DISSERTATION

THE EFFECTS OF HEAT STRESS ON HYDRATION STATUS, PHYSIOLOGICAL STRAIN, AND COGNITIVE FUNCTION IN HUMANS

Submitted by

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ABSTRACT

THE EFFECTS OF HEAT STRESS ON HYDRATION STATUS, PHYSIOLOGICAL STRAIN, AND COGNITIVE FUNCTION IN HUMANS

The following dissertation is comprised of a series of experiments with the overall aim of determining the change in hydration status, cardiovascular and thermal strain (i.e. physiological strain), and cognitive function of healthy individuals in response to bouts of both short (<60 minutes) and long-duration (>120 minutes) passive and active heat stress. Increasing core temperatures that occur from the imbalance of heat gain to heat loss, termed uncompensable heat stress, is the primary reason for succumbing to heat related illnesses in many different athletic and occupational settings. However, appreciating the differences in the effects of short and long-duration passive and active heat stress on the hydration status, physiological strain, and cognitive function in humans, have yet to be fully explained in the literature. Understanding the differences in these physiological metrics, especially cognitive function, could lend itself to the establishment of specific techniques and treatments (clinically and field-based) to mitigate physical and cognitive decline following bouts of both short or long-duration heat stress.

To better uncover these physiological changes, we simulated active and passive heat stress conditions within a controlled laboratory environment, using steady-state workloads, ad-libitum drinking patterns, and a set temperature and relative humidity to reduce fluctuating environmental and working conditions that could adversely influence

primary outcome variables. We hypothesized that short and long-duration bouts of heat stress, both active and passive, will (1) decrease measures of hydration status; (2) increase markers of physiological strain; and (3) decrease measures of cognitive function. Furthermore, we hypothesize that the declines in cognitive function will be unaffected due to fluid supplementation type and primarily occur secondary to hyperventilation-induced hypocapnia reducing global cerebral blood flow through the internal carotid artery.

The primary finding of this dissertation is that cognitive function declines following long-duration bouts of heat stress and that this degradation is independent to the type of heat stress (passive or active) or the variety of fluid supplementation. However, contrary to our hypothesis, this decline in cognitive function, although associated with decreases in global cerebral blood flow that are stimulated by hyperventilation-induced hypocapnia, can be significantly blunted if individuals consume fluids supplemented with exogenous glucose.

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CHAPTER I – INTRODUCTION AND EXPERIMENTAL AIMS

Driven by human-induced greenhouse gas emission, largescale shifts in weather patterns have caused significant changes in the global climate, generating record high temperatures in many parts of the world. When paired with an increasing public interest in extreme endurance sports (ultra-marathons, ruck marches, and Ironman triathlons) or with occupations deployed within harsh environmental settings (military and emergency response personnel), these austere environmental transformations have amplified the worldwide frequency of heat related illnesses induced by uncompensable heat stress. Heat stress occurs secondary to both passive (environmental) and active (exercise) settings but is subsequent to a rise in core temperature from sustained heat storage within the body. In these circumstances, the imbalance of heat gain to heat loss generally results from (1) exercise-induced metabolic heat production; (2) unrelenting external environmental conditions such as increasing temperature and relative humidity; or (3) the wearing of nonpermeable clothing, which can limit heat and water vapor dissipation from the body. Since the early 20th century, the various effects of hyperthermia on human physiological processes have been major areas of study in the scientific community, leading to a vast appreciation for the major risks associated with heat related illnesses as well as methods to mitigate uncompensable heat stress. Certainly, decades of scientific resolve have contributed to these various advancements within the world of environmental and occupational physiology, however many gaps within this immense body of literature still remain elusive.

The systemic physiological responses to heat stress that occur within the human body vary greatly when exploring the differences in both passive or exercise-induced heat stress. For example, compared to normothermic exercise conditions, it has been shown that long-duration exercise (≥120 minutes) in hot and humid environments can decrease hydration status (i.e. changes to body mass and plasma osmolality), exercise capacity, and neuromuscular function. These physiological declines are vastly different from shorter bouts of exercise in the heat (≤60 minutes), which has been demonstrated in our lab to have no significant difference to any of these measures. Similarly, bouts of long-duration passive heat stress have demonstrated impairments in both orthostatic tolerance as well as global and regional cerebral blood flow secondary to peripheral venous pooling, decreases in cardiac output, and hyperventilation-induced hypocapnia. Indeed, the duration of the heat stress, be it from passive or active stimuli, play a major role in the human body's continuous struggle to maintain thermoneutrality. However, a major gap in both passive and exercise heat stress literature still remains largely inconclusive, which is the study of the differences of long-duration heat stress (passive vs. active) on hydration status and cognitive function.

Therefore, the primary objectives of this dissertation were to (1) identify the changes in hydration status and cognitive function following both long-duration passive and exercise heat stress; and (2) attempt to elucidate the physiological mechanism(s) involved in these changes to hydration and cognition.

Specific Aims

Experiment #1: to determine the effects of ad libitum flavor and fluid intake on changes in body mass and physiological strain during short-duration, moderate intensity exercise in the heat.

Experiment #2: to determine if supplementation with electrolytes alone or electrolytes + carbohydrates versus plain water would limit declines in hydration status and cognitive function during long-duration, moderate intensity exercise in the heat.

Experiment #3: to determine if long-duration passive heat stress attenuates global cerebral blood flow and if so, (1) does this attenuation correlate with decreases in both cognitive function and arterial tension of carbon dioxide; and (2) is the decline in cognitive function blunted with exogenous glucose supplementation.

This collection of work provides a novel perspective on the physiological differences in both passive and active heat stress during differing lengths of moderate-intensity exercise. Furthermore, our conclusions offer the first experimental evidence that glucose supplementation may in fact blunt dehydration-induced cognitive decline that occurs secondary to both long-duration passive and active heat stress. Finally, these findings have the capacity to be immediately translated to military, athletic, and emergency response personnel during endurance based field operations in austere climates as techniques to mitigate heat related illness. To date, the preponderance of literature has examined how both short-term passive and exercise heat stress effects

cognitive function. To our knowledge, the studies within this dissertation are the first experiments to (1) compare both passive and active heat stress on hydration status and cognitive function; (2) illustrate potential physiological mechanisms that have the ability to defend against heat-induced cognitive decline; and (3) provide heat-stress mitigation techniques that can be promptly implemented in many occupational roles.

CHAPTER II – MANUSCRIPT I[1]

Self-Selected fluid volume and flavor strength does not alter fluid intake, body mass loss, or physiological strain during moderate-intensity exercise in the heat

Summary

The purpose of this study was to determine the effects of ad libitum flavor and fluid intake on changes in body mass (BM) and physiological strain during moderate intensity exercise in the heat. Ten subjects (24±3yrs, 7M/3F) performed 60 minutes of treadmill walking at 1.3 m/s and 7% grade in an environmental chamber set to 33°C and 10% relative humidity while carrying a 22.7 kg pack on two different occasions. Subjects consumed either plain water or water plus flavored (Infuze), ad libitum, at each visit. Pre and post exercise, fluid consumption (change in fluid reservoir weight) and BM (nude) were measured. During exercise, heart rate (HR), systolic blood pressure (SBP), rate of perceived exertion (RPE), oxygen consumption (VO_2), respiratory exchange ratio (RER), co[1]re temperature (T_C), and physiological strain index (PSI) were recorded every 15 mins during exercise. No significant differences were observed for fluid consumption between fluid conditions (512±97.2 mL water vs. 414.3±62.5 mL Infuze). Despite a significant decrease from baseline, there were no significant differences in overall

[1]This chapter was accepted for publication in the *Journal of Thermal Biology* online on March 17, 2020. Details of the publication are listed below:
Self-Selected Fluid volume and flavor strength does not alter fluid intake, body mass loss, or physiological strain during moderate-intensity exercise in the heat
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change of BM (Δ –1.18 vs. -0.64 Kg) or percent body weight loss for water and Infuze conditions, respectively (1.58±0.6 and 0.79±0.2 %). Furthermore, there were no significant differences in HR (144±6 vs. 143±8 bpm), SBP (157±5 vs. 155±5 mmHg), RPE, VO_2 (27.4±0.9 vs. 28.1±1.2 ml/Kg/min), RER, T_C (38.1±0.1 vs. 37.0±0.1 °C), and peak PSI (5.4±0.4 vs. 5.7±0.8) between conditions. Offering individuals the choice to actively manipulate flavor strength did not significantly influence ad libitum fluid consumption, fluid loss, or physiological strain during 60 minutes of moderate intensity exercise in the heat.

Introduction

The hydration state of an individual is important during both short and long term physical activity, particularly in environments with high ambient heat and low relative humidity (Nolte *et al*., 2019). With exercise, total body water can fluctuate up to 0.5% of an individual's body mass through sweating as a means to effectively cool. This may increase further if the exercise is performed in severe climates (Cheuvront *et al*., 2003). If a euhydrated state is not maintained and substantial dehydration ensues, subsequent increases in core body temperature (T_C) could occur (Montain *et al*., 1995) leading to increased risk of heat related illness such as heat exhaustion (Wallace *et al*., 2005), heat stroke (Chao *et al*., 1981), and rhabdomyolysis (Wappler *et al*., 2001). Furthermore, multiple studies have demonstrated that dehydration, whereby individual losses of >2% of body mass, consistently leads to impaired aerobic exercise performance (Nybo *et al*., 2001; Sawka, 2012; Ely *et al*., 2010; Cheuvront *et al*., 2003). This impairment in aerobic performance is attributed to sweat rates that accelerate loss

of body water leading to decreases in plasma volume (Cheuvront *et al*., 2003), which subsequently decreases stroke volume and an inability to maintain adequate cardiac output. Additionally, there is an increase in myocardial work arising secondary to decreased stroke volume and a compensatory increase in heart rate (HR), known as cardiac drift (Gonzalez-Alonso, 1997; Dawson, 2005). Therefore investigating hydration strategies to diminish occurrences of hypohydration during activity and exercise, especially in challenging environments, is vital for mitigating heat related injury and maintain aerobic performance.

Studies in various populations (pediatric, military, and athletics) suggest that palatable fluids (i.e. beverages that taste “good”) will increase ad libitum drinking compared to consumption of plain water alone (Hubbard *et al*., 1984; Szlyk *et al*, 1991; Maughan, 1994; Santana *et al*., 1995; Harper *et al*, 2017). Conversely, other studies have shown no significant differences in palatable beverages on fluid consumption in similar populations (Seidman *et al*., 1991; Burstein *et al*., 1994; Byrne *et al*, 2005; Cesarz *et al*, 2017). The difference in the findings related to ad libitum drinking and perceived beverage palatability may be attributed to environmental factors, work conditions, individual flavor preferences and a limited offering of flavors from researchers. These studies utilized hydration reservoirs (i.e. Camelbak® bladders) or bottles filled with different solutions such as plain water or a combination of either electrolytes and/or carbohydrates (for flavor) and focused on the effect of electrolyte concentration on fluid consumption (Byrne *et al*., 2005; Cesarz *et al*., 2017). More importantly, all of these studies utilized solutions with a fixed concentration, which did not allow the subject to actively change flavor strength during exercise to meet their

individual flavor preference (Szlyk *et al*., 1991; Maughan, 1994; Harper *et al*., 2017, Byrne *et al*., 2005; Cesarz *et al*., 2017).

Fluid reservoirs are commonplace during long-duration endurance events and many military training environments. Although the utility of fluid reservoirs during exercise may seem like a suitable way to carry multiple liters of fluid, there have also been cited complications associated with reservoir usage. When operating in austere environments, many military populations do not have ample time to properly clean their hydration reservoirs after drinking fluids other than plain water. Additionally, the supplementation of carbohydrates to fluid reservoirs has been shown to increase bacterial growth and waterborne illnesses such as Eschirichia coli (Helmes *et al*., 2010). In these particular situations, having a reservoir system that could be used without unnecessarily contaminating the entire hydration unit would be of operational utility for users. The Hydro M1™ (Infuze L.L.C., Logan, UT) is a patent-pending device that mixes water from any reservoir with an externally fixated flavor cartridge. The cartridge can be filled with any pre-made concentrate and the Hydro M1™ can be manipulated to provide more or less concentrate to meet any flavor preference. Finally, the Hydro M1™ is outfitted with a backflow preventer that inhibits flavor (i.e. sugars and electrolytes) from traveling back into the reservoir, allowing the user to consume flavored water while also keeping the reservoir itself clean.

