not reflect the same relative physiological stress. Therefore, adjusting TTE speed allowed us to preserve comparable relative intensity pre- and post-intervention.

Within each durability test, the fatiguing trial was required to elicit >94% of maximal heart rate and >80% VO_2max (determined during VO_2max testing). Trials were terminated at 40 minutes if volitional exhaustion had not occurred. Based on these criteria, no individual tests were removed from the dataset. However, one participant did not meet the physiological criteria but reached the 40-minute cap. Participants who reached 40 minutes without volitional exhaustion were terminated at that time. Consequently, for these individuals, the termination point may not reflect a uniform physiological endpoint compared to participants who fatigued earlier. This introduces potential limitations to internal validity when interpreting durability estimates derived from time-to-fatigue measures. The imposed 40-minute ceiling may also limit generalizability, as individuals capable of sustaining exercise beyond 40 minutes may exhibit

different physiological strain profiles compared to those who fatigued earlier (n = 2, one in each group). These two participants ran both TTE trials for 40 min at a similar intensity, meaning the relative intensity of the test within subject was similar, and therefore these participant's fatigue characteristics should be similar within participant. The durability comparisons should be interpreted within the constraints of the experimental design.

Between durability tests, trials were considered comparably difficult if VO_2 at the end of the fatiguing bout was within 10% between tests and end-exercise heart rate was within 7 beats per minute. Based on these criteria, one participant's durability data (n = 1; control group) was excluded from analysis due to failure to meet between test comparability thresholds.

RESULTS

Heat Acclimation.

Body Core Temperature. End exercise body core temperature pre-intervention was similar between the control and heat groups ($p = 0.84$). Post-intervention, end exercise body core temperatures were similar between groups ($p = 0.59$). End exercise core body temperature was similar in the control group before and after the intervention with an average change of -0.01°C ($p = 0.47$). There was a significant reduction in end exercise body core temperature in the heat acclimation group post intervention compared to pre intervention resulting in an average reduction of -0.3°C ($p < 0.01$) (Figure 5.3).

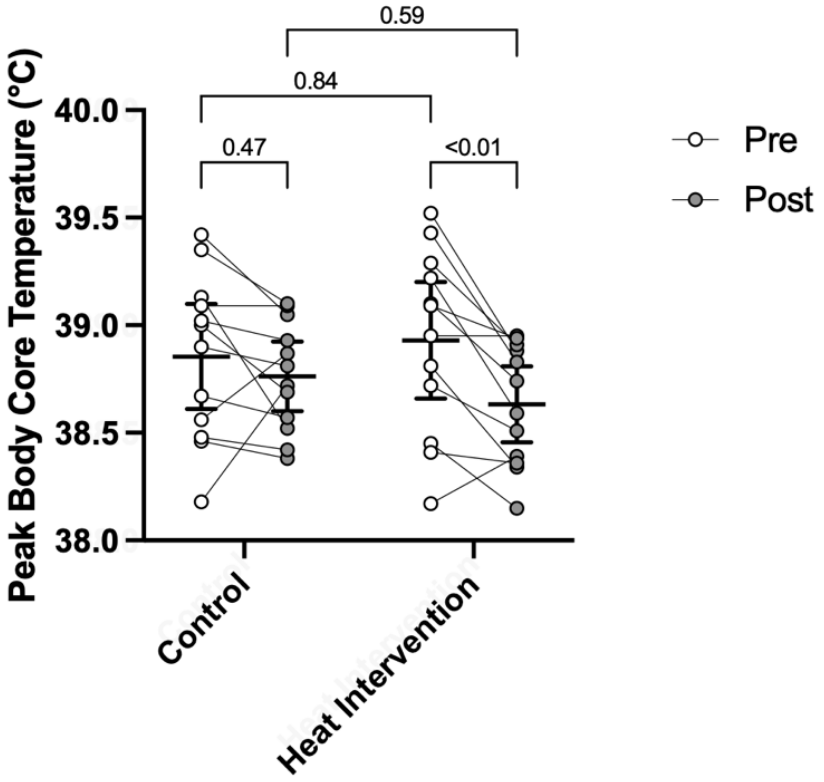


Figure 5.3. Peak body core temperature (°C) pre and post intervention in the control and heat group during the heat tolerance test.

Heart Rate. End exercise heart rate pre-intervention was similar between the control and heat groups. End exercise heart rate was similar in the control group before and after the intervention 177 (170, 185) BPM, 175 (167, 182) BPM; respectively, p = 0.40). End exercise heart rate was lower in the heat group before and after the intervention 183 (176, 191) BPM, 177 (169, 186) BPM; respectively, p = 0.03). There was a significant -6 BPM reduction in end exercise heart rate in the heat acclimation group post intervention (Figure 5.4). There was not a significant difference in post-intervention end exercise body core temperatures between groups (p = 0.78).

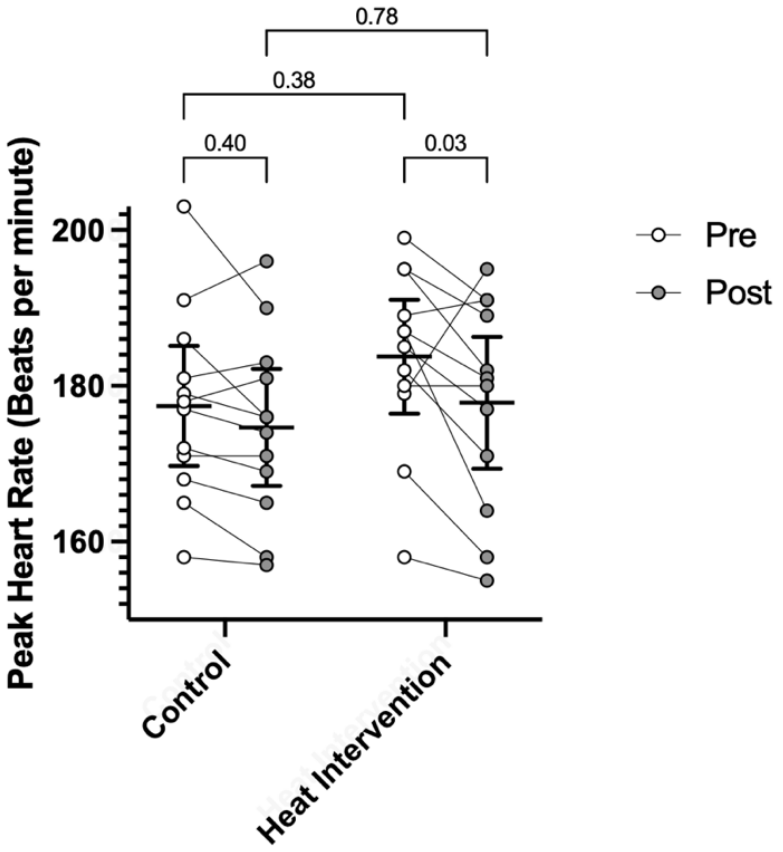


Figure 5.4. Peak heart rate (beats per minute) pre and post intervention in the control and heat group during the heat tolerance test.

Sweat Loss. Sweat loss pre-intervention was similar between the control and heat groups. Sweat loss was similar in the control group before and after the intervention 650 mL (555, 747), 660 mL (561, 759); respectively, $p = 0.70$). Sweat loss was greater in the heat group after the intervention (pre: 697mL (596, 798) post: 810 (689, 931); $p < 0.01$). There was a significant +113 mL increase in sweat loss in the heat acclimation group post intervention (Figure 5.5). The heat group post intervention had a great sweat loss than the control group post intervention ($p = 0.03$).

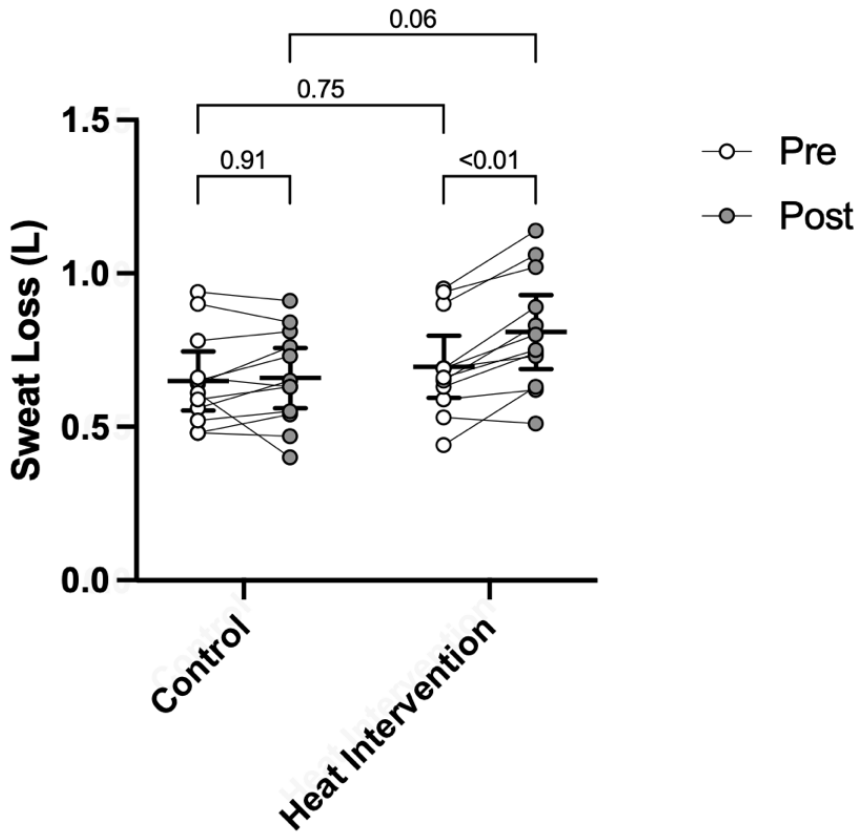


Figure 5.5. Total sweat loss (L) pre and post intervention in the control and heat group after the heat tolerance test.

Perception. Rating of perceived exertion was similar between groups at all both timepoints. Thermal perception was similar between the control and heat groups pre intervention. Thermal perception in the control group was similar before and after the intervention ($p = 0.46$). There was a significant -1 reduction in thermal perception in the heat acclimation group post intervention (**Table 5.3**). The heat group post intervention had a lower thermal perception than the control group post intervention ($p = 0.02$).

Table 5.3. Peak Rating of Perceived Exertion (RPE) and Thermal Perception (TP) during the heat tolerance testing pre and post intervention. * Denotes a difference from pre intervention within group. ** denotes a difference from the control group at the same timepoint.

Variable and timepoint	Control (*n* = 12)		Heat (*n* = 12)	
	Pre	Post	Pre	Post
Heat Tolerance Test				
RPE	14 (12, 16)	14 (13, 15)	13 (12, 14)	12 (10, 14)
TP	6 (5, 8)	7 (6, 8)	6 (5, 7)	5 (4, 5)*,**

Values are means (95% confidence limits).

Durability

Test Details. Both groups exhibited similar zero-slope testing speed stages (Table 5.4). Both groups had similar time to exhaustion trial speeds, times, distances and energy expenditure for both the pre and post intervention durability tests (Table 5.5).

Table 5.4. Speeds determined for the zero-slope protocol. Speeds were selected based on the results of the graded exercise test in Visit 1 to attain gas exchange threshold (GET), respiratory compensation point (RCP), and max speed (MAX). Speed spans all exercise domains: moderate (90% GET and Δ50% GET & RCP), heavy (RCP) and severe (Δ20% RCP & MAX).

Variable	Control	Heat
Speed at 90% GET ($km \cdot h^{-1}$)	8.9 (8.1, 9.8)	8.9 (8.3, 9.5)
Speed at Δ50% GET & RCP ($km \cdot h^{-1}$)	11.3 (10.4, 12.2)	11.3 (10.7, 12.0)
Speed at RCP ($km \cdot h^{-1}$)	12.7 (11.8, 13.6)	12.7 (12.2, 13.3)
Speed at Δ20% RCP & MAX ($km \cdot h^{-1}$)	13.0 (12.1, 13.9)	13.0 (12.4, 13.6)

Participant characteristics by group. Values are means (95% CI).

Table 5.5. Time to exhaustion trial results pre and post intervention. Speed post intervention was adjusted based on the critical speed calculation prior to the exercise test.

Variable and timepoint	Control (n = 12)		Heat (n = 12)	
	Pre	Post	Pre	Post
Time to Exhaustion Trial				
Speed ($km \cdot h^{-1}$)	12.6 (11.5, 13.6)	12.6 (11.7, 13.6)	12.1 (11.1, 13.1)	12.3 (11.3, 13.2)
Time (min)	21.6 (16.9, 26.4)	21.3 (14.6, 28.0)	21.5 (14.4, 28.6)	22.4 (15.7, 29.0)
Distance (km)	4.5 (3.7, 5.2)	4.6 (3.2, 5.9)	4.1 (3.0, 5.2)	4.4 (3.3, 5.4)
Energy Expenditure (kcal)	299 (242, 356)	288 (208, 369)	283 (198, 369)	306 (218, 394)

Values are means (95% confidence limits).

Critical Speed. Unfatigued critical speeds were similar in the control group and heat group before the intervention (11.9 $km \cdot h^{-1}$ (10.8, 12.9), 11.3 (10.0, 12.6), respectively; $p = 0.84$). Before the intervention, both the control group and heat group had a reduction in critical speed post fatiguing exercise compared to the rested state critical speed estimate ($p < 0.01$, $p = 0.03$, respectively) of similar magnitude in between groups ($p = 0.96$). After the intervention, pre-exercise (rested state) critical speeds were similar between the control and heat group (12.0 $km \cdot h^{-1}$ (11.0, 13.1), 11.6 $km \cdot h^{-1}$ (10.7, 12.6), respectively; $p = 0.80$). After the intervention, post fatigue critical speed was reduced in the control group (11.5 $km \cdot h^{-1}$ (10.2, 12.8); $p = 0.03$), but similar in the heat group (11.6 (10.6, 12.5); $p = 0.96$) compared to pre fatigued critical speed estimates. Fatigued critical speed was similar between the control and heat groups after the intervention ($p = 0.98$; Figure 5.6).

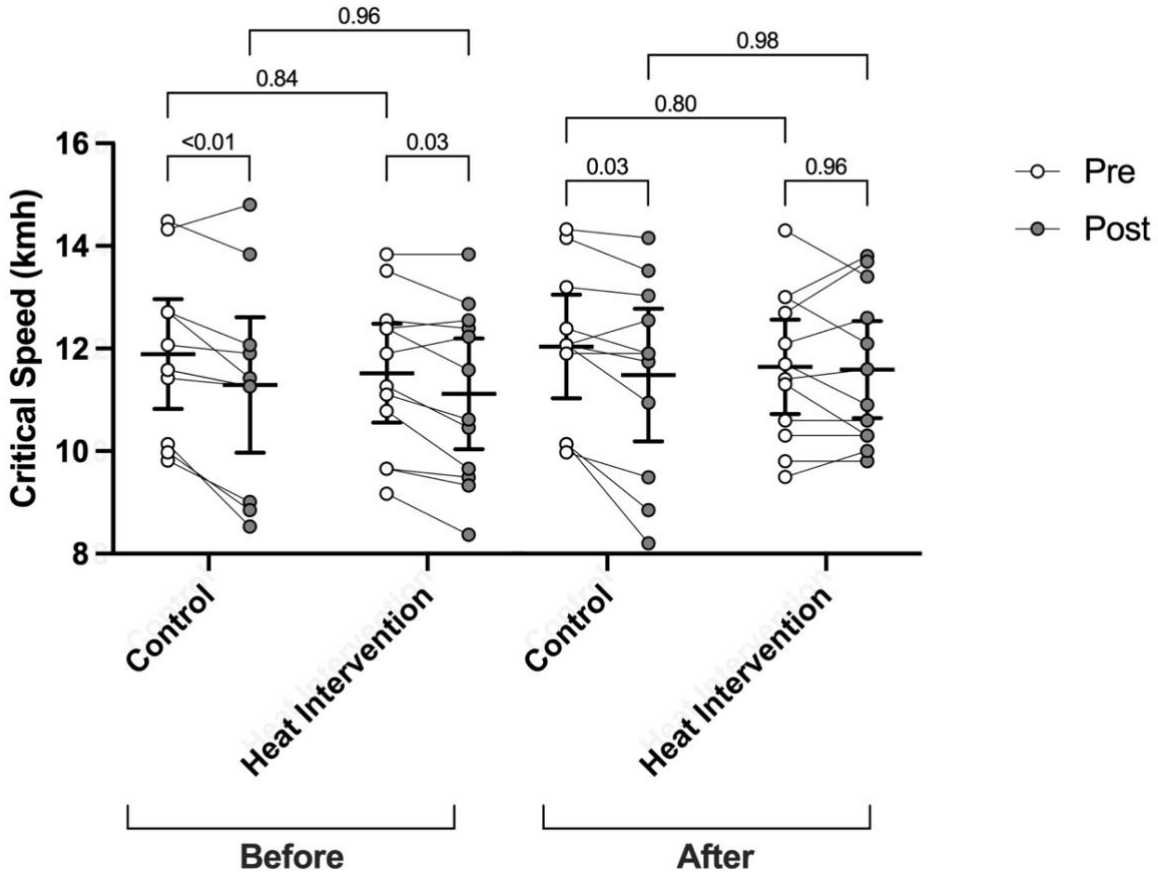


Figure 5.6. Critical speed ($km \cdot h^{-1}$) from each durability test pre and post fatiguing exercise before and after the intervention for the control and heat groups.

Durability. Maintenance (%) was similar between the control and heat group before the intervention (94% (90.0, 98.2), 96.0% (93.2, 98.8), respectively; p = 0.71). After the intervention, the heat group (99.3% (95.5, 103.1) is trending towards improved maintenance compared to the control group (93.5% (88.2, 98.9), respectively, p = 0.06). The control group performed similarly during both durability tests resulting in similar levels of critical speed maintenance (p = 0.93). However, post intervention, the heat group is trending towards improved maintenance compared to pre intervention (Figure 5.7; p = 0.09).

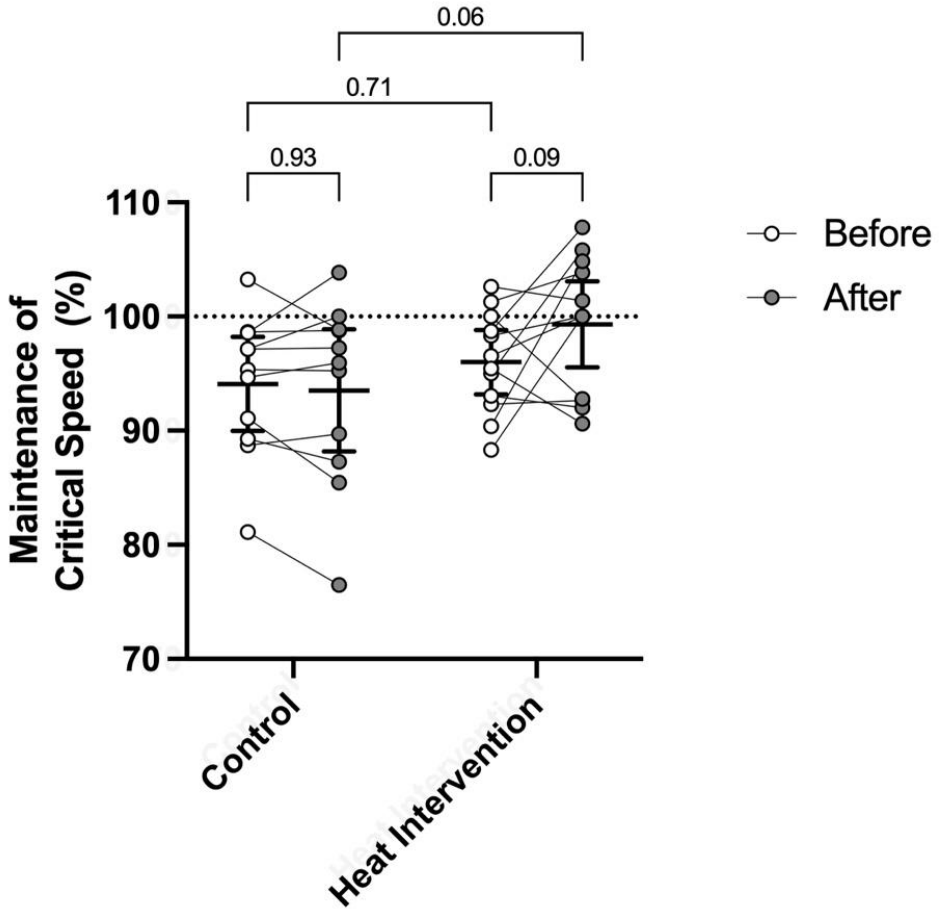


Figure 5.7. Maintenance of critical speed (%) before and after the intervention in the control and heat groups.