As such, the purpose of this study was to determine the effects of ad libitum drinking of a non-carbohydrate flavored solution, where the amount of flavor could be actively controlled by the subject during exercise, on fluid consumption during laboratory controlled exercise in the heat. We also determined the effects on changes in body

mass and physiological strain (measured via cardiovascular work and thermal stress). We hypothesized that fluid intake would be greater when subjects are allowed to actively control the flavor of the solution, and that this would be associated with less of a reduction in body mass and increases in physiology strain during exercise in the heat.

Methods

Subjects

After providing informed, written consent and completing a health history questionnaire, 10 healthy, recreationally active men (n = 7) and women (n = 3) between the ages of 18-35 years old were enrolled in the study (Table 1). Subjects were normotensive, nonsmokers, not taking medications that could limit thermoregulatory capacity, and without any known autoimmune disorders. The study was approved by the Colorado State University Institutional Review Board (#18-8168) and in coordination with the Declaration of Helsinki.

Study protocol

Each subject presented to the Human Performance and Clinical Research Laboratory at Colorado State University for their initial screening visit and two study day visits. The screening visit began with the health history and screening questionnaires. Once completed, the subjects had a Dual-Energy X-ray Absorptiometry (DEXA; Hologic, Bedford, MA, USA) scan to assess lean body mass and fat mass. The subjects then underwent a VO_{2max} test on a treadmill (Quinton TM65, Mortara Instruments Inc., Wilwaukee, Wisconsin) via a Bruce Protocol utilizing a metabolic cart (Parvo Medics,

Sandy, Utah) to assure the subjects were adequately fit to partake in the study. After a rest period, the subjects performed a “study day familiarization protocol” where they walked on a treadmill for 10 minutes at 1.3 m/s and 7% grade wearing a pack that weighed approximately 22.7 kg. The speed, grade, and load of this familiarization protocol was chosen to closely mimic occupations were load carriage over several miles is required, such as military populations (special operators, security forces, orienteering teams, and trainees), wildland firefighters, and athletes (Sol et al, 2018; Nolte *et al*., 2019; Beis *et al*., 2005).

For the 2 subsequent study day visits, the subjects ingested a T_C sensing pill (CorTemp, Palmetto, Florida) at least 4 hours prior to initiating exercise, allowing T_C to be monitored throughout the exercise bout. Subjects were also asked to wear identical light-weight, moisture wicking garments for both study day visits. Subjects were not required to arrive in a fasted state and had no dietary restrictions in preparation for the study day visits. Dry weight was measured nude using a digital platform scaling system (MedWeigh MS-2510). Subjects were asked to void both their bowel and bladder prior to dry weight measurement. Measurement of body mass change was chosen because it is a simple and rapid assessment of hydration status. Additionally, body mass as a confounding factor secondary to potential body composition changes, a cited disadvantage of using the metric to measure hydration status, was not relevant because the study periods were no more than 1 week apart (Cheuvront & Sawka, 2005). Once these pre-exercise measurements were completed, the subject entered an environmental chamber set to 33°C and under 10% relative humidity and donned a HR monitor chest strap (Polar Electro Inc., Bethpage New York) as well as the 22.7 kg

pack. The temperature and humidity settings were chose to mimic harsh environmental conditions that some populations and occupations may encounter (Cuddy *et al*., 2008; Nolte *et al*., 2019). The pack gave the subjects access to a Camelbak® bladder (Petaluma, California) filled with ambient temperature fluid, which was weighed prior to exercise. To maintain adequate hydration, subjects were instructed to "drink as much and as often as they wanted" (ad libitum). The Hydro M1™ (Logan, Utah) was attached to the mouthpiece of the Camelbak® drinking tube. Once this gear had been donned appropriately, baseline measurements were taken to include HR, blood pressure (BP), rate of perceived exertion (RPE), oxygen consumption (VO_2), respiratory exchange ratio (RER), and T_C. Following these baseline measurements, the subject began the exercise protocol.

Exercise was performed for 60 minutes on a treadmill. The above measurements (HR, BP, RPE, VO_2, RER, and T_C) were taken every 15 minutes. HR was measured via a Polar watch that had been previously synced to the HR monitor strap. BP was measured by manual auscultation while RER and VO_2 were measured through metabolic cart. To decrease the disruption of ad libitum fluid consumption, VO_2 was measured for 3 minutes in the middle of each 15 minute interval (7.5, 22.5, 37.5, and 52.5 minutes). We elected to measure VO_2 for two reasons. The first was to quantify the absolute and relative oxygen requirements of performing this type of work in each individual. The second was to determine whether any VO_2 drift was evident at this work rate in these simulated environmental conditions, an outcome observed in previous longer duration, laboratory controlled studies of similar intensity (Périard *et al*., 2010). RPE was measured via Borg Rating of Perceived Exertion Chart. Each subject

performed this protocol twice on separate days, once drinking water and once drinking flavored water, the order of which was randomized and counter-balanced. For the flavored fluid, a sugar-free flavored concentrate from Infuze L.L.C. called Elixer™ (Infuze condition) was used. For water, no flavor cartridge was needed however the Hydro M1™ was still used for standardization purposes. Following exercise, the subjects voided both their bowel and bladder prior to dry weight measurement. Additionally, the Camelbak® bladder was weighed to measure total fluid consumption. These weights were used track any change in body mass and the amount of fluid consumed during the exercise bout. These measurements allowed quantification of hydration status and detection of any differences between the two fluid conditions. Finally, the measurement of PSI was calculated using the following equation established by Moran and colleagues (1998):

$$PSI = 5(T_{ret} - T_{re0}) * (39.5 - T_{re0})^{-1} + 5(HR_t - HR_0) * (180 - HR_0)^{-1}$$

where T_{ret} and HR_t are measurements taken at predetermined points throughout an exercise or event, while T_{re0} and HR_0 are the initial measurements for T_C and HR before the exercise was initiated. For our study design, T_{ret} and HR_t were taken at 15, 30, 45, and 60 minutes of exercise (Moran *et al*., 1998).

Statistical analysis

Data was group averaged and the two conditions were statistically compared using 2-tailed T-tests and 2-way repeated measures ANOVA. Repeated measures ANOVA was used to assess the presence of main and interaction effects between

conditions throughout the exercise bout at each of the 4 exercise time points (condition versus time). Statistical significance was set to $P < .05$ *a priori*.

Results

Fluid consumption and body mass

The amount of fluid intake was not different between fluid conditions for pre to post exercise (512 ± 97 mL versus 414 ± 63 mL for water and Infuze, respectively). There were no significant reductions in body mass after exercise in either fluid condition (Table 2). There was a main effect for time ($P = 0.0164$), however, a main effect for condition was not found (Time x Condition).

Heart rate and blood pressure

As expected, both HR and SBP increased during exercise, however no significant differences between fluid conditions were observed (Figure 1, Panels A. and B., respectively). There was a main effect for time for both HR and SBP.

Rating of perceived exertion

Throughout exercise, RPE was 12.4 ± 0.1 and 12.2 ± 0.1 for water and Infuze conditions, respectively (Figure 3). There was a main effect for time but no interaction effect was found.

Oxygen consumption and respiratory exchange ratio

Throughout exercise, VO_2 was 27.4 ± 0.9 and 28.0 ± 1.2 mL/kg/min for water and

Infuze conditions, respectively. On average, VO_2 was 53% of VO_{2max} for both fluid conditions. There were no significant differences in absolute or relative oxygen consumption at baseline or at any time point during exercise between fluid conditions (Figure 4). No main or interaction effects were found in either fluid condition. RER was 0.84 ± 0.01 and 0.83 ± 0.02 for water and Infuze conditions, respectively. There were no significant differences in RER at baseline or at any time point during exercise between fluid conditions, nor were there any main or interaction effects found (Figure 4).

Core temperature and physiological strain index

There was no significant difference in T_C at baseline or at any time point during exercise between fluid conditions (Figure 2, Panel A.). A main effect for time was found for both fluid conditions, however, there was no interaction effect between conditions. There was no significant difference in PSI between fluid conditions. Although no interaction effect was seen, there was a main effect for time within both fluid conditions (Figure 2, Panel B.).

Discussion

The primary aim of the present study was to determine whether, compared to plain water, ad libitum drinking of a flavored solution (Infuze) would lead to greater fluid consumption if the amount of flavor was actively controlled by the subject during exercise. The secondary aim was to examine whether any increase in fluid consumption among the flavored condition would attenuate the reduction in body mass and yield lower measures of both cardiovascular work and thermal stress, as assessed by the

physiological strain index (PSI). Contrary to our hypothesis, we failed to observe any significant differences in ad libitum fluid consumption, body mass change, or PSI. The negligible differences in fluid consumption, body mass and physiological variables between conditions indicate that there was no effect of flavor, even when the subject was given the ability to actively manipulate the strength of flavor through the Hydro M1™.

Fluid consumption and body mass

In the present study, overall fluid consumption for both conditions was not significantly different. To our knowledge, the current study is the only laboratory-based study where environmental conditions and work rate were controlled while studying the differences of subject-controlled flavor strength on fluid consumption. Many reports in the literature have examined the influence of flavor (from added carbohydrates), with and without electrolytes, on fluid consumption during extended durations (>60 mins) of field-based military operations (Seidman *et al*., 1991; Burstein *et al*., 1994; Byrne *et al*., 2005) and similar to the present study, failed to observe an effect of flavor (from carbohydrates) on fluid consumption.

Interestingly, a 2008 study by Cuddy and colleagues found that the addition of unflavored electrolytes to water significantly reduced the amount of fluid consumed over a 15 hour work shift compared to plain water alone. Despite these subjects consuming a reduced amount of fluid, they remained appropriately hydrated as assessed by nude body mass and urine specific gravity (Cuddy *et al*., 2008). In that study, the authors attributed the reduced fluid intake to greater fluid retention and decreased urine

production and suggested that secondary to electrolytes (specifically sodium) expanding plasma volume and decreasing urine output, the subjects who consumed the electrolyte solution experienced an increased rate of rehydration. In the present study, the flavored beverage adopted for the Hydro MI™ system did not have added sugar. Overall, the flavor concentrate (Elixir™) contained only 10mg of sodium, significantly less than that which was utilized in the Cuddy et al study (123mg sodium) and amounts characteristically found in commercially available sports drinks such as Gatorade (270mg sodium).

In contrast to Cuddy and colleagues (2008) reporting decreased fluid consumption following unflavored electrolyte ingestion, other studies have shown that ad libitum fluid intake can be increased when an unflavored electrolyte solution is supplemented with flavor from carbohydrates (Hubbard *et al*., 1984; Szlyk *et al*., 1991). Hubbard (1984) and Szlyk (1991) studied untrained males in an environmental chamber (with a fixed temperature, speed, and grade for 6 hrs of simulated hiking) and Army personnel (performing normal operational duties for 12 hrs), respectively. Similar to Cuddy, both found that when unflavored electrolytes were added to water, fluid intake significantly decreased. Interestingly though, both studies (Hubbard *et al*., 1984; Szlyk *et al*., 1991) found that when that same fluid had added flavoring in the form of carbohydrate (sucrose and glucose, respectively), fluid consumption increased. Thus, it appears as though flavor (from carbohydrates) may be increasing fluid consumption when added to unflavored electrolytes and may also be offsetting the decreased fluid consumption observed from drinking unflavored electrolyte solutions. To our knowledge, there has not been a study that has independently examined the role of flavor, without

added carbohydrates on overall fluid consumption during exercise in the heat. In the present study, we elected to control for both work output and environmental conditions in order to isolate the effects of flavor on overall fluid consumption. The data from the present study suggest that, during short-duration exercise (≤ 60 minutes), fluids flavored without carbohydrates and negligible amounts of sodium, do not lead to significant increases in ad libitum fluid consumption.