DISCUSSION

The purpose of this research was to explore a novel heat acclimation protocol and to assess performance durability in female runners. The mixed-method intermittent heat acclimation protocol was effective in heat acclimating female runners in our investigation. Furthermore, this outcome validates the mixed method protocol's effectiveness for heat preparedness in female runners. Heat acclimation benefits did not clearly extend statistically relevant performance benefits but may improve running durability in female runners.

Heat Acclimation Protocol.

Our results indicate that the mixed method heat acclimation protocol was successful in evoking a heat acclimated phenotype in endurance trained female runners. Reductions in peak heart rate, peak body core temperature, and thermal perception scales with an increase in sweat rate during the heat tolerance test highlights a heat acclimated phenotype. A recent meta-analysis by McDonald and colleagues examined the average change across 211 heat acclimation protocols (McDonald et al., 2025). Mean protocol characteristics of 8±4 exposures, 90±36 minutes per exposure at 39.1± 4.8°C resulted in an average change in end exercise core temperature of -0.42 °C, end exercise heart rate of -17 beats per minute and increased whole-body sweat rate by 163 mL per hour. Our protocol characteristics were 12 exposures, 30 minutes per exposure, averaging 40°C over the three-week period. Our protocol resulted in a -0.3 °C reduction in end exercise core temperature, end exercise heart rate -6 beats per minute, and increased whole-body sweat rate by 226 mL per hour. The core body temperature and heart rate adaptations in our study are smaller in magnitude than described by McDonald and colleagues. This may be due to the total amount of time spent in heat exposure during the intervention.

Participants had only two hours of heat exposure each week for three weeks. In total, only six hours of heat exposure were administered, which is vastly different from the aggregated average presented by McDonald and colleagues. Despite a low number of exposure hours, the relative exercise intensity in the heat was high (running at 70% VO_2max), likely playing a key role in producing meaningful adaptation. Heat exposure intensity, frequency, and modality are all important considerations when developing a heat acclimation protocol (Richey et al., 2026). Research should continue to elucidate how different modalities and temporalities of heat acclimation may lend to specific adaptations.

Using a mixed method approach to heat acclimation, there may be specific advantages to using both heating modalities. Limited research is available on how modalities of heat acclimation may lead to unique adaptation profiles. Most heat acclimation studies focus on the presence of adaptation rather than how different modalities may lead to adaptation via distinct mechanisms and result in an adaptation profile that is distinct. Combining both methods may lead to a more well-rounded heat acclimation adaption profile. Additionally, the length of the protocol may lead to increased sweating rate, but diminished plasma volume expansion. We saw a robust increase in sweat rate in our participants, likely due to the overall length of the protocol provided adequate time for sudomotor adaptations. However, this may come at the expense of plasma volume expansion. Our protocol was longer, and intermittent heat exposures may lend to less cardiovascular adaptation and elevated, but diminished plasma volume expansion.

Additional consideration must be made when contextualizing a heat acclimation protocol for athletes. Adding heat acclimation into the training plan of an athlete requires consideration for downstream effects of heat exposure and additional stress on the athlete. Athletes must maintain effective training and recovery while heat acclimating. Heat exposure can affect

sleeping habits, nutrition (hunger levels), and energy levels – all of which can jeopardize training. Traditional 10-day heat acclimation protocols are disruptive to ongoing training plans and limit their applicability to training and competing athletes. With these considerations in mind, we sought to develop a heat acclimation protocol for athletes in the midst of training and competing. The incorporation of a mixed-method heat acclimation protocol was to ensure athletes would have adequate rest between training and active heating sessions. The order of participant's visits each week were at their discretion and based on training schedule. Participants were encouraged to incorporate passive heating on higher volume or intensity training days (e.g. long runs, interval workouts). While this study was not designed to assess the minimal effective dose of to incur heat adaptations, we have shown that this specific heat dosage was effective at improving measures of heat acclimation.

Female Specific Considerations.

There is a dearth of research studies exploring running for active heat acclimation in female only cohorts. A recent meta-analysis highlighted an absence of active running heat acclimation protocols including female athletes performed (Kelly et al., 2023). This area research is imperative for informing female athletes, their coaches and sport practitioners of effective heating protocols. Despite anatomical differences and historical biases, females exhibit similar thermal tolerance to males (Yanovich et al., 2020). However, some research suggests that females may need a longer time course in order to acclimate (N. V. Kirby et al., 2019; Mee et al., 2015). Therefore, utilizing a heat acclimation protocol with a longer time course for adaptation may be advantageous for female athletes.

Durability.

Our results indicate that heat acclimation may improve running durability in female athletes. Prior to the intervention, decrements in critical speed following fatiguing exercise were similar between groups. However, after the intervention period, the heat acclimated group did not experience the reduction in critical speed observed in the control group or in their own pre-intervention trial. The lack of fatigue in the heat group after intervention suggests there is potential benefit of heat acclimation for durability. Our durability metric (% maintenance of critical speed) provided partial support for this interpretation, demonstrating a trend in the expected direction compared to the heat group before the intervention ($p = 0.09$) and compared to the control group after the intervention ($p = 0.06$). Overall, these findings align with our hypothesis that a heat-acclimated phenotype would enhance the maintenance of critical speed, although the results were not entirely conclusive.

Improvements in performance metrics after heat acclimation have been attributed to the systemic adaptations in cardiovascular stability, thermoregulation and muscle metabolism. Plasma volume expansion during exercise results in lower heart rate at the same work rate (Daanen et al., 2018; Wyndham, 1951) and increased maximal cardiac output (Lorenzo et al., 2010). Improvements in thermoregulation manifest as a lower sweat onset and increased sweat rate (Nielsen et al., 1993; Roberts et al., 1977). Improved sweat response lends to lower skin temperatures, creating a larger skin to core temperature gradient, thereby enhancing heat dissipation. Heat acclimation has also been shown to improve skeletal muscle metabolism. Enhanced glycogen sparing in the muscle (King et al., 1985; Kirwan et al., 1987), increase muscle contractility (Racinais, Wilson, & Périard, 2017), and improved metabolic efficiency result in lower muscle and blood lactate concentrations during exercise (Young et al., 1985). The

myriad of local and systemic changes to thermoregulation and metabolic optimization support the rationale as to why heat acclimation may improve running durability. Specifically, in this study with the use of NIRs sensors, a heat acclimated phenotype may have provided adequate blood flow to working muscles for longer during the latter stages of the durability test where thermoregulatory and cardiovascular drift occur. An additional hypothesis is that this improved blood flow may result in further carbohydrate sparing and ultimately lower lactate accumulation, allowing participants to maintain high intensity for longer.

Despite the myriad of adaptations to heat, it is possible that the benefits of heat acclimation do not extend to submaximal exercise tests or short duration exercise testing. Many heat acclimation investigations assess performance using maximal or near-maximal exercise tasks, where participants approach maximal capacity in the later stages of testing. Under these conditions, the physiological adaptations induced by heat acclimation may exert a more substantial and detectable influence. Alternatively, longer exercise tests may manifest improved performance as heat adaptations in thermoregulation or blood volume expansion play a larger role as cardiovascular drift begins to limit performance.

Additionally, submaximal exercise improvements in performance after heat acclimation may be harder to elucidate than other performance metrics. The primary adaptations of heat acclimation that lead to improved performance are cardiovascular adaptations. However, at submaximal exercise intensities such as critical speed, oxygen delivery and muscle blood flow may not be sufficiently challenged to reveal measurable performance benefits. If blood flow or oxygen delivery were not strained, it may be hard to parse apart the influence of our intervention. Perhaps if the durability trials were in hot environmental conditions, posing additional blood flow constraints, we may have seen more prominent changes in muscle blood delivery at critical

speed. Together, these findings indicate that the performance enhancing effects of heat acclimation may be intensity dependent, and more pronounced during exercise that substantially challenges oxygen delivery and maximal aerobic capacity.

Experimental Considerations.

To our knowledge, this is the first study aimed at assessing reductions of critical speed via NIRs in female runners. The use of NIRs is non-invasive and reproducible, a test structure similar to this could be a good standard practice to assess durability as the Moxy sensor is accessible, and the zero-slope protocol is an established method. Additionally, the zero-slope protocol uses four submaximal exercise stages to estimate critical speed and may be less susceptible to motivation-based estimations of critical speed such as the three-minute all-out protocol (Matthews et al., 2023). An additional strength the of durability test design was our decision to re-calculate the time to exhaustion trial speed for the post intervention testing to remove the confounding effects of training induced adaptation during a participant's enrollment in the study or daily fluctuations in performance. Current investigations of durability have not changed the testing intensities post-intervention, limiting the interpretations of their results.