In the current study, changes in body mass (pre versus post exercise) were utilized to assess changes in hydration status (Cheuvront *et al.*, 2004; Cheuvront & Sawka, 2005). While we failed to observe significant differences between conditions, following exercise, there was a significant decrease in overall body mass ($P < 0.002$) following exercise. Our subjects demonstrated a 1.58 ± 0.6 and 0.79 ± 0.2 % body mass change (Table 2) for the plain water and flavored water trails, respectively. Earlier studies indicate that as little as 2% reduction in body mass can elicit significant reductions in cardiac output (Grandjean *et al*., 2002) and increases in core body temperature secondary to inadequate fluid consumption and imbalances in plasma electrolyte concentrations (Cheuvront *et al*., 2004). Additionally, numerous studies have shown reductions in muscular endurance and maximal aerobic power (VO_{2max}) following body water losses of 2-4% (Sawka and Pandolf, 1990; Montain *et al*., 1998b; Sawka *et al*., 1996a) that are further exacerbated in hot climates (Armstrong *et al*., 1985). Our study, which specifically examined the novel use of the Hydro M1™ to allow subject control of flavor strength, suggests that varying flavor strength does not aid in mitigating significant losses in body mass (between or within fluid conditions) even during shorter duration exercise bouts.

Physiological strain index

As anticipated, all subjects demonstrated an increase in HR and T_C in response to exercise and the average PSI was 5.6 throughout both fluid conditions. Secondary to the increases in both HR and T_C, PSI increased in a time dependent manner during exercise. The PSI values observed in the current study indicate that short-duration ($\leq$ 60 minutes), moderate intensity load carriage activities performed in austere climates, can elicit higher CV and thermal strain values that equal some published literature studying firefighter and athlete populations (Rodríguez-Marroyo *et al*., 2011; Cuddy *et al*., 2015; Bergeron *et al*., 2009). However, because there were no significant differences in PSI between fluid conditions, our data suggests that the ability to actively manipulate flavor strength during a 60 minute bout of exercise does not attenuate measures of CV or thermal stress.

As previously mentioned, the average calculated PSI for both fluid conditions was 5.6, which exceeds the published values of Yakota and colleagues (2002) who studied PSI changes in Marine Corps infantry personnel during simulated military field activities (Yakota *et al*., 2002). Yakota et al found that, on average, only ~10% of their subjects demonstrated PSI values above 4.0 during higher intensity events such as simulated fighting and rucking activities. This PSI value (4.0) falls considerably short of the average PSI found during the current study (5.6, Table 3) as well as other publications studying actual military rucking events (Palmer, 2017). Unlike many field-based experiments where subjects had the ability to adjust their work rate throughout their duty day (Yakota *et al*., 2002; Cuddy *et al*., 2008), the current study was laboratory-based where the speed, grade, temperature, humidity, and load were fixed,

which is likely why our PSI value is greater than those reported from field-based studies (Budd, 2001).

Limitations

One limitation of our study was that the data was collected under simulated, laboratory-based conditions. This clearly limits the inferences that can be made to real-world populations where fluctuating workloads are the standard. The current study utilized consistent steady-state workloads, which are difficult and inappropriate to compare to most occupations (Budd, 2001) however, the controlled laboratory design reduces the fluctuating environmental and working conditions that could influence drinking patterns. Accordingly, many field-based studies that have reported an effect of flavor (via carbohydrate) on fluid consumption have extended work periods of hours to days (Hubbard *et al*., 1984; Bergeron *et al*., 2006; Palmer *et al*., 2017). Within these field-based studies, despite the relative work rate being lower when performed over an extended period of time, there is a greater period of time for an individual to ingest fluids, allowing overall fluid intake to be greater (0.5L in this study vs. 1.7L in other studies) (Bergeron *et al*., 2006). Therefore, it remains possible that flavor alone may influence fluid consumption when exercise duration is extended.

A second limitation of our study was the relatively acute exposure of our participants to these exercise conditions. Many professional athletes are routinely performing these types of physical tasks every day, for multiple days, or months at a time. Anecdotally, it is not uncommon for some groups to report “water fatigue” in the latter part of their work season (Bergeron *et al*., 2006). In these instances, offering a

variety of flavors and flavor strengths may increase fluid consumption for these individuals over a longer period of time.

Finally, we did not account for the amount of self-selected flavor amounts in the Infuze condition. This makes the exact quantification of electrolytes impossible. Future studies focusing on flavor amount and hydration status should measure the amount of flavor additive.

Conclusions

The results of the present investigation indicate that the ability to control flavor strength does not increase fluid consumption during 60 minutes of moderate intensity aerobic exercise in the heat, nor does it impact changes in body mass and physiological responses to exercise. Other factors such as temperature of the fluid as well as carbohydrate and electrolyte concentrations have been shown to increase ad libitum fluid consumption during short and long-duration exercise bouts (Hubbard *et al*., 1984; Szlyk *et al*., 1991; Maughan, 1994; Santana *et al*., 1995; Harper *et al*., 2017) and thus may play a more important role in determining fluid ingestion. We conclude that, during a 60 minute, moderate intensity steady-state bout of exercise in the heat, the ability to actively change flavor strength does not significantly change fluid consumption, changes in body mass, or measurements of CV and thermal strain in young healthy humans. However, it remains to be determined whether subject-controlled flavor strength can increase ad libitum fluid consumption during longer duration (>60 minutes) exercise bouts in both laboratory-controlled and real-world environments, as well as over extended periods of time. Subject-controlled flavor strength should be considered

in future studies to observe whether it will increase ad libitum fluid consumption during longer duration (>60mins) exercise bouts in both laboratory-controlled and real-world environments.

Table 2.1: Subject Characteristics (mean ± SD)

	Total	Male (n = 7)	Female (n = 3)
Age (years)	24 ± 3	25 ± 3	21 ± 3
Height (cm)	178.9 ± 12.9	184.1 ± 9.4	163.7 ± 6.7
Weight (kg)	76.6 ± 18.1	85.2 ± 13.5	56.5 ± 6.9
Body Mass Index (kg/m^2)	24.2 ± 4.4	25.6 ± 4.6	20.9 ± 0.9
% Fat	22.3 ± 4.4	20.8 ± 4.1	25.9 ± 3.7
VO_{2max} (L min^{-1})	3.9 ± 0.9	4.3 ± 0.8	3.2 ± 0.4
VO_{2max} (mL kg^{-1} min^{-1})	52.2 ± 5.7	50.6 ± 5.4	56.1 ± 5.0
VO_{2max} (mL kg FFM^{-1} min^{-1})	67.2 ± 7.5	64 ± 4.8	74.8 ± 7.8
HR_{max} (bpm)	190 ± 11	189 ± 13	193 ± 4

Table 2.2: Body mass change as a marker of hydration status over 1 hr of moderate intensity exercise (mean ± SD)

	Water	Infuze	Overall
Pre-exercise Body Mass (kg)	75.7 ± 13.6	76.0 ± 13.2	75.9 ± 13.4
Post-exercise Body Mass (kg)	74.5 ± 12.9[#]	75.4 ± 12.9[#]	75.0 ± 12.9*
% Reduction	1.58 ± 0.6	0.79 ± 0.2	1.2 ± 0.4

* $P < 0.05$, main effect for time from pre-exercise to post-exercise; [#] $P < 0.05$, significantly different pre-exercise to post-exercise within condition.

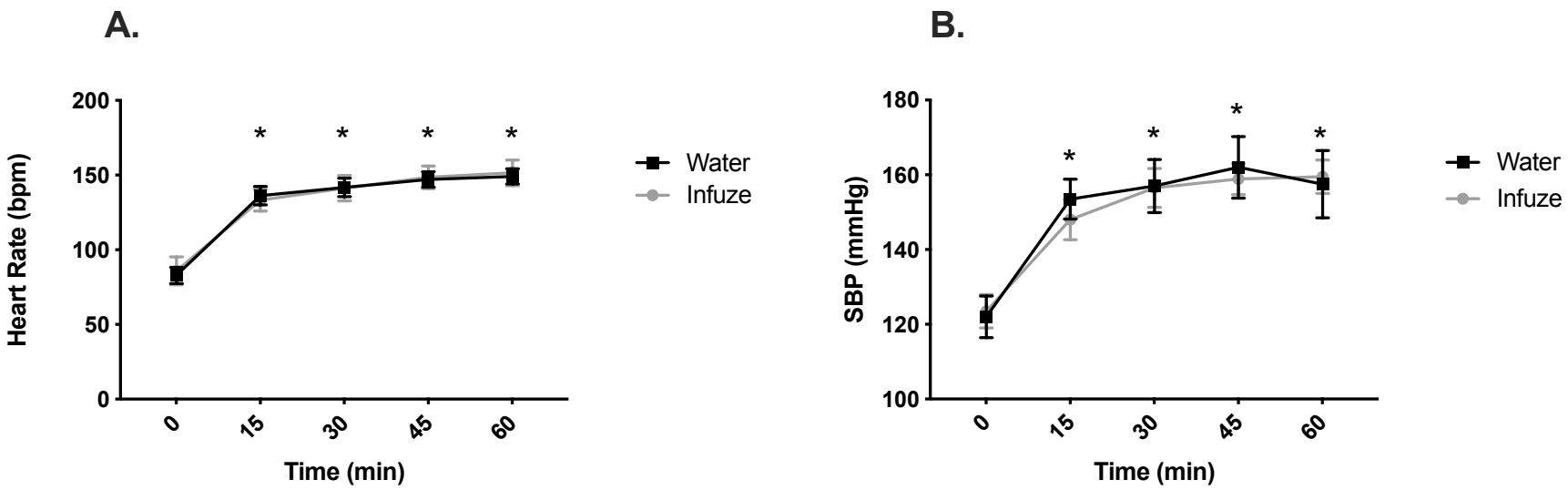

Figure 2.1: The effects of water versus Infuze on heart rate and systolic blood pressure during exercise in the heat

HR (*A.*) and SBP (*B.*) were measured every 15 minutes during moderate intensity exercise in the heat. Both HR and SBP increased from baseline, however there were no differences observed between water and Infuze (self-selected flavor strength) conditions. All differences are significant at $P < 0.05$, * main effect for time, all time points different from 0 min for both water and Infuze conditions.

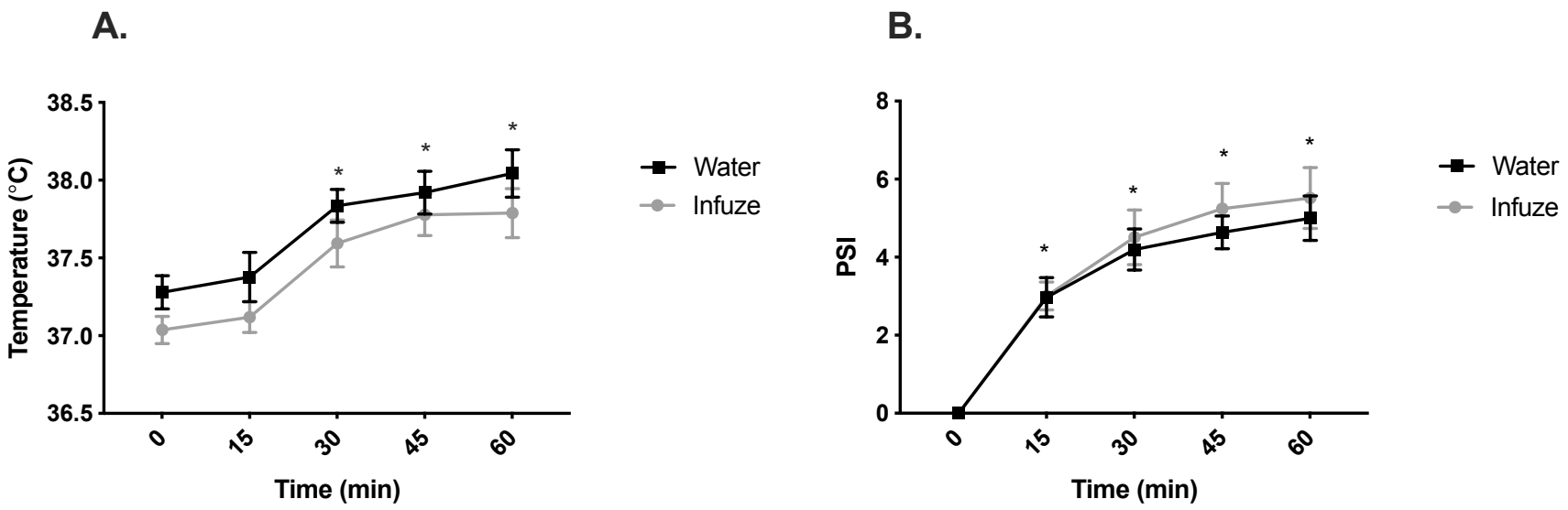

Figure 2.2: The effects of water versus Infuze on core temperature and physiological strain index during exercise in the heat

Core temperature (T_C) (*A.*) was recorded every 15 minutes during moderate intensity exercise in the heat. Physiological Strain Index (PSI) (*B.*) was calculated via T_C and HR at each 15 minute interval during the 60 minute exercise bout. T_C and PSI increased from baseline, however there were no differences observed between water and Infuze (self-selected flavor strength) conditions. All differences are significant at $P < 0.05$, * main effect for time, all time points different from 0 min for both water and Infuze conditions.

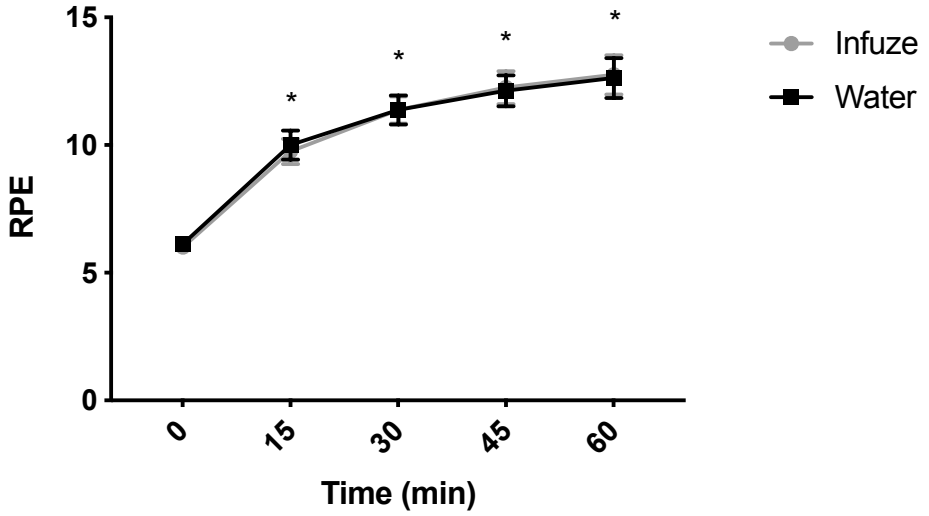

Figure 2.3: The effects of water versus Infuze on rate of perceived exertion during exercise in the heat

Rate of Perceived Exertion (RPE) was recorded every 15 minutes during moderate intensity exercise in the heat. RPE increased from baseline, however there were no differences observed between water and Infuze (self-selected flavor strength) conditions. All differences are significant at $P < 0.05$, * main effect for time, all time points different from 0 min for both water and Infuze conditions.

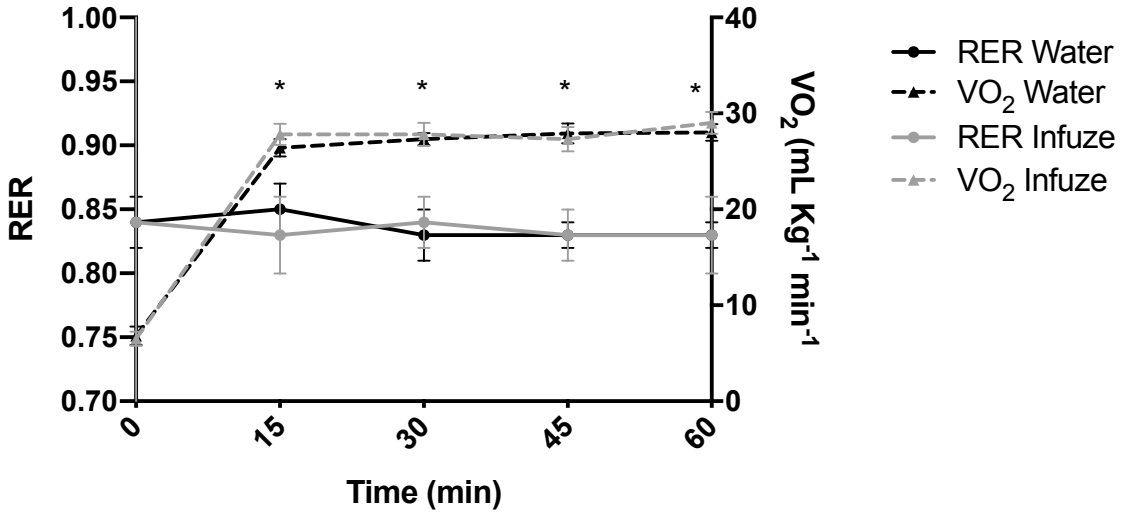

Figure 2.4: The effects of water versus Infuze on respiratory exchange ratio and VO_2 during exercise in the heat

Respiratory Exchange Ratio (RER) and VO_2 was recorded via metabolic cart every 15 minutes during moderate intensity exercise in the heat. Both RER and VO_2 increased from baseline, however there were no differences observed between water and Infuze (self-selected flavor strength) conditions. All differences are significant at $P < 0.05$, * main effect for time, all time points different from 0min for both water and Infuze conditions in RER and VO_2.

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CHAPTER III – MANUSCRIPT II[2]

Carbohydrate Ingestion Attenuates Cognitive Dysfunction Following Long-Duration Exercise Heat Stress In Humans

Summary

The purpose of this study was to determine if electrolyte or carbohydrate supplementation vs. water would limit the magnitude of dehydration and decline in cognitive function in humans following long-duration hyperthermic-exercise. Twenty-four subjects performed 3 visits of 2hrs walking (3mph/7% grade) in an environmental chamber (33ºC/10% relative humidity). In random order, subjects consumed water (W), electrolytes (Gatorade Zero; E), or electrolytes+carbohydrates (Gatorade; E+C). Throughout exercise (EX), subjects carried a 23kg pack and drank ad-libitum. Pre-and post-EX, body mass (BM) and plasma osmolality (pOsm) were measured. Physiological Strain Index (PSI) and core temperature (T_C) were recorded every 15mins. Plasma glucose (GLU) was measured every 30m[2]ins. Cognitive processing (SCWT) was measured post-EX and compared to baseline (BL). A subset of 8 subjects performed a normothermic (N) protocol (21ºC/ambient humidity) to ascertain how the exercise stimulus influenced hydration status and cognition without heat. There were no

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Carbohydrate Ingestion Attenuates Cognitive Dysfunction Following Long-Duration Exercise In The Heat In Humans
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significant differences between fluid conditions (W, E, E+C) for BM loss (Δ2.5±0.2, 2.5±0.2, 2.3±0.2kg), fluid consumption (1.9±0.2, 1.9±0.2, 1.8±0.2L), pOsm (Δ1.5±2.7, 2.2±2.4, 2.0±1.5mmol/L), peak-PSI (7.5±0.4, 7.0±0.6, 7.9±0.5), and peak-T_C (38.7±0.1, 38.6±0.2, 38.8±0.2ºC). GLU decreased significantly in W and E, whereas it increased above BL in E+C at 60, 90, and 120mins ($P<0.05$). Compared to BL values (43.6±26ms), SCWT performance significantly decreased in all conditions (463±93, 422±83, 140±52ms, $P<0.05$). Importantly, compared to W and E, the impairment in SCWT was significantly attenuated in E+C ($P<0.05$). As expected, when compared to the heat-stress protocol (W, E, E+C), N resulted in lower BM loss, fluid consumption, and peak-PSI (1.1±0.1kg, 1.2±0.7L, 4.8, respectively), and improved SCWT performance. These data are the first to suggest that, independent of supplementation variety, cognitive processing significantly decreases immediately following long-duration exercise in the heat in healthy humans. Compared to water and fluids supplemented with only electrolytes, fluids supplemented with carbohydrates significantly blunts this decrease in cognitive function.

Introduction

During exercise, total body water (TBW) fluctuates with alterations in workload, evaporative cooling (i.e. sweating), volume of fluid consumption, and environmental conditions (Popowski et al., 2001). Multiple studies have defined dehydration as individual TBW losses of ≥2% body mass (Nybo et al., 2001; Cheuvront et al., 2003) and reductions of this magnitude consistently lead to impairments in aerobic exercise performance, such as VO_{2max} and time to exhaustion (Ely et al., 2010; Sawka et al.,

2012). There is evidence that these decrements in aerobic performance occur from dehydration and TBW loss that decreases plasma volume, which subsequently attenuates stroke volume during exercise (Gonzalez-Alonso et al., 1997; Cheuvront et al., 2003; Sawka et al., 2012). Further, when exercise is performed in hot conditions for prolonged durations (>30 minutes), several studies report declines in cognitive function following exercise (Wittbrodt et al., 2018; Otani et al., 2017; Piil et al., 2019). The cause of this decline in cognitive function has been attributed to a number of possible factors including dehydration (Wittbrodt et al., 2018; Piil et al., 2019), reductions in cerebral blood flow (Ide et al., 2000; Ogoh et al., 2009; Sato et al., 2009), and alterations in brain neurotransmitter function (Edvinsson et al., 1975; Mitchell et al., 2009). These findings related to cognitive decline following exercise in the heat are in contrast to the positive effect of short-duration (normothermic) exercise on cognitive function in healthy (Hogan et al., 2013; Langlois et al., 2013) and clinical populations (Quaney et al., 2009; Cruise et al., 2011; Motl & Sandroff, 2015).

When individuals perform exercise in hot environments, a number of hydration strategies are adopted to mitigate the risk of dehydration and heat related injury. Despite these strategies, mild dehydration appears to be inevitable during long-duration exercise in the heat despite adequate fluid ingestion throughout the exercise bout (Sawka et al., 2007). Furthermore, for some occupations, such as military (aircrew, special operations, trainees, orienteers, and astronauts) and emergency response personnel (structural and wildland firefighters, paramedics, and hazardous material personnel), field operations may span up to 24 hours in hot (>30ºC) and dry (<10% relative humidity) environments for several weeks in a row. As such, adopting common

hydration strategies may be cumbersome and not operationally realistic to these subject populations (Greenleaf & Fortney, 1992; Hunt et al., 2016; Schlader et al., 2020). However, some field based studies have reported that ingesting fluids containing either electrolytes or carbohydrates can attenuate TBW loss, potentially by preserving plasma osmolality (pOsm) (Cuddy et al., 2008) or by increasing fluid consumption (Hubbard et al., 1984; Szlyk et al., 1991). Furthermore, carbohydrate ingestion in the form of a commercially available sports drink prevented post-exercise decline in cognitive function following an outdoor European Football match in warm temperatures (Bandelow et al., 2010). However, in this study, exercise duration and intensity were not controlled.

Given the above information as background, we aimed to determine whether ingestion of electrolytes+carbohydrates would attenuate dehydration and cognitive dysfunction following prolonged exercise in the heat in humans. We hypothesized that compared to plain water or electrolytes alone, changes in 1) body mass and plasma osmolality (hydration status) and 2) cognitive function would be attenuated when subjects consumed beverages supplemented with electrolytes+carbohydrates during prolonged exercise in the heat.