Our participants were chosen based on Tier 2 and 3 athletes as described by McKay and colleagues (McKay et al., 2022). We did not have parameters that limited inclusion based on factors such as weekly mileage, race time qualifications, or VO_2max cut offs. While the study does include endurance trained individuals, the event specific training of our participants varied from 5k efforts up to ultramarathoners. This could have impacted the variance in durability between our participants. Training volume also greatly varied between our participants. Participants were asked to maintain normal training outside of the research study and to record

their training during the intervention period of the study. Training volume is highly correlative to durability and may be an important screening criteria for future durability research studies (Zanini, Folland, & Blagrove, 2025a). Future work should consider having a similar event-specific pool of athletes and designing the length of the TTE to that event for more consistent results and better applicability.

Another important consideration is the protocol and durability test administered. This is the first study to use the zero-slope protocol before and after fatiguing exercise. The goal of our protocol was to assess decrements in critical speed based on the estimations of muscle oxygenation using NIRs sensors. We saw ~6% decrements in critical speed during a durability test which proved to be meaningful reductions in performance capacity. Recent work on the reliability of zero-slope estimates highlights meaningful change as >3% (Hudgins et al., 2025) highlighting the viability of using NIRs in future durability testing efforts. Our fatiguing exercise protocol (105% estimated critical speed) was chosen to elicit fatigue at close to a 5,000 meter effort. Thus, our durability protocol was shorter in length than previous investigations (TTE: ~21 minutes) but had an overall running time of 90+ minutes. A longer and lower relative intensity TTE may produce larger drops in critical speed (Hunter et al., 2025). However, performance fatigue at shorter length exercise bouts (e.g. 5,000m efforts) and longer exercise bouts (e.g. marathon) should both be studied to understand more about the interplay of intensity and fatigue when quantifying durability. Durability protocols should be titrated to specific race length events or intervals to improve their validity to race day outcomes. We believe additional consideration should be taken for shorter durability based events and how to address, quantify and define them (Lucernoni et al., 2025; Meixner et al., 2025).

CONCLUSION

Mixed method intermittent heat acclimation was successful in producing heat acclimation phenotypes and indicated heat preparedness in female athletes. Durability, as defined in this research study as % maintenance of critical speed did not statistically improve with heat acclimation, but raw critical speed estimates were improved after fatiguing exercise following heat acclimation. Our results indicate a trending improvement in durability after a 3-week heat acclimation protocol. Future studies should continue to elucidate the mechanistic underpinnings of durability and utilize ecologically valid heat acclimation protocols for athletes.

CHAPTER VI

EFFECT OF HEAT ACCLIMATION ON IRON REGULATION IN FEMALE ATHELTES

INTRODUCTION

Iron absorption is tightly regulated as there is no direct excretory pathway. Hepcidin, a peptide endocrine hormone, is a key iron regulator. The primary function of hepcidin is to block absorption of iron in the intestines by binding to ferroportin channels on the basolateral side of enterocytes (Nemeth, Tuttle, et al., 2004). Hepcidin is elevated as a part of the acute phase immune response post-exercise and is triggered predominately by muscle derived Interluekin-6 (IL-6). IL-6 is an important part of the inflammation cascade that acts to produce and release hepcidin (Nemeth, Rivera, et al., 2004; Nemeth & Ganz, 2021). It responds in a dose-dependent manner following varying intensities of cycling and running (Sim et al., 2013). Hepcidin levels can remain elevated for more than 24 hours (Ishibashi et al., 2022). Thus, athletes who train frequently are likely seeing chronic elevations in hepcidin (Ishibashi et al., 2022; Peeling et al., 2009a). Chronically elevated hepcidin has been targeted as the main cause of iron deficiency in athletes (Peeling, 2022; Peeling et al., 2008). Therefore, strategies are needed that may mitigate the rise in hepcidin for athletes.

One potential mitigation strategy that has been explored is the application of environmental stressors. Badenhorst and colleagues exposed male participants to normobaric hypoxia after completing high intensity intervals resulting in successful -1.98 nM mitigation of hepcidin concentration (Badenhorst et al., 2014). Hypoxia activates hypoxia inducible factor pathways leading to the production of erythropoietin, both of which are modulators of hepcidin

(Pak et al., 2006; Peyssonnaux et al., 2008). Heat exposure may be another potential strategy, similar to hypoxia, that could mitigate hepcidin concentration post-exercise.

Heat exposure may evoke a similar reduction in post exercise hepcidin due to the relative hypoxic-like effects of heating. Heat exposure can result in a redistribution of blood flow that may challenge oxygen delivery during exercise or reduced oxygen tension at the level of the kidney. Acute bouts of passive heating result in transient rises in erythropoietin (DiMarco et al., 2024). Recent research has demonstrated the hemopoietic potential of prolonged (at least 5 weeks) heat acclimation leading to elevated levels of erythropoietin and hemoglobin mass (Cubel et al., 2024; Oberholzer et al., 2019; Rønnestad et al., 2021). Repeated heating and exercise bouts may lead to improved iron absorption and utilization to support improvements in hemoglobin mass.

Most of the current understanding regarding iron regulatory biomarkers post exercise is based on all male or mixed sex cohorts (McKay et al., 2021; Peeling et al., 2009b, 2009a, 2017). Although these studies were the first to demonstrate the effect of exercise on iron regulation, it is still unclear how heat acclimation may improve iron regulation in a cohort of only females. Female athletes at all levels, but especially highly competitive athletes, frequently experience iron deficiencies (Cowell et al., 2003). There is a need to target specific interventions towards the female athletes to address issues pertinent to them. The purpose of this research is to investigate heat acclimation as a potential modulator of iron regulation in female athletes. We hypothesize that female runners who have undergone three weeks of heat acclimation will have reduction in hepcidin concentration.

METHODS

Participants. This study was approved by the Institutional Review Board at the University of Oregon and conformed to the principles of the Declaration of Helsinki except for registration in a database. Twenty-four healthy endurance trained female athletes participated in this research study (Table 6.1). Participants provided written informed consent prior to research activities. Inclusion into the study was limited to Tier 2 and Tier 3 endurance trained athletes as described by McKay and colleagues (McKay et al., 2022). Most (n = 19) participants reported normal, ~28 day menstrual cycles and about half reported hormonal contraceptive usage (n = 7 oral contraceptives, n = 6 intrauterine devices). We recorded contraceptive type and use and menstrual cycle phase across participants, and we did not find any systematic impacts on our data or findings.

Table 6.1. Participant Anthropometrics.

Variable	Control (*n* = 12)	Heat (n = 12)
Age (y)	29 ± 5	25 ± 5
Height (m)	1.7 ± 0.04	1.7 ± 0.04
Body mass (kg)	61.9 ± 8.6	65.0 ± 7.4
Body mass index (kg m^{-2})	22.6 ± 2.7	22.8 ± 2.1
VO_{2max} (ml kg^{-1} min^{-1})	52.9 ± 6.3	52.9 ± 5.6
Running Speed @ 70% max (km·h^{-1})	9.7 ± 2.2	9.5 ± 1.8

Participant characteristics by group. Values are means (± SD).

Protocol Overview. This study was part of a larger project examining heat acclimation for performance benefits. After consent, participants completed two experimental visits, an intervention period (3 weeks), then one final experimental visit. For experimental visits,

participants were asked to abstain from eating or drinking 2 hours prior to the visit and vigorous exercise, supplements, alcohol and recreational drugs for 24 hours prior to the testing visit.

Visit 1: Graded Exercise Test. A graded exercise test was administered to assess VO2max. Participants were asked to follow the abstentions of the experimental visits. Speed was increased on a treadmill by 1 $km \cdot h^{-1}$ every three minutes with grade kept constant at 1%. Expired gases were collected and analyzed via indirect calorimetry (Parvo Medics, True One 2400) for oxygen uptake (VO_2), carbon dioxide production (VCO_2), and Ventilation (VE). The test ended when the participant reached volitional exhaustion. Participants were given a 10-minute break before completing a supramaximal verification. Participants completed another trial to volitional exhaustion set to 110% of the final speed achieved during the graded exercise test (Poole & Jones, 2017). VO_2max was determined at the highest 30 second average during the ramp protocol. Results of the VO_2max test were used to block randomize participants into the control or heat group for the intervention period.

Visit 2: Pre Heat Tolerance Test. Participants were asked to ingest a core temperature pill (e-Celsius performance pill, BodyCap) at least 4 hours prior to the experimental session and provide at 24-hour dietary recall prior to the start of the visit. When participants arrived at the laboratory, they provided a urine sample to ensure euhydration (urine specific gravity > 1.020; Atago; Master-URC/NM) and a nude body weight. If urine specific gravity was greater than 1.020, participants were given 5 mL water per kg body weight prior to exercise as no fluid intake was allowed during the test. Participants provided a blood sample prior to exercise. Participants exercised in an environmental chamber set to 38°C and 40% relative humidity for 30 minutes at

the speed associated with 70% of VO_2max. Perceptual scales were given throughout the trial. Heart rate was measured via chest strap heart rate monitor (Model H10, Polar Electro). If a participant's body core temperature exceeded 39.5°C before the end of the test, then the test was ended (n = 1 at minute 26). Immediately after exercise, an intravenous catheter was placed, and a blood sample was collected. Participants then provided a second nude weight to calculate total body water loss. Blood sampling continued once every hour for three hours after exercise.

Intervention. The intervention period consisted of three weeks. Participants were block-randomized into the control or heat acclimation group based on VO_2max results.