Methods

Subjects

Following informed, written consent and the completion of a health history questionnaire, a total of 24 subjects (18 men, 6 women) were enrolled in the present study. Subjects were between the ages of 18-35 years, healthy, recreationally active, normotensive, nonsmokers, and without any comorbidity or autoimmune disorder that

could limit thermoregulatory capacity (Table 1). This study was approved by the Colorado State University Institutional Review Board (#18-8168) and in accordance with the Declaration of Helsinki.

Screening and Familiarization Protocol

Subjects presented to the Human Performance and Clinical Research Laboratory at Colorado State University for the initial consent and screening visit and the 3 study day visits. The consent and screening visit was used to brief the subjects on the study timeline and participant expectations. Following this, a health history and screening questionnaire was completed. The subjects then underwent a Dual-Energy X-ray Absorptiometry (DEXA; Hologic, Bedford, MA, USA) scan to assess lean body mass, fat mass, and bone mineral density. Subsequently, the subjects completed a VO_{2max} test on a treadmill (Quinton TM65, Mortara Instruments Inc., Milwaukee, Wisconsin) via a Bruce Protocol utilizing a metabolic cart (Parvo Medics, Sandy, Utah). In order to assure that the subjects were adequately fit to partake in the present study, all subjects were required to achieve a VO_{2max} of ≥50 mL/kg/min. Following the VO_{2max} test and a brief rest period, each subject completed a "study day familiarization protocol" to assure that carrying a weighted pack would not cause any musculoskeletal aggravation. This protocol required the subjects to walk on a treadmill for 10 minutes at 1.3 m/s and 7% grade while wearing a pack that weighed approximately 22.7 kg. The speed, grade, and load of this protocol was identical to a study previously performed in our laboratory (Deming et al., 2020) and was chosen to simulate occupations were load carriage (60% VO_{2max}) over several kilometers is required, such as many military populations (aircrew,

special operators, security forces, orienteering teams, and trainees), emergency personnel, and athletes (Sol et al, 2018; Nolte et al., 2019; Beis et al., 2005).

Exercise Heat-Stress Study Protocol

For the 3 study day visits, the subjects ingested a core temperature (T_C) sensing pill (CorTemp, Palmetto, Florida) 4 hours prior to the visit allowing T_C to be monitored throughout the exercise bout. Subjects wore a standardized outfit consisting of a long-sleeved shirt, pants, and athletic shoes for all study day visits. No dietary restrictions were set in preparation for the study day visits nor were subjects required to arrive in a fasted state. The dry weight of the subject was measured nude using a digital platform scale (MedWeigh MS-2510). Subjects voided both bowel and bladder before nude weight was measured. Following the completion of these pre-exercise measurements, subjects entered an environmental chamber that was set to 33°C and ≤10% relative humidity, which are conditions that mimic harsh conditions encountered by many military and emergency personnel (Cuddy et al., 2008; Nolte et al., 2019). Inside the chamber, they donned a heart rate (HR) monitor chest strap (Polar Electro Inc., Bethpage, New York) and a pack that weighed 22.7 kg. This pack also gave the subject access to a Camelbak® reservoir (Petaluma, California) filled with fluid of ambient temperature. The reservoir was weighed prior to exercise. Subjects were requested to "drink as much and as often as they wanted" (ad libitum). Baseline measurements were taken for HR, blood pressure (BP; manual auscultation), rate of perceived exertion (RPE), and T_C. A small sample of venous blood (~10 mL) was collected through an antecubital vein to assess pre-exercise blood glucose (GLU), calcium (Ca^{2+}), chloride

(Cl^-), sodium (Na^+), and potassium (K^+) via Piccolo Xpress Chemistry Analyzer (Abaxis, Inc., Union City, California). The measurement of pre- and post-exercise pOsm concentration was calculated using the following equation established by Purssell and colleagues (Purssell et al., 2001):

$$2\ Na^+\ (mEq/L) + [Urea\ (mg/dL)/2.8] + [Glucose\ (mg/dL)/18]$$

Following the above measurements, subjects began the exercise protocol. The chosen exercise stimulus for this study was 120 minutes on a treadmill. HR, BP, RPE, and T_C were taken every 15 minutes (Deming et al., 2020). Glucose was assessed every 30 minutes via finger prick and Contour® Next EZ Meter analysis (Ascensia Diabetes Care, Parsippany, New Jersey). Each subject performed the above protocol 3 separate times separated by at least 7 days to limit temperature and humidity acclimation (Cheung et al., 2000). The 3 fluid conditions were plain water (W), water supplemented with electrolytes (Gatorade Zero; E), and water supplemented with electrolytes and carbohydrates (Gatorade; E+C). Gatorade Zero was chosen as a fluid condition to observe whether electrolyte supplementation alone (without carbohydrate) would alter fluid consumption amount and maintain pOsm concentrations during long-duration, steady-state exercise in the heat (Cuddy et al., 2008; Deming et al., 2020). The order of fluid condition was randomized and counter-balanced and the subjects were not informed of which fluid they were assigned. Gatorade Zero contained 0.46 mg/mL Na^+ and 0.14 mg/mL K^+ while Gatorade contained 0.46 mg/mL Na^+, 0.13 mg/mL K^+, and 60.9 mg/mL of carbohydrates (mixture of glucose and dextrose). After the 120 minute

exercise bout was complete, the subjects voided their bowel and bladder prior to dry weight measurement in the nude. Total fluid consumption was measured by subtracting the post-exercise Camelbak® reservoir weight from the pre-exercise reservoir weight. Post-exercise pOsm was assessed identical to the pre-exercise technique via comprehensive metabolic panel. The amount of fluid consumed through drinking, fluid lost through sweating, and change of pOsm concentrations allowed the evaluation of hydration status. Cardiovascular and thermal strain was measured via the Physiological Strain Index equation established by Moran and colleagues (1998):

$$PSI = 5(T_{ret} - T_{re0}) * (39.5 - T_{re0})^{-1} + 5(HR_t - HR_0) * (180 - HR_0)^{-1}$$

where T_{ret} and HR_t are measurements for core body temperature and HR taken at predetermined points throughout an exercise stimulus, while T_{re0} and HR_0 are the initial measurements for core body temperature and HR immediately prior to the initiation of exercise (Moran et al., 1998). For the present study protocol, T_{ret} and HR_t were assessed at 15, 30, 45, 60, 75, 90, and 120 minutes of exercise.

Normothermic Exercise Study Protocol

To determine how the selected long-duration exercise stimulus would influence hydration status and cognitive function without any additional confounding variables, such as heat, low humidity, or the supplementation of electrolytes and carbohydrates, a subset of 8 subjects of the original 24 performed a control exercise trial under normothermic conditions (N). The N condition was identical to the above study day visits

except for the temperature of the environmental chamber (21ºC and ambient humidity) and the fluid condition (only W). All other variables during exercise (HR, BP, GLU, RPE, and T_C) as well as pre and post exercise (pOsm, fluid consumption, and body weight) were measured in an identical fashion to the other 3 fluid conditions in the heat.

Cognitive Function Battery

A series of cognitive tests were utilized to measure reaction time, processing speed, short to long-term memory conversion, and mood state. Baseline values of the tests were measured during the consent and screening visit following the health history questionnaire. On each of the study day visits the tests were repeated following the hyperthermic exercise stimulus for all fluid conditions as well as the normothermic exercise control group. The Stroop Color and Word Test (SCWT) was used to evaluate the reaction time of the subject as well as their ability to inhibit cognitive interference, together known as the Stroop Effect (Scarpina & Tagini, 2017).

Conversion of short to long-term memory was evaluated through a word-list recall test (Labban & Etnier, 2011). The test displayed 15 different words to the subject on a computer screen. Each of the 15 words were individually displayed on the computer screen for 1 second each. Following that 1 second of display time, the word would disappear and the next word would be displayed. The 15 words did not repeat themselves. After the 15 words were displayed, the subject had 60 seconds to record as many of the words as they could remember. After the 60 seconds concluded, the amount of correctly recalled words was summed as the recall score.

The mood state of the subject was evaluated via a 65-item assessment called the Profile of Mood States (POMS). This test measures 6 subscales of mood, 1) tension-anxiety, 2) depression-dejection, 3) anger-hostility, 4) vigor-activity, 5) fatigue-inertia, and 6) confusion-bewilderment. Each subscale was individually scored and combined to make a final total mood disturbance score (Berger & Motl, 2000). We also performed a time control condition for the cognition tests to expose the possibility of a "learning effect" for the chosen cognitive function tests. This was completed by 6 of the 24 subjects, who took the full battery of cognition function tests once per week for a total of 3 weeks (Table 4).

Statistical Analysis

Data for each fluid condition within hyperthermic exercise, and that for the normothermic exercise control group, were group averaged and statistically compared using a 2-way, repeated measures ANOVA and multiple comparisons. When appropriate, individual fluid conditions were compared to baseline values via 2-tailed T-tests. The presence of main and interaction effects between conditions were determined with repeated measures ANOVA at each of the exercise time points (condition versus time). Statistical significance was set at $P < 0.05$ (*a priori*).

Results

Body Mass

Body mass was significantly reduced from baseline in all 3 fluid conditions following the exercise heat-stress protocol, with no differences between conditions

(Table 2 and Figure 1). Following exercise in normothermic conditions, body mass was also significantly reduced from baseline, although this was attenuated compared with exercise in hyperthermic conditions.

Fluid Consumption

During hyperthermic exercise, there were no significant differences in total fluid consumed during the 120 minute bout of exercise (Figure 2) between the 3 fluid conditions. On average, subjects consumed approximately 1.8 L of fluid for the water, electrolytes alone, or electrolytes+carbohydrates supplementation conditions (1.9 ± 0.2, 1.8 ± 0.2, and 1.8 ± 0.2 L, respectively). Compared to hyperthermic exercise, subjects performing normothermic exercise (N) consumed significantly less fluid (1.2 ± 0.1 L; $P < 0.05$).

Cardiovascular and Thermal Strain

As expected, there was a main effect of time on cardiovascular and thermal strain (i.e. PSI) during hyperthermic exercise ($P < 0.05$). However, there were no significant differences between the 3 fluid conditions in cardiovascular or thermal strain at any point throughout the 120 minute bout of exercise. When compared to hyperthermic exercise, PSI was significantly attenuated during normothermic exercise at every time point ($P < 0.05$; Figure 3).

Hematological Measures

At baseline (pre-exercise), there were no significant differences in pOsm or electrolyte concentration between any condition. Further, there were no significant changes in pOsm or electrolyte concentration after hyperthermic exercise in any fluid condition, nor were there changes in response to normothermic exercise (Table 3). Baseline glucose ranged from ~85-100 mg/dL, and was similar across conditions (Figure 4). During hyperthermic exercise, blood glucose significantly decreased over time in the water and electrolyte only conditions ($P < 0.05$ vs. baseline). In contrast, glucose significantly increased over time in the electrolyte+carbohydrate condition, and was significantly greater than levels observed during all other conditions from minute 60 until the end of exercise. Normothermic exercise did not impact blood glucose.

Cognitive Battery

Compared to baseline, there were no significant differences between the word-list recall test and total mood disturbance score of the POMS following the 120 minute bout of hyperthermic exercise (Figures 5 and 6, respectively). Contrary to this finding, compared to baseline, word-list recall scores were significantly greater after the 120 minute bout of normothermic exercise. Following hyperthermic exercise, the POMS fatigue-inertia subscale (i.e. decreased physical energy level) was significantly decreased in all fluid conditions ($P < 0.05$) as well as the vigor-activity subscale (i.e. increased physical energy level) in the electrolyte+carbohydrate condition only. Finally, following hyperthermic exercise and compared to baseline, there was a significant increase in the Stroop Effect (i.e. reaction time and ability to inhibit cognitive

interference) in all fluid conditions ($P < 0.05$) (Figure 7), indicating impaired cognitive function. Importantly, although increased from baseline, this effect was significantly attenuated (~65%) in the electrolyte+carbohydrate condition compared to both the water and electrolyte only conditions. In contrast to hyperthermic exercise and compared to normothermic baseline, the Stroop Effect was significantly reduced after normothermic exercise (i.e. improved function). Finally, in the subset of subjects who underwent the time-control experiments, there were no significant differences between trial number for any measure of cognitive function, indicating that a learning effect did not occur over time (Table 4).