Heat Group. Participants completed four heating sessions per week for three weeks (12 visits total). Heating sessions included two active running sessions in an environmental chamber and two passive hot water immersion sessions weekly. Active heating sessions were 30 minutes of running at 70% VO_2max starting at 38°C and 40% relative humidity. Each week the active heating sessions progressed to 40°C and 40% relative humidity, ending with 42°C and 40% relative humidity. Passive sessions consisted of 30 minutes of hot water immersion to neck-deep submersion in 41°C water. Weekly, the ordering of the visits was at the discretion of the participants. Participants were encouraged to do passive heating on days with higher training volumes.

Control Group. Participants completed two visits per week for three weeks (six visits total). Participants ran on a treadmill at room temperature with a fan on for 30 minutes at 70% VO_2max.

Visit 3: Post Heat Tolerance Test. Participants repeated the heat tolerance test identical to Visit 2. Participants were asked to match their dietary intake 24 hours prior to the experimental visits and reported to the lab at the same time of day as the initial heat tolerance test to minimize circadian influence.

Blood Analysis. Iron status (serum iron, transferrin, ferritin) blood analyses were conducted by a clinical laboratory (Quest Diagnostics). Interluekin-6 and hepcidin were analyzed using enzyme linked immunosorbent assays (Quantikine HS ELISA Human IL-6 (SS600C), Quantikine HS ELISA Human Hepcidin (DHP250); respectively). Assay samples were conducted in duplicate for all subjects at all time points. One IL-6 sample is missing from the data set due to being over the limit of detection of the assay.

Statistical Analysis. Values are expressed as means (95% Confidence Intervals) unless stated otherwise. Alpha was set to 0.05 for all statistical inferences, including familywise error rates. Fixed effects were time (before and after intervention), timepoint (baseline, 0h, 1h, 2h, and 3h post exercise) and group (control and heat). Inferences were drawn from mixed-effects models for measurements across timepoints, before and after the intervention and between groups. (Prism 10, GraphPad, Boston, MA, USA). Inferences regarding changes over time within each group were drawn from Dunnett's multiple comparison test versus baseline, with reported p-values and confidence intervals adjusted for multiplicity. Inferences regarding the difference between pre and post intervention within each group were drawn from Šidák's multiple comparisons test, restricted to comparisons at the same timepoint between groups. Inferences

regarding the difference between groups were drawn from Šidák's multiple comparisons test, restricted to comparisons at the same timepoint between group.

Reproducibility and Rigor An a prioi sample size calculation was made using the expected variation and meaningful change in hepcidin concentration based on the findings of previous research (Badenhorst et al., 2014). Using an anticipated meaningful change of 2 nM in mean concentration, reported standard deviation of hepcidin from Badenhorst and colleagues (SD = 0.82 pg/mL), an α of 0.05, and β of 0.15 (power = 0.85) we calculated the necessary sample size to be 24 participants in a continuous endpoint, two independent sample groups study.

The largest limitation of the current investigation surrounds menstrual cycle and oral contraceptive use of our participants. Since the aim of the project was not to assess how the menstrual cycle effects hepcidin we did not stagger enrollment to align menstrual cycle phase within the study. Previous research suggests that menstrual cycle does not impact hepcidin concentrations (Alfaro-Magallanes et al., 2021). While we did not stagger participants based on cycle, heat tolerance tests were 4 weeks apart. Nineteen participants self-reported normal 28-day menstrual cycles (n = 10 control group; n = 9 heat group), so most participants were in the same phase for both testing periods. Additionally, we had participants self-report menstrual cycle status and did not see any systematic effects of menstrual cycle phase on our data. We did not limit inclusion into the study based on contraceptive use as oral contraceptive use does not seem to impact hepcidin or IL-6 concentrations post exercise (Sim et al., 2015). Additionally, we did not exclude participants based on low iron status to improve the ecological validity of our assessment. Participants were asked to provide a 24-hour dietary recall prior to the pre intervention heat tolerance test and asked to match their food intake for the post intervention heat

tolerance test to ensure similar carbohydrate and micronutrient intake prior to testing. Heat tolerance tests occurred at the same time of day to minimize the influence of circadian rhythm.

RESULTS

Heat Acclimation.

Body Core Temperature. Peak body core temperatures were similar pre intervention between the control and heat groups ($p = 0.84$). After the intervention, peak body core temperatures were lower in the heat group compared to before intervention (-0.3 °C; $p < 0.01$), but similar in the control group (-0.1°C; $p = 0.47$; Table 6.2). There were no differences after the intervention between the control and heat group ($p = 0.59$).

Sweat Loss. Sweat loss was similar pre intervention between the control and heat groups ($p = 0.75$). After the intervention, sweat loss was greater in the heat group compared to before intervention (+113 mL; $p < 0.01$), but similar in the control group (+9 mL; $p = 0.91$; Table 6.2). There were no differences after the intervention between the control and heat group ($p = 0.06$).

Heart Rate. End exercise heart rate was similar pre intervention between the control and heat groups ($p = 0.38$). After the intervention, heart rate was lower in the heat group compared to before intervention (-6 beats per minute; $p = 0.03$), but similar in the control group (-3 beats per minute; $p = 0.40$; Table 6.2). There were no differences after the intervention between the control and heat group ($p = 0.78$).

Perceptual Scales. End rating of perceived exertion (RPE) was similar between groups before ($p = 0.80$) and after the intervention ($p = 0.07$; Table 6.2). The control and heat group had similar end ratings of perceived exertion from before to after the intervention ($p > 0.99$, $p = 0.18$; respectively). End exercise thermal perception was similar before the intervention for both groups ($p = 0.69$). However, thermal perception was lower after the intervention in the heat group compared to pre intervention ($p = 0.02$) and was lower than the control group after the intervention ($p = 0.02$; Table 6.2).

Table 6.2. Heat acclimation characteristics before and after the intervention for both groups. * Denotes a significant difference from pre intervention within the same group. ** Denotes a significant difference from the control group at the same time point.

Variable and timepoint	Control (*n* = 12)		Heat (*n* = 12)	
	Pre	Post	Pre	Post
End exercise Body Core Temperature (°C)	38.9 (38.6, 39.2)	38.8 (38.6, 38.9)	38.9 (38.7, 39.2)	38.6 (38.5, 38.8) *
End exercise Heart Rate (beats per min)	177 (170, 185)	174 (167, 182)	184 (176, 191)	177 (169, 186) *
Sweat Loss (L)	0.7 (0.6, 0.7)	0.6 (0.6, 0.8)	0.7 (0.6, 0.8)	0.8 (0.7, 0.9) *
End exercise rating of perceived exertion	13.8 (11.9, 15.7)	13.9 (12.7, 15.1)	13.3 (12.1, 14.3)	11.8 (10.1, 13.6)
End exercise thermal perception	6.4 (5.3, 7.6)	6.8 (5.7, 7.9)	5.8 (4.5, 7.1)	4.8 (3.7, 5.0) *, **

Values are means (95% confidence limits).

Iron Regulation.

Iron Status. Serum iron, transferrin and ferritin were stable within and between groups before and after the intervention period (Table 6.3).

Table 6.3. Serum iron, transferrin and ferritin of participants immediately prior to the heat tolerance tests before and after the intervention.

Variable and timepoint	Control (*n* = 12)		Heat (*n* = 12)	
	Pre	Post	Pre	Post
Serum Iron (mcg/dL)	112.8 (95.2, 130.3)	110.8 (78.5, 143.0)	111.4 (89.5, 133.3)	108.8 (86.4, 131.2)
Serum Transferrin (mg/dL)	286.1 (254.8, 317.3)	288.6 (252.8, 324.4)	302.8 (281.6, 324.1)	308.3 (289.6, 327.1)
Serum Ferritin (ng/mL)	33.0 (25.5, 40.5)	32.3 (22.4, 42.1)	29.3 (22.4, 36.3)	26.6 (18.5, 34.7)

Values are means (95% confidence limits).

Interluekin-6. Interluekin-6 was similar at baseline in both groups, before ($p > 0.99$) and after ($p > 0.99$) the intervention. The rise in IL-6 post exercise was similar in both groups immmediately post exercise, 1 hour, 2 hour and 3 hours post exercise, peaking similarly at 3 hours post exercise before ($p > 0.99$) and after ($p = 0.42$) the intervention between the control and heat groups (Figure 6.1).

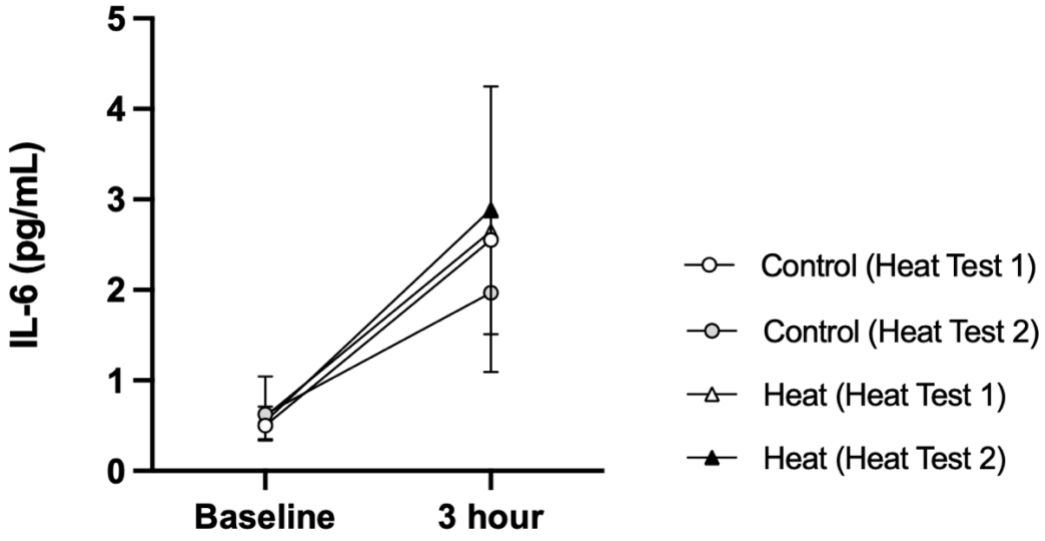


Figure 6.1. Interleukin-6 concentration (pg/mL) at baseline, pre heat tolerance test and 3 hours post heat tolerance test in the control (circles) and heat group (triangles).

Hepcidin. Hepcidin was similar at baseline in both groups, before (p = 0.26) and after (p = 0.71) the intervention. The rise in hepcidin post exercise was similar in both groups immmediately post exercise, 1 hour, 2 hour and 3 hours post exercise, peaking similarly at 3 hours post exercise before (p = 0.66) and after (p = 0.67) the intervention between the control and heat groups (Figure 6.2).

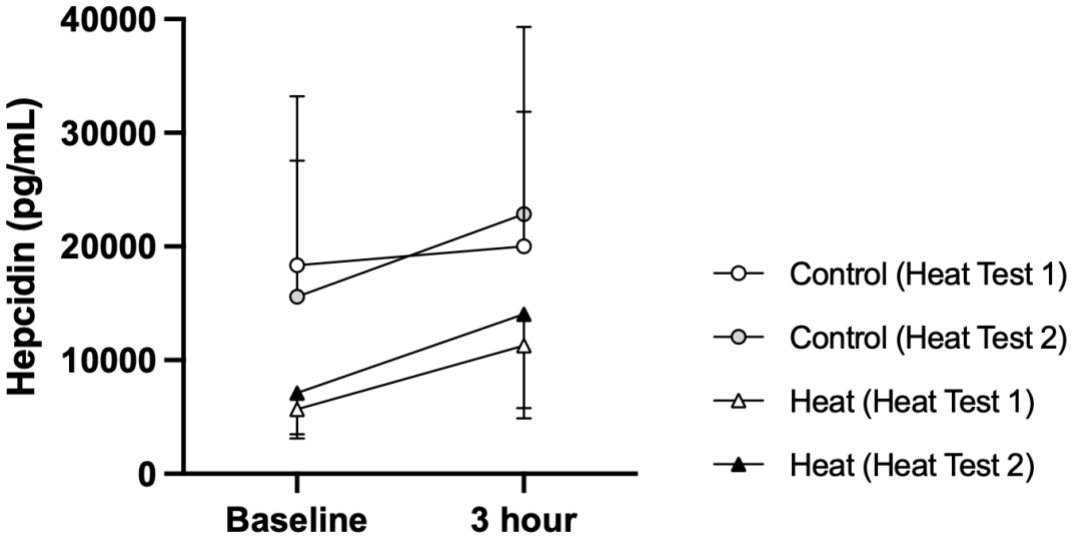


Figure 6.2. Hepcidin concentration (pg/mL) at baseline, pre heat tolerance test and 3 hours post heat tolerance test in the control (circles) and heat group (triangles).

DISCUSSION

The purpose of the research study was to explore if heat acclimation can dimmish post exercise IL-6 and hepcidin concentrations and improve overall iron status in endurance trained female runners. Our previous investigations on acute heat exposure and hepcidin demonstrated exacerbated release of hepcidin and IL-6 post exercise (Chapter IV). We hypothesized that heat acclimation would lead to mitigated hepcidin release and improved iron status after heat acclimation via mechanisms of reduced IL-6 release post exercise and potential hemopoietic effects of longer heat acclimation protocols. The results of the study indicate that heat acclimation did not result in changes to iron regulation as hepcidin and IL-6 were similarly elevated pre and post intervention in both groups over the 3-hour post exercise measuring period.

Heat & Biomarkers

Our intervention was successful in producing hallmark adaptations of heat acclimation (reduced end exercise body core temperature, reduced end exercise heart rate and increased sweat loss). However, the overall amount of heat exposure (6 hours total over 3 weeks) was low compared to other protocols (McDonald et al., 2025). Minimal heat exposure time can be advantageous as it reduces athlete burden, but disadvantageous if the heat exposure was not robust enough to modulate other factors. There is a need to balance the potency of the heat acclimation protocol while mitigating additional stressors placed on a training athlete. Our heat acclimation intervention was successful in producing a maintainable heat acclimated phenotype, but this did not translate to improvements in iron regulation. Interluekin-6 and hepcidin concentrations were unaffected by heat acclimation. There is conflicting literature on the ability

of heat acclimation to reduce inflammatory responses (Costello et al., 2018; B. J. Lee & Thake, 2017). Not all heat acclimation protocols result in lowering inflammation. Previous research has demonstrated that non-heat interventions that successfully modulated IL-6 resulted in reduced hepcidin concentration (Badenhorst et al., 2014, 2015). One other study has observed heat acclimation as a potential modulator of iron regulation in males (Sumi et al., 2022). Sumi and colleagues performed a 10-day consecutive active heating protocol consisting of 60 minutes of running at 60% VO_2max at 35 °C and 50% relative humidity. Post intervention, there were hallmark signals of heat acclimation, but there was no change in hepcidin concentration post exercise in hot environmental conditions. The male cohort had high iron status (average ferritin ~100 ng/mL), and the authors suggest that the intervention would be effective if targeting individuals with low iron status and a more robust heating protocol. While the current investigation addresses these points via a predominately low iron status cohort with a longer-term heating protocol, there was no modulation of post exercise iron regulation after heat acclimation. Alternatively, increases in overall training volume in both groups may have resulted in blunted IL-6 and hepcidin release, as previous studies have demonstrated that chronic training reduces the post exercise IL-6 responses (Nash et al., 2023).

Heat & Iron Cross Talk

Heat exposure as an acute and chronic stimulus have overlapping mechanistic pathways as iron regulation. In light of the growing interest in prolonged heat acclimation interventions and their reported effects on hemoglobin mass, greater attention should be given to iron balance, particularly within athletic populations. The cross talk between heat and iron regulation is an under-researched area, where there are many potential mechanisms that interact that may be

leveraged to improve the viability of interventions to serve as both performance and health benefits.

Hemoglobin mass gains after prolonged heat acclimation are of great interest, however the mechanisms remain uncertain . The current hypotheses center around acute blood volume contractions followed by chronic expansion, resulting in erythropoietic stimulus, or activations of hypoxia inducible factors. Both stimuli are thought to be sensed at the kidney and result in chronic elevations of erythropoietin, resulting in hemoglobin mass expansion. It has been purported that distribution of blood flow during heat stress and expression of heat shock proteins can stabilize hypoxia inducible factor (HIF) pathway (Ely et al., 2014; Li et al., 2025; Maloyan et al., 2005). HIF1α is stabilized during conditions of hypoxia resulting in the modulator of protein expression, resulting in hematopoietic signals in the kidneys and the production of erythropoietin (Semenza, 2023). If a heating protocol is prolonged, chronic elevations in erythropoietin can result in red blood cell development and increases in hemoglobin mass (Oberholzer et al., 2019). Erythropoietin (and its precursor erythroferrone) are inhibitors of hepcidin release (Pak et al., 2006). This mechanism is in place to increase dietary absorption of iron during a period of red cell development. Additionally, the stabilization of the HIF pathway has downstream effects on cellular expression of ferritin and transferrin. The HIF pathways can activate iron response proteins and iron response elements that modulate the expression of transferrin and decrease the expression of ferritin (Galy et al., 2024). In a hypoxic state, increasing transferrin would increase total iron binding capacity in the blood for iron, and increased systemic availability of iron. We did not see these phenotypic expression changes in our iron status variables pre-to-post intervention in our heat group. In the present study, we saw a small, nonsignificant reduction in mean ferritin in the heat group, potentially pointing to the effects of heat as reducing stored iron

and utilizing it. Our heating stimulus may not have been long enough to see changes in iron regulation or iron status as the current work around hemoglobin mass changes with heat acclimation have been longer interventions (5 weeks) (Cubel et al., 2024; Oberholzer et al., 2019; Rønnestad et al., 2021). Prolonged heat acclimation warrants careful monitoring of iron status to evaluate potential risks associated with its implementation. It is possible that heat acclimation could result in worsened iron status if an athlete is unable to increase dietary iron absorption. This should be further explored as more athletes are seeking heat acclimation for competition. There is a need for further investigations to provide clear recommendations surrounding iron management for athletes undergoing prolonged heat acclimation, similar to what is recommended for altitude.

Iron Status & Sex differences

Most of the current research on iron regulation in exercising athletes has been conducted on predominately male cohorts. While this research provides us a foundational understanding of the implications of exercise on iron regulation; it remains unclear if this is similar in female athletes. Only a few studies have explored female only cohorts (Alfaro-Magallanes et al., 2021; Ishibashi et al., 2017, 2022; Sim et al., 2014). Females tend to struggle more with iron deficiency than male athletes and the magnitude of the hepcidin released post exercise by females is lower than previously shown in the male cohort studies (Chapter IV). This is likely due to sex differences in baseline ferritin, as ferritin is highly correlative to hepcidin release (Peeling et al., 2017). Females tend to have lower ferritin and therefore lower post exercise hepcidin production, but this is not well documented in the literature.

This study did not screen out for people of low iron status; therefore, the cohort of participants represent a more realistic reflection of the population of endurance athletes. Based on the criteria by Peeling and colleagues, most of our female athletes were iron deficient (serum ferritin < 30 ng/mL; n = 7 in control group, n = 8 in heat group; pre intervention) or sub-optimal iron status (serum ferritin = 30 – 50 ng/mL; n = 3 in control group, n = 4 in heat group; pre intervention) (Peeling et al., 2014). Serum ferritin values post intervention continued to follow a similar pattern. Iron deficiency and anemia are associated with attenuated hepcidin responses, suggesting that iron regulatory signals may override inflammatory stimuli—potentially mediated by low-grade hypoxia (Pagani et al., 2019). Given that several participants in this cohort presented with suboptimal or iron-deficient status, the lack of change in hepcidin concentration may be attributable to their overall low ferritin levels. In iron-deficient states, the physiological drive to enhance iron absorption and mobilization may suppress hepcidin release, even in the presence of exercise-induced inflammation. This may help explain why previous investigations conducted in male cohorts with higher baseline ferritin concentrations have been more successful in demonstrating modulation of post-exercise hepcidin responses (Badenhorst et al., 2014, 2015). Collectively, these findings underscore the importance of examining female specific physiology, as iron status and regulatory responses may differ substantially from those observed in male populations.