Discussion

The principle aim of this study was to determine the effects of 3 different fluid conditions (water, electrolytes alone, and electrolytes+carbohydrates) on hydration status and cognitive processing following a bout of long-duration, moderate-intensity hyperthermic exercise. Our hypotheses were that, compared to the water and electrolytes alone conditions, both 1) hydration status (measured via ad libitum consumption of fluid, reductions in body mass, and changes in pOsm) and 2) cognitive function (measured via SCWT, memory recall, and POMS) would be enhanced in the electrolyte+carbohydrate condition secondary to the supplementation of carbohydrates during the exercise bout. The primary findings of this study are that (1) hydration status was not different among the different fluid conditions, and (2) compared to the water and electrolyte only conditions, cognitive processing (via Stroop Effect) was less impaired in the carbohydrate condition. Furthermore, the Stroop Effect was actually

reduced (i.e. improved processing) from baseline after normothermic exercise, indicating that the impairment in cognitive processing is not attributable to long-duration exercise *per se.*

Fluid Consumption, Body Mass, and Physiological Strain

Fluid consumption throughout the 120 minute bout of hyperthermic exercise was not significantly different between the 3 fluid conditions, despite electrolyte or carbohydrate supplementation. Compared to hyperthermic exercise, fluid intake was lower during normothermic exercise suggesting that the difference in fluid intake was secondary to the addition of heat to the exercise stimulus. Previous studies regarding the effects of electrolyte and carbohydrate supplementation on hydration status have yielded equivocal results. Our findings are in agreement with other reports specific to many military populations such as orienteers, aircrew, and trainees (Burstein et al., 1994; Byrne et al., 2005) whereby there was no hydration advantage with electrolyte or carbohydrate supplementation during long-duration exercise or passive heat-stress. Other published reports have shown that ad libitum fluid consumption is in fact increased when water was flavored with a carbohydrate and electrolyte mixture in untrained males during a 6 hour simulated hiking event in civilian and military populations, respectively (Hubbard et al., 1984; Szlyk et al., 1991). These results were validated in subsequent studies in trained endurance and military males during differing lengths of exercise intensities (Seidman et al., 1991; Burstein et al., 1994; Byrne et al., 2005). In contrast, Cuddy and colleagues demonstrated that the addition of unflavored electrolytes during a long work shift in the heat (15 hours) significantly reduced the

amount of fluid consumed, but maintained hydration status when compared to a plain water condition (Cuddy et al., 2008). Although unclear, the differences observed between studies may be related to the duration and intensity of exercise, as well as different environmental factors (heat/humidity).

Similar to fluid consumption, there were no significant differences in reductions in body mass during hyperthermic exercise among the 3 fluid conditions. Independent of electrolyte and carbohydrate supplementation during the hyperthermic exercise protocol, subjects lost ~3% of their body mass from pre- to post-exercise (Table 2). The reduction in body mass due to exercise was significantly less in the normothermic condition (~1.1% from baseline) compared to the hyperthermic condition. Our data is consistent with prior studies that demonstrate when fluid consumption is similar, there are no differences in body mass reductions when consuming water, electrolytes, or carbohydrates during prolonged exercise in the heat (Burstein et al., 1994; Byrne et al., 2005; Deming et al., 2020).

Aside from the anticipated increase in both HR and T_C, cardiovascular and thermal strain (i.e. Physiological Strain Index, or PSI) were not significantly different between fluid conditions during hyperthermic exercise. PSI increased in a time-dependent manner during the 120 minute bout of exercise, reaching its peak of 7.5, 7.0, and 7.9 for water, electrolyte, and electrolyte+carbohydrate conditions, respectively. A peak of 4.8 was observed during normothermic exercise, a value significantly lower than any fluid condition within the heat-stress protocol. The PSI values obtained during hyperthermic exercise are comparable to similar studies examining changes in cardiovascular and thermal strain secondary to load carriage during challenging

environmental conditions (Nunneley et al., 2002; Yokota et al., 2002; Rodriguez-Marroyo et al., 2011; Bergeron et al., 2009, Deming et al., 2020). Despite the substantial increase in PSI during hyperthermic exercise, the negligible differences noted between fluid conditions within the heat-stress protocol suggest that neither the supplementation of electrolytes or carbohydrates significantly attenuate cardiovascular or thermal strain during long-duration exercise-heat stress. The average peak PSI observed in this study (7.5 in the heat-stress protocol) was higher than other published reports citing this metric during field-based experiments (Yakota et al., 2002; Cuddy et al., 2008; Palmer et al., 2017). This is understandably so as the subjects in the present study were exercising at a fixed speed, grade, temperature, humidity, and load which is dramatically different than subjects studied in field-based environments where they can alter and adjust their work intensity to mitigate dangerous rises in physiological strain (Budd, 2001).

Hematological Measures

No significant changes were observed from baseline in response to either hyperthermic and normothermic exercise for pOsm or plasma electrolytes. Although this may appear somewhat surprising given the significant reduction in body mass after exercise (i.e. dehydration), these data are consistent with other studies indicating that 120 minutes of hyperthermic exercise may not be long enough to induce significant changes in pOsm or electrolytes particularly when subjects are allowed to consume fluids ad libitum (Greenleaf et al., 1983; Del Coso et al., 2015). Blood glucose decreased during hyperthermic exercise in the water and electrolytes only conditions

and remained stable during normothermic exercise (Figure 4). In contrast, blood glucose increased during exercise in the electrolyte+carbohydrate condition and was significantly greater than all other conditions. These findings are consistent with previous studies demonstrating decreases in blood glucose during long-duration hyperthermic exercise (Nielsen et al., 1990; Hargreaves et al., 1996) unless supplemented exogenously (King et al., 1985; Mudambo et al., 1997). The increase in blood glucose was expected in the electrolyte+carbohydrate condition as this fluid was the only option that offered the participants additional carbohydrate during the exercise stimulus, and importantly, may offer protective effects on cognitive function (see below).

Cognitive Battery

Profile of Mood States

Compared to baseline measures, no significant differences were observed between the 3 hyperthermic exercise conditions for both the memory recall test and for 4 of the 6 subscales of the POMS from pre- to post-exercise. The tension-anxiety, depression-dejection, anger-hostility, and confusion-bewilderment subsections of the POMS were all similar to baseline measures indicating that mood remained relatively comparable independent of fluid condition. Compared to baseline however, the fatigue-inertia subscale score was significantly higher in all hyperthermic exercise fluid conditions (6.2, 11.1, 10.3, and 10.0 for water, electrolytes only, and electrolyte+carbohydrate conditions, respectively) and the vigor-activity subscale score was significantly lower in the electrolyte+carbohydrate condition only (15.0 and 10.5 for baseline and electrolyte+carbohydrate, respectively). This increase in fatigue (denoted

by an increased fatigue-inertia score) following exercise has been previously observed and documented under similar occupational and exercising conditions (McMorris et al., 2006).

Memory Recall

There were no effects of hyperthermic exercise on memory recall scores for any fluid condition. Further, although baseline measures were slightly higher for the subgroup who underwent the normothermic exercise protocol, memory recall scores we also unaffected (Figure 5). This has been studied previously, whereby normothermic short-duration exercise may in fact augment memory formation (Davy, 1973) and that when heat is added, memory formation becomes inhibited (Cian et al., 2000).

Stroop Effect

Following hyperthermic exercise, all subjects demonstrated significantly increased Stroop Effect scores, independent of fluid condition (21.5, 462.9, 421.8, and 215.6 ms for baseline, water, electrolytes only, and electrolytes+carbohydrates, respectively). When compared to both the water and electrolyte only conditions, the electrolyte+carbohydrate condition demonstrated a significantly attenuated (~65%) Stroop Effect score (Figure 7). The Stroop Effect assesses reaction time and cognitive processing speed as well as the frontal lobe's ability to provide selective attention and some executive control processes (Zomeren, 1992). It is also an indirect measure of the brain's ability to inhibit cognitive interference (Macleod, 1991). Short-duration (<60 minutes) bouts of normothermic exercise are reported to decrease Stroop Effect scores

allowing individuals to react and process information faster than they would during resting conditions (Sibley et al., 2006; Yanagisawa et al., 2010; Byun et al., 2014; Crush et al., 2017), and our data following 120 minutes of moderate-intensity normothermic exercise support these findings (Figure 7). This suggests that the attenuation in cognitive processing speed and reaction time observed in the hyperthermic trials are specific to long-duration exercise in the heat and not just long-duration exercise *per se*. When examining the 3 fluid conditions in the heat, electrolyte+carbohydrate condition had a significantly smaller impairment in Stroop Effect compared to the water and electrolyte only conditions, indicating that elevation of blood glucose has some protective effect on cognitive function during long-duration exercise in the heat. In fact, our data suggest that reductions in blood glucose during hyperthermic exercise may be more mechanistically-linked with impaired cognitive function than the heat-stress itself. Our current findings are in agreement with Gagnon et al. (2010) who demonstrated that independent of hyperthermic exercise, glucose ingestion increased attentional control as measured by the Stroop Effect, in healthy, fasted adults. Additionally, increased plasma glucose levels secondary to fluid supplementation have been shown to improve cognitive function following a period of hypoglycemia in diabetic populations (Punthakee et al., 2012), during a bout of short-term moderate intensity normothermic exercise (Sünram-Lea et al., 2012), and during intermittent aerobic hyperthermic exercise (Bandelow et al., 2010). However, the mechanism(s) by which increased plasma glucose attenuates cognitive dysfunction following hyperthermic exercise is presently unknown.

Time-Control

Time-control data performed in a subgroup of subjects demonstrated no significant differences in the 6 subscales of the POMS, memory recall, or Stroop Effect scores (Table 4), suggesting that a “learning” or “practice” effect did not occur and therefore does not impact the interpretation of our findings.

Potential Mechanisms

The mechanism(s) by which preserving or increasing blood glucose during hyperthermic exercise attenuates certain measures of cognitive dysfunction are presently unknown. Given the reliance of the brain on glucose for metabolism, hypoglycemia (independent of heat stress) has been shown to impair cognitive function (Lacy et al., 2020; Punthakee et al., 2012). With respect to hyperthermic exercise, several studies have shown data reductions in both global (Nybo et al., 2001; Nielsen & Nybo, 2003; Ainslie et al., 2009) and regional (Nunneley et al., 2002; Qian et al., 2014) cerebral blood flow, a response thought to be secondary to hyperthermia-induced hyperventilation (Nybo et al., 2002). Importantly, reductions in cerebral blood flow have been linked with impaired cognitive function (Ide et al., 2000; Ogoh et al., 2009; Sato et al., 2009). In the current study, we did not measure cerebral blood flow, however if attenuated, supplementation of glucose may have provided greater concentrations of glucose and greater cerebral glucose delivery allowing for less perturbation in cerebral metabolism. To the best of our knowledge, the present study is the first to quantify these detriments in humans following a bout of long-duration, moderate-intensity hyperthermic exercise.

Limitations

There are a few limitations of the present study that deserve mention. First, the experiments in the laboratory were highly controlled and thus the subjects exercised at a constant workload under consistent environmental conditions. Therefore, our conclusions are specific to the experimental conditions employed, and we acknowledge that any inferences to real-world occupations are somewhat limited (Budd, 2001). A second limitation is that we were unable to measure global or regional cerebral blood flow to gain insight in whether reductions in cerebral perfusion is related to impaired cognitive function during long-duration hyperthermic exercise. This would have allowed for an enhanced understanding of changes in cerebral blood flow and glucose delivery secondary to the combined stress of hyperthermia and exercise and potentially allow for the comprehension of mechanisms directly involved in cognitive control.