CONCLUSION

A three-week intermittent mixed method heat acclimation protocol was successful in heat acclimating endurance trained female athletes. However, heat acclimation did not lead to changes in iron status (ferritin, transferrin and serum iron) nor post exercise IL-6 and hepcidin

concentrations. Heat acclimation represents a potential mediator of iron status, particularly as its use becomes increasingly prevalent among training and competitive athletes. Future research should continue to evaluate female specific iron regulation and potential interventions that may mitigate hepcidin release post exercise.

CHAPTER VII

SYNTHESIS & FUTURE DIRECTIONS

REVIEW OF FINDINGS

The overall goal of the projects was to explore heat in both acute and chronic settings and contextualize these stimuli to iron regulation-- an issue pertinent to the female athlete. Running durability after heat acclimation was also assessed via a novel methodology and metric. Chapter IV addressed Aim 1 where we successfully characterized the inflammatory and iron regulatory response to an acute bout exercise in hot environmental conditions in female athletes. Our results indicate that acute heat exposure during exercise resulted in greater elevations in IL-6 and hepcidin release post exercise compared to a cool exercise condition. Chapter V addressed Aim 2 where we assessed the effects of an intermittent mixed method heat acclimation protocol on performance durability in endurance trained female athletes. Our results indicate that our novel heat acclimation protocol was successful in resulting in heat preparedness in female athletes by exhibiting hallmark adaptations to heat, and these benefits may extend to improved durability in thermoneutral conditions. Chapter VI addressed Aim 3 where we explored the use of the intermittent mixed method heat acclimation protocol to assess if it was a viable intervention to improve iron regulation post exercise and iron status in endurance trained female athletes. Our results indicate our novel heat acclimation protocol was unsuccessful in modulating the post exercise IL-6 and hepcidin responses to exercise in hot environmental conditions.

POTENTIAL APPLICATIONS

Many aspects of these aims were novel, and the project summary above highlights an applied and integrative approach to leveraging heat exposure for both performance enhancement

and health optimization in female athletes. Collectively, these investigations provide new insights and methodological approaches that advance the field and hopefully stimulate future research in areas we believe represent meaningful and impactful directions.

Novel Heating Protocol

Given the history of heat acclimation and roots in occupational safety, it is important that researchers move away from these traditional consecutive day heating models when developing a heat acclimation protocol for training and competing athletes. Traditional protocols require athletes to make changes to their training volume or intensity to incorporate heating. Moving towards intermittent, mixed method, or fully passive heating protocols may be more advantageous for training and competing athletes.

The decision to develop a mixed-method intermittent protocol over the span of three weeks arose from the idea to make a protocol specifically geared towards allowing athletes to continue normal training during the acclimation period. Active and passive heating visits allowed the athletes to disperse heating stimuli at their discretion and match passive heating visits with higher effort training days (Pryor et al., 2019). Our heat acclimation protocol resulted in meaningful heat preparedness, with similar magnitudes of adaptation to more robust, consecutive day heating protocols. The novel heating protocol's overall heat exposure was modest compared to previous investigations and breaks away from the mold of "traditional" heat acclimation protocols. While this protocol was not designed to investigate the minimal effective dose, compared to a recent review, our protocol resulted in similar heat adaptation in half the amount of heat exposure time as continuous heat acclimation studies (McDonald et al., 2025).

Despite our protocol being effective for heat preparedness, these benefits did not extend to changes in iron regulation, and it is unclear if durability was effectively altered. While the

protocol has strengths for heat preparedness by minimizing athlete burden, the results did not translate to all of the cellular adaptations and phenotypic expressions we hypothesized. Heat acclimation is a coordinated effort between local cellular and systemic adaptations but exploring the cellular and systemic differences between continuous and intermittent heating strategies may provide important insight in future investigations. While both approaches are valid for heat preparedness, variations in protocol frequency could be leveraged to target distinct goals. Intermittent heating may be sufficient to enhance heat preparedness, whereas meaningful cellular adaptations may require more consistent, novel daily thermal stimuli to drive cellular adaptations. Local cellular responses to heat are mediated by transient cellular signaling, meaning adaptation may occur on shorter timelines, but depend on repeated exposure. While systemic adaptations may tolerate longer intervals between stimuli, these adaptations are driven predominately through hormonal pathways (i.e. aldosterone, EPO). This proposed phenomenon raises an interesting question about the possibility of the same cumulative heat load distributed differently over time resulting in divergent adaptations. While previous investigations have explored systemic acclimation differences between continuous and intermittent heating protocols (Fein et al., 1975; N. Gill & Sleivert, 2001; Sunderland et al., 2008), differences between cellular mechanisms and systemic mechanisms remain to be elucidated clearly.

Another interesting consideration is the use of a mixed-method passive and active heating protocol. We have previously determined that common passive heating modalities exert different magnitudes of acute physiological stress (Atencio et al., 2025), suggesting these differences across heating modalities may manifest as distinct adaptation profiles. Despite this, a growing body of work suggests similar systemic adaptations regardless of common passive (hot water immersion or traditional sauna) and active heating modalities in diverse environments. For

example, passive heating has been shown to be as effective as active heating (McIntyre et al., 2021). Similarly, different forms of active heating (dry versus humid active heat acclimation) produced comparable heat adaptation and cross-adaptation in both interventions (McDonald et al., 2026).

While different heating stimuli may ultimately produce similar adaptations, a recent meta-analysis suggests there remains uncertainty regarding the viability of passive heating compared to active heating intervention (Solomon & Laye, 2025). Although previous investigations indicate overall similarity between interventions, uncertainty persists regarding whether one modality may be more effective than another. Given the remaining uncertainty, combining passive and active heating strategies may produce more robust acclimation if meaningful differences between modalities are identified. Future investigations should continue comparing heating modalities under equivalent prescribed heat loads to determine whether distinct approaches elicit unique physiological adaptations.

Current investigations are moving towards fully passive heating protocols for athletes due to practical and economic limitations (Heathcote et al., 2018), despite mixed results of the viability compared to active acclimation (Solomon & Laye, 2025). Passive protocols may be advantageous from the standpoint of mitigated additional stress, easy implementation, and adherence (Ashworth et al., 2023; Zurawlew et al., 2018, 2018). However, it is still unclear if there are additional benefits to be derived from active heating specifically. Active heating may be advantageous not only as a very potent heating stimulus due to the combination of stressors from exercise and heat, but also from a psychological or neural adjustment. Hyperthermia acutely impairs maximal voluntary contraction associated with a reduction in voluntary activation and onset of central fatigue (Nybo & Nielsen, 2001). However both central and

peripheral adaptations to heat may preserve central motor output during prolonged exercise in hot ambient conditions by delaying the onset of thermally mediated central fatigue (Armstrong & Stoppani, 2002; Racinais, Wilson, Gaoua, et al., 2017). Further, heat acclimation has been proposed to mitigate the deleterious effects of psychological impact of heat on mood, motivation and performance (Acevedo et al., 2025; Donnan et al., 2023; Naito et al., 2024). It remains unclear whether these benefits are specific to particular types of heat acclimation or apply broadly across all acclimation strategies. From an applied perspective, athletes may therefore be encouraged to perform acclimation protocols that more closely replicate the environmental conditions and exercise modality of competition, allowing these stressors to be strategically integrated into training and preparation. Additionally, the extent to which athletes may benefit from sport-specific training performed in heated conditions is not well understood. Although these effects are difficult to quantify, they would provide meaningful insights and help guide recommendations for athlete-specific heating protocols.

Heat and Iron Cross Talk

There is a dearth of information about the overlapping mechanisms of heat acclimation and iron regulation. Further, there is a lack of information regarding female athlete iron regulation. With the growing popularity of heat acclimation for performance benefit, there is a need to understand the potential implications on iron regulation. A small, but growing body of research has demonstrated increases in hemoglobin mass to prolonged heat acclimation (Cubel et al., 2024; Lundby et al., 2023; Oberholzer et al., 2019; Rønnestad et al., 2021). While these investigations purport different mechanisms for the elevation in hemoglobin mass that stem from

heat acclimation, all authors agree that heat stimulus results in a hypoxic state. Plasma volume contraction (acutely) or expansion (chronically) and stabilization of hypoxia inducible factor (HIF) pathways linked to heat acclimation are hypothesized to result in the production erythropoietin (EPO) (Jenkins et al., 2025).

The predominant hypotheses surrounding increases in hemoglobin mass rely on the generation of a hypoxic stimulus and subsequent EPO release (Semenza, 2023). Prolonged heat acclimation protocols may implicate iron status and raise important considerations regarding iron regulation. Iron availability is essential for erythropoiesis, and hypoxia exerts well-established effects on iron metabolism through mechanisms involving iron regulatory proteins, iron response elements, and suppression of hepcidin (Galy et al., 2024; Peyssonnaux et al., 2008). Together these manifest as increased total iron bunding capacity in circulation, minimized iron storage, and improved dietary iron absorption capabilities (Haase, 2010).

However, the interaction of heat and hypoxia exposure adds complexity. Acute heat stress in females appears to transiently exacerbate inflammatory signaling and elevate hepcidin, which could impair iron availability in female athletes (Chapter IV). Yet with chronic heat acclimation, at least over a three-week period, this negative effect does not appear to persist (Chapter VI). (Importantly, differences in baseline iron status between our two studies limit the direct translatability of findings and should be considered when interpreting these results). It is possible that, over time, the erythropoietic drive associated with a hypoxia stimulus derived from heat exposure begins to outweigh the acute inflammatory response to heat. This may reflect a classic stress–adaptation process, though the specific mechanisms remain unclear and warrant further investigation.

Notably, there are currently no heat acclimation studies extending beyond three weeks that comprehensively assess iron regulation and iron status. Prolonged interventions may result in changes to iron regulation, particularly if the balance between hematopoietic stimuli and heat-induced inflammation shifts over time. If an athlete is unable to absorb adequate dietary iron due to training and/or heat exposure, it is possible that prolonged heating may result in iron deficiency or increased iron demand as seen in altitude research (Koivisto-Mørk et al., 2021; Nolte et al., 2025). Future investigations should consider monitoring iron status or add iron supplementation during prolonged heating investigations. These findings may provide insight into equipping athletes with clear recommendations surrounding iron management while undergoing prolonged heat acclimation.

Further, there is still inadequate information about the differences in iron regulation and iron status between males and females. In the current investigations, serum ferritin is markedly lower than previous male dominant cohorts or male only cohorts and plays a critical role on responses to interventions. Interventions that may improve iron handling in female athletes are of great interest. The use of normobaric hypoxia post exercise has been shown to be effective in reducing hepcidin concentrations post exercise in males (Badenhorst et al., 2014), but this has yet to be demonstrated in a female cohort. Additionally, other hypoxia inducing methods post exercise may lead to similar reductions in hepcidin. Unexplored avenues such as post-exercise carbon monoxide inhalation (hypoxemia) or hot water immersion (reduced oxygen tension at the level of the kidney) could potentially modulate iron regulation. Both stimuli could introduce sufficient stimuli to attenuate exercise-induced increases in hepcidin while simultaneously promoting performance adaptations. We have previously demonstrated that carbon monoxide exposure and hot water immersion, independently or in combination, elicit comparable acute

elevations in erythropoietin (EPO) in females (DiMarco et al., 2023), which may contribute to transient suppression of hepcidin (Pak et al., 2006). Notably, in the present investigation, females exhibited a greater erythropoietic response to these stimuli, suggesting potential sex-specific adaptations that warrant further exploration. Future research should prioritize both performance enhancement and athlete health and well-being. Efforts to optimize performance should not outpace foundational considerations such as iron status and overall physiological resilience, particularly when modifiable factors remain unaddressed.

Novel Durability Methods

To our knowledge, this is the first study to use the zero-slope protocol twice in one testing session to assess durability. The zero-slope method has been validated for delineating sustainable from unsustainable exercise efforts across running and cycling modalities (Forot et al., 2026; Hudgins et al., 2025; Matthews et al., 2023). While this method has been used for estimating critical speed, this new application of the zero-slope protocol highlights the ability of the method to assess nuance dynamics changes in maximal metabolic steady state within a single exercise bout, but may face challenges when utilizing this method over the course of an intervention, or between day comparison trials. The use of the NIRs devices and the zero-slope protocol provides some distinct advantages compared to other methods used to assess durability.

The largest advantage of using zero-slope protocol is that it can be used in a lab setting on a treadmill in a single testing session. Other methods to quantify critical speed require multiple trials or multiple days, a self-propelling treadmill or outdoor track/GPS – all of which must be maximal exercising efforts by participants. Multiple day time-trials make it impossible to assess the impact of fatigue over the course of one running session. Additionally, all

quantifications of critical speed rely heavily on participant motivation and the participant's ability to give an all-out effort without subconscious pacing strategies. While all out efforts may be less of an issue in the elite and sub elite categories, it will become an issue when studying recreationally active individuals. The zero-slope protocol minimizes motivation from this assessment and relies on running at four submaximal stages, providing an estimate of critical speed in approximately 20 minutes. Assessing durability of critical power on a bike has been done before, but it is logistically easier with 3 minute all out trials on a cycle ergometer. To our knowledge, this is the first study to quantify the durability of critical speed in runners.

To better understand the reliability of our durability test, and given that it has not been previously implemented, we conducted a post hoc analysis to assess its repeatability after data collection. Data from the durability tests of the control group were used before and after the intervention to assess how repeatable the durability test was. It is important to note that the control group functioned as a sham condition, as participants increased their training volume and completed an intervention period (i.e. they were not a true time control group). A small bias of -0.56% exists from the first to second durability test, and all observations fell within the repeatability limit (11.3 %; Figure 7.1). These data suggest that while durability was assessed accurately, the precision of the durability metric may be limited. This may be a fundamental characteristic for all durability testing as this metric has already been determined to be highly variable. The relatively large repeatability limit may explain some of the difficulty in reaching statistical significance in Chapter V, as the expected changes (~6%) are smaller than the determined repeatability limit. Consequently, the measurement variability may obscure small but potentially meaningful physiological changes. Future work aiming to apply the zero-slope protocol in this manner should further validate the protocol by incorporating a true time-control

condition and conducting multiple trials within the same individuals to better characterize measurement variability. We believe this application of the zero-slope protocol has merit but would benefit from more rigorous validation testing before broader implementation in durability research. Despite limitations in precision, the test appears to provide reasonable assessments of durability and offers valuable insight into the nuanced, dynamic changes in muscle oxygenation during fatigue as well as the associated systemic responses.

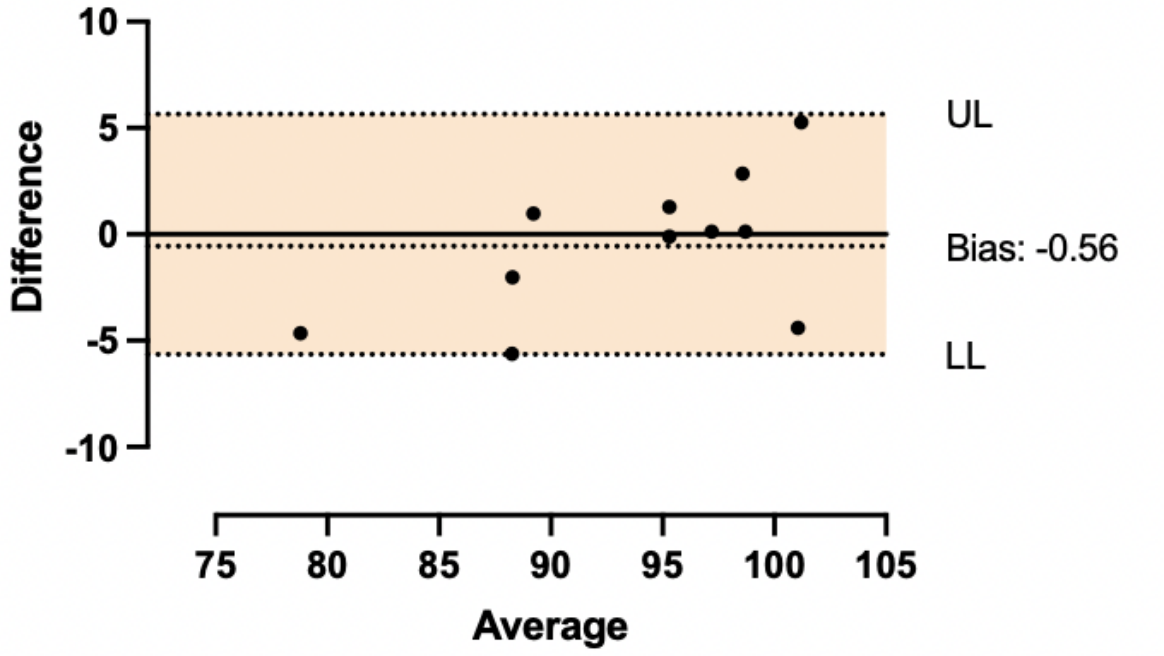


Figure 7.1. Bland-Altman plot showing the agreement between repeated measurements of durability (% maintenance) in the control group (functioning as a sham group) before and after the intervention. The difference between trials is plotted against the mean of the two measurements. The dashed lines represent the mean bias, as well as the upper and lower repeatability limit.

Aside from methodological concerns, we provide several recommendations for this method and durability trials in general. The durability test should be honed to a specific type of athlete, and screening protocols should attempt to screen based on event specific runners, event

specific performance (time ranges), and weekly training volume. We believe these screening questions are a few of the key factors that introduce a lot of the variability we saw in our data. However, this variability may be inherent to the durability metrics as previous investigations have also noted large variability in durability (3 to -32%) (Clark et al., 2018), which we also saw in our data (5 to -23%). In the honing of the durability test to a specific athlete, the TTE can be titrated to the target population. Choosing a TTE that reflects a specific race length or intensity would provide meaningful application and results for both the researcher and athlete. For example, a 5000m compared to a marathon runner would train and compete differently, likely resulting in very different intensity and duration testing metrics. Differing intensity and duration TTE trials during the durability test warrants further investigation, longer TTE trials may result in larger decrements in estimated critical speed and therefore provide clearer results, as a more potent reduction in critical speed may minimize the amount of noise in the sample.

Interestingly, compared to Clark and colleagues, we observed similar reductions in critical power despite markedly different trial duration and intensity (2 hours of heavy-domain cycling reduction; versus ~20 min of severe-domain running) (Clark et al., 2018). This highlights the complexity of durability, as both forms of fatiguing exercise appear to capture distinct aspects of durability that warrant further defining, investigating, and characterizing (Lucernoni et al., 2025; Meixner et al., 2025). Together, these observations suggest durability may manifest similarly in distinctly different exercise intensities and durations that warrant future investigations.

CONCLUSION

These findings support the complexity of heat exposure showing the beneficial effects for heat preparedness, deleterious effects on acute iron regulation, and potential influence of heat on durability in endurance trained female athletes. These findings extend previous work on heat acclimation, providing a novel "athlete centric" model for heat acclimation. Additionally, these findings open new avenues of research focused on the interaction between heat exposure and iron regulation and provide a novel use of methodology for assessing durability in runners. Future investigations should continue to explore these avenues through an applied and integrative lens, pursuing both performance optimization and health.

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