Conclusions

To our knowledge, these data are the first to demonstrate that independent of electrolyte and carbohydrate supplementation, the ability to inhibit cognitive interference (Stroop Effect) and reaction time significantly decreases immediately following long-duration ($\geq$120 mins), moderate-intensity hyperthermic exercise in healthy humans. When compared to consuming either plain water or water supplemented with electrolytes, carbohydrate supplementation significantly blunts this impairment in cognitive function. However, the addition of carbohydrates does not completely attenuate all cognitive impairments secondary to heat stress found in this study. Maintaining normal cognitive processing speeds and reaction times are vital

characteristics in many military, athletic, and first-responder occupations during sustained periods of cardiovascular and thermal stress. These data are the first to demonstrate that, even during long-duration moderate intensity hyperthermic exercise, glucose supplementation may indeed attenuate declines in cognitive function, specifically that of processing speed and reaction time. Therefore, targeting the systems and structures assessed by the Stroop Effect may be an effective strategy in mitigating the observed decline in cognitive processing speed during this type of exercise stimulus and must be considered in future investigations.

Table 3.1: Subject characteristics (mean ± SD)

	n = 24
Age (years)	29 ± 3
Body Mass (kg)	80.1 ± 4.3
BMI (kg/m^2)	24.4 ± 0.8
% Fat	22.4 ± 1.1
VO_{2max} (L min-1)	3.9 ± 0.2
VO_{2max} (mL kg^{-1} min^{-1})	51.8 ± 1.8
VO_{2max} (mL kg FFM^{-1} min^{-1})	68.1 ± 2.4
HR_{max} (BPM)	180 ± 5

Table 3.2: Body mass change as a marker of hydration status over 2 hours of moderate-intensity exercise in hyperthermic and normothermic conditions (mean ± SD)

	W	***E***	***E+C***	***N***
Pre-exercise Body Mass (kg)	78.4 ± 13.3	78.5 ± 13.6	78.7 ± 13.2	83.7 ± 11.4
Post-exercise Body Mass (kg)	75.8 ± 12.7#	75.9 ± 13.2#	76.3 ± 12.9#	82.6 ± 11.3#
Body Mass Reduction (%)	3.2 ± 0.9*	3.0 ± 0.8*	3.0 ± 1.1*	1.1 ± 0.1^

* Indicates main effect for time from pre to post-exercise vs zero ($P < 0.05$)
# Indicates within condition difference pre to post-exercise ($P < 0.05$)
^ Indicates difference vs. W, E, and E+C conditions ($P < 0.05$)
W = water; E = electrolytes only (Gatorade Zero); E+C = electrolytes+carbohydrates (Gatorade; all exercise in the heat; n = 24); N = normothermic exercise with water only (n = 8)

Table 3.3: Plasma Osmolality and electrolyte concentrations in hyperthermic and normothermic exercise conditions (mean ± SD)

		W	***E***	***E+C***	***N***
Pre-exercise	**pOsm (mmol/L)**	287.1 ± 2.2	287.2 ± 1.7	289.4 ± 1.5	281.2 ± 0.9
	[Na^+] (mmol/L)	138.4 ± 1.7	138.6 ± 0.8	139.4 ± 0.7	135.9 ± 0.3
	[Ca^{2+}] (mmol/L)	9.7 ± 0.1	9.5 ± 0.1	9.7 ± 0.1	9.7 ± 0.1
	[Cl^-] (mmol/L)	105.1 ± 0.7	106.9 ± 0.6	105.5 ± 0.5	107.6 ± 0.7
	[K^+] (mmol/L)	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.2
		W	***E***	***E+C***	***N***
Post-exercise	**pOsm (mmol/L)**	288.6 ± 1.8	289.4 ± 1.5	291.4 ± 1.5	286.8 ± 1.4
	[Na^+] (mmol/L)	138.9 ± 0.8	139.6 ± 0.7	139.7 ± 0.7	138.9 ± 0.7
	[Ca^{2+}] (mmol/L)	10.2 ± 0.1	9.9 ± 0.1	9.8 ± 0.1	9.9 ± 0.1
	[Cl^-] (mmol/L)	102.4 ± 0.7	103.6 ± 0.8	103.8 ± 0.6	105.5 ± 0.9
	[K^+] (mmol/L)	4.1 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.5 ± 0.3

pOsm = plasma osmolality
W = water; E = electrolytes only (Gatorade Zero); E+C = electrolytes+carbohydrates (Gatorade; all exercise in the heat; n = 24); N = normothermic exercise with water only (n = 8)

Table 3.4: Time-control data for the cognitive battery (n = 6, mean ± SD)

	Stroop Effect (ms)	*Word-List Recall*	*TMD POMS*
Trial 1	40.1 ± 8.8	9 ± 2	3 ± 2
Trial 2	34.8 ± 15.8	10 ± 2	9 ± 1
Trial 3	44.1 ± 13.1	9 ± 2	7 ± 5

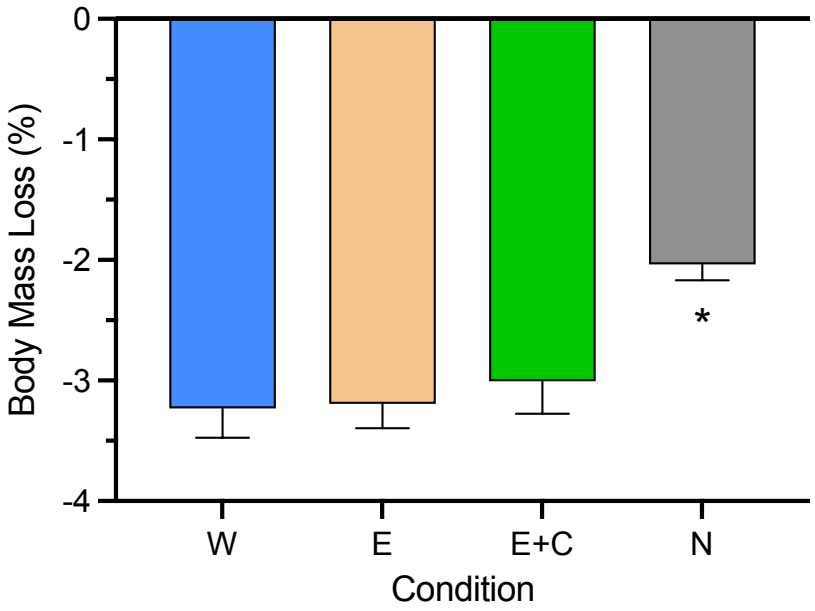

Figure 3.1: Changes in body mass after 120 minutes of moderate-intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8).
* Indicates difference compared to W, E, and E+C ($P < 0.05$).

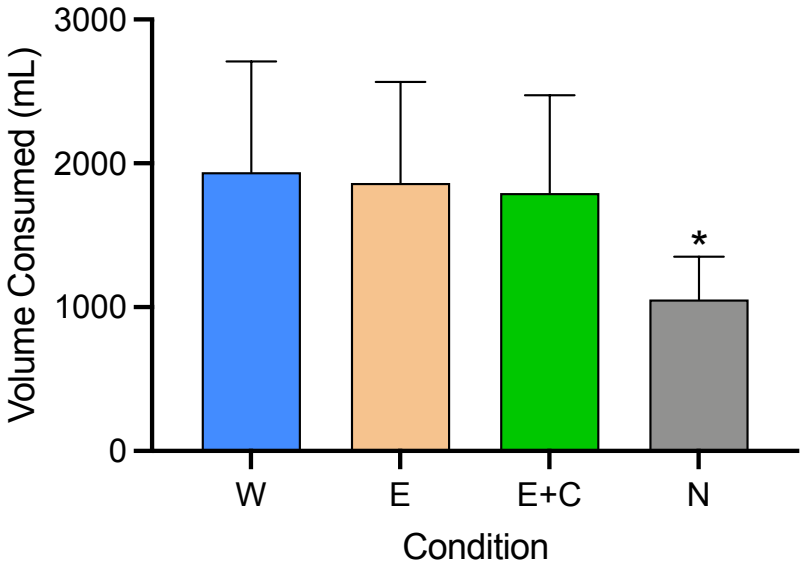

Figure 3.2: Total fluid consumption over 120 minutes of moderate intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8)
* Indicates difference compared to W, E, and EC ($P < 0.05$)

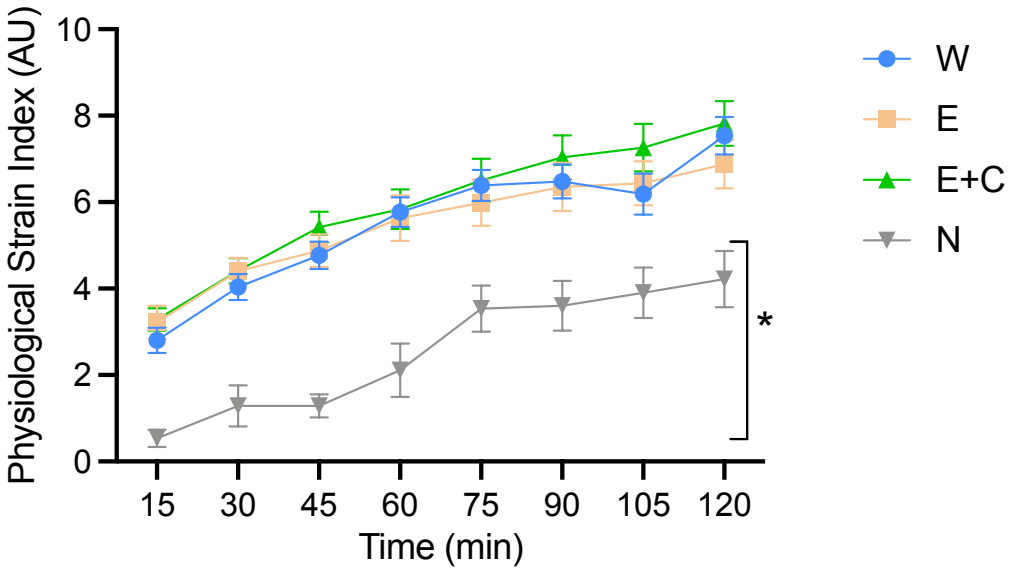

Figure 3.3: Physiological strain index (PSI) measured at 15 minute intervals over a 120 minute bout of moderate intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8)
* Indicates difference compared to W, E, and E+C at all time points ($P < 0.05$)

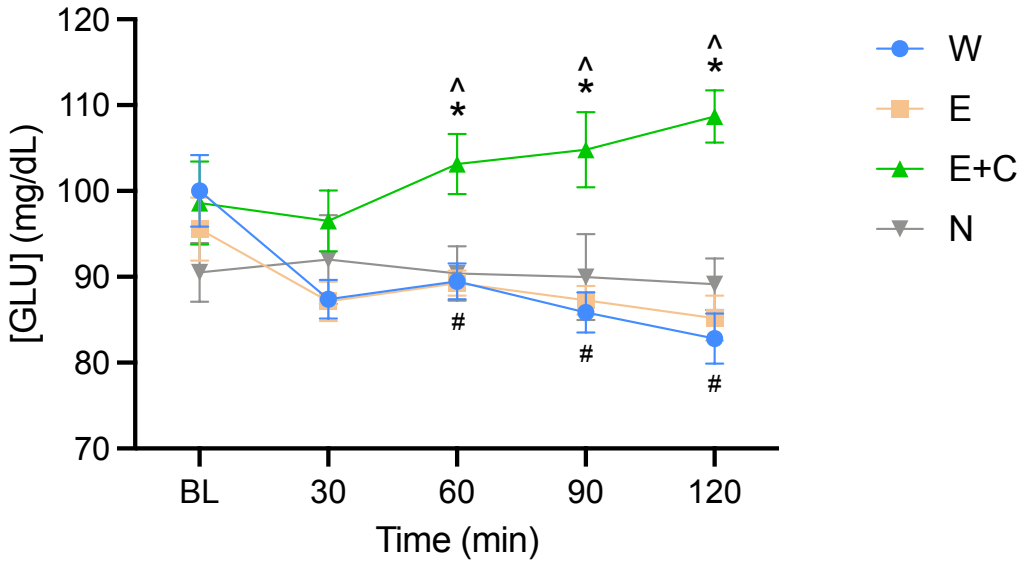

Figure 3.4: Blood glucose concentration (GLU) at baseline (BL) and 30 minute intervals over a 120 minute bout of moderate intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8).
***** Indicates significant compared to all other conditions at 60, 90, and 120 minute time points ($P < 0.05$)
# Indicates difference compared to BL for W and E at 60, 90, and 120 minute time points ($P < 0.05$)
^ Indicates difference compared to BL at 60, 90, and 120 minute time points ($P < 0.05$)

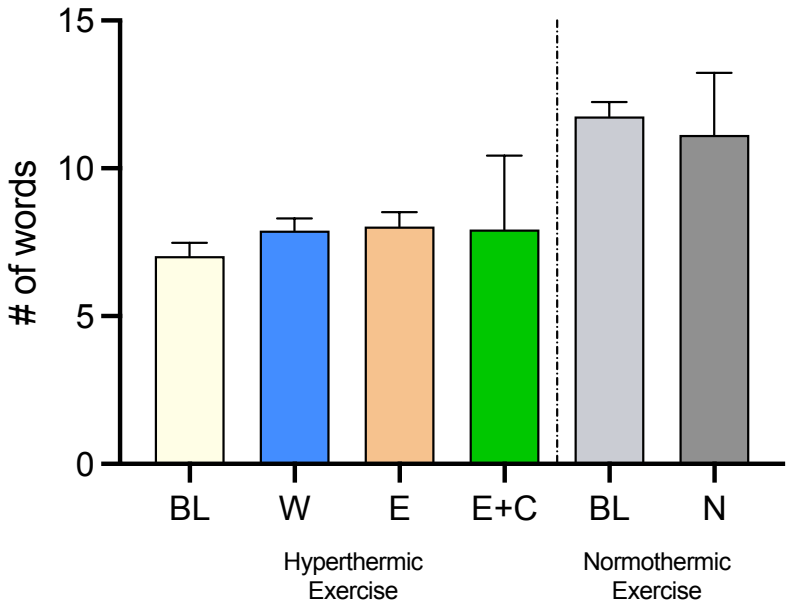

Figure 3.5: The effects of electrolyte and carbohydrate supplementation on short-term to long-term memory conversation following a 120 minute bout of moderate intensity exercise in hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8).

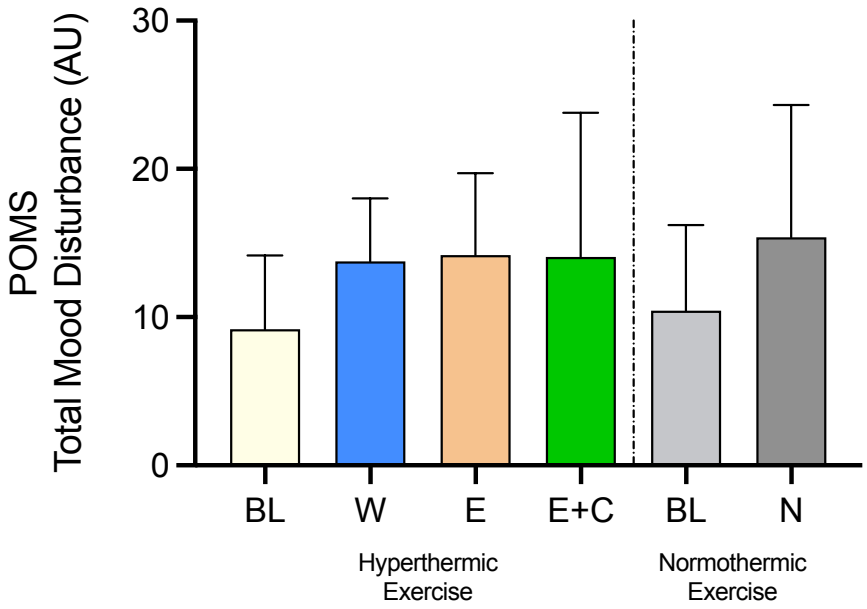

Figure 3.6: The change in Profile of Mood States (POMS) total mood disturbance score following a 120 minute bout of moderate intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8).

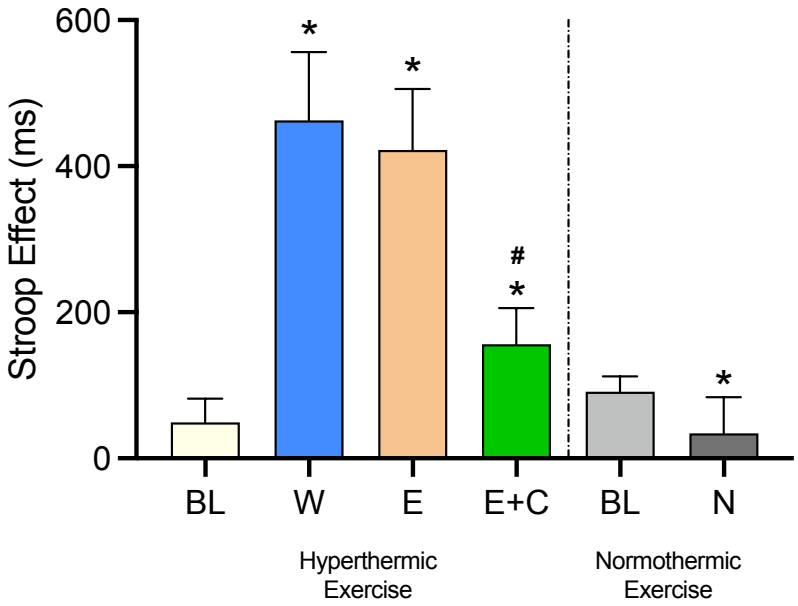

Figure 3.7: The change in Stroop Effect scores following a 120 minute bout of moderate intensity hyperthermic exercise while subjects ingested water (W), electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 24) and a normothermic exercise condition (water only; n = 8)
* Indicates difference compared to BL within condition ($P < 0.05$)
# Indicates difference compared to W and E ($P < 0.05$)

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CHAPTER IV – MANUSCRIPT III

Glucose Ingestion Attenuates Cognitive Dysfunction Following Long-Duration Passive Heat Stress In Humans

Summary

The purpose of this study was to determine if electrolyte or carbohydrate supplementation would reduce markers of cognitive decline in humans following long-duration passive heat stress. Fifteen subjects performed 2 visits of 120 minutes of passive heat stress wearing liquid perfused suits infused with 50ºC water. In random order, subjects consumed fluids supplemented with electrolytes (E) or electrolytes+carbohydrates (E+C). Pre- and post-heat stress, body mass (BM) and plasma osmolality (pOsm) were measured. Heat rate, blood pressure, Physiological Strain Index (PSI), core temperature (T_C), plasma glucose, plasma lactate, respiration rate, end-tidal CO_2, internal carotid artery (ICA) velocity, and ICA diameter were recorded every 15mins. Cognitive function was assessed via the Automated Neurophysiological Assessment Metric (simple vs. complex cognitive tasks) at 30 minutes and at 120 minutes. There were no significant differences between fluid conditions (E & E+C) for BM loss (0.33±0.2 & 0.22±0.2kg), pOsm (Δ3.2±8.0 & 4.8±6.0mmol/L), peak-PSI (6.2±0.3 & 5.9±0.3), peak T_C (38.9±0.1 38.9±0.1ºC), and ICA diameter (0.42±0.01 & 0.42±0.01 cm). Plasma glucose was significantly higher in the E+C condition at 60, 75, 90, 105, & 120 minutes ($P<0.05$). Importantly, compared to the E condition, the E+C condition demonstrated a significant increase in ICA blood velocity and blood flow at the 90, 105, and 120 minute time points ($P<0.05$). There were

no significant differences between the 2 fluid conditions for the simple cognitive tasks. Compared to the E condition, the E+C condition demonstrated improved performance on the complex cognitive tasks ($P<0.05$). These data are the first to suggest that glucose may have a protective effect on declines in cognitive function following long-duration passive heat stress. Furthermore, these data propose a novel mechanism of glucose modulating the sympathetic nervous system, allowing the preservation of blood flow through the ICA.

Introduction

During heat stress, the human body's thermoregulatory reflexes function to redistribute blood flow to dissipate heat to the environment in an effort to avoid dangerous rises in core temperature (Cheung et al., 2000; Wittbrodt et al., 2018). This automatic redistribution shunts blood from the core to the periphery, thereby causing cutaneous vasodilation for heat dissipation as well as decreases in venous return and cardiac output (Gonzalez-Alonso et al., 1997). During exercise and passive heat stress, the primary purpose of the redistribution of blood flow occurs to maintain adequate perfusion and nutrient supply to the working muscle (Nielsen et al., 1990) as well as to prevent uncompensable heat stress (Cheung et al., 2000), respectively. Uncompensable heat stress occurs when the body's heat production or storage exceeds that of heat loss, ultimately causing an imbalance in the heat balance equation (Moran et al., 1998). In a passive heat stress setting, where heat gain occurs not from metabolic heat production but from environmental factors (heat/humidity), heat stress commonly occurs in elderly or clinical populations (Armstrong & Kenny, 1993; Schlader

et al., 2015) as well as in auto racing sports (NASCAR, motorcycle, and boat), emergency response personnel (hazardous material personnel) and many military populations (aviators, navigators, aircrew, astronauts, and decontamination teams) (Greenleaf & Fortney, 1992; Ozaki et al., 2005; Gania et al., 2011; Hunt et al., 2016; Schlader et al., 2020).

When a passive heat stress stimulus is endured for short periods of time (<30 minutes), numerous studies report no change in cognitive function, both for simple and complex tasks (Racinais et al., 2008; Simmons et al., 2008; Schlader et al., 2015; Mollica et al., 2019; Piil et al., 2019). However, if the passive heat stress stimulus persists for a longer duration (>30 minutes), definite conclusions on the effects of said heat stress to cognitive function become equivocal (Radakovic et al., 2007; Schlader et al., 2015; Nakata et al., 2015; Kingma et al., 2020). In the studies that have observed cognitive decline following a longer bout of passive heat stress, the source of cognitive deterioration has been attributed to several causes to include reductions in hydration status (i.e. declines in total body water, plasma osmolality, and electrolyte concentrations) (Wittbrodt et al., 2018; Piil et al., 2019), attenuation in cerebral blood flow (Nybo et al., 2001; Nielsen & Nybo, 2003; Ainslie et al., 2009), hyperventilation-induced hypocapnia (Nybo et al., 2002; Nelson et al., 2011), and to a lesser extent, shifts in regional cerebral neurotransmitter utility (Edvinsson et al., 1975; Mitchell et al., 2009). Our lab recently completed studies focusing on both short (Deming et al., 2020) and long-duration (120 minutes) exercise heat stress and the associated cognitive changes (Deming et al., 2021). We found that, independent of electrolyte or carbohydrate supplementation, cognitive function was significantly attenuated

immediately following the long-duration exercise heat stress stimulus. Interestingly, we observed that in the cohort who supplemented with exogenous glucose during the bout of exercise heat stress, their associated cognitive decline was significantly attenuated to that of supplementation with water or electrolytes alone. We agree that the stimulus of long-duration passive heat stress physiologically differs from that of exercise heat stress, however, the protective role of glucose supplementation on cognitive decline following a long-duration passive heat stimulus has, to our knowledge, not yet been elucidated.

Using the background information above, we aimed to determine whether the consumption of exogenous glucose during a bout of long-duration (120 minutes) passive heat stress would reduce markers of cognitive dysfunction for both simple and complex cognitive tasks. As such, we hypothesized that, compared to consuming electrolytes alone, cognitive decline would be significantly attenuated when subjects ingested fluids supplemented with glucose during a bout of prolonged passive heat stress.

Methods

Ethical Approval and Human Subjects

Following informed, written consent and after completing a health and exercise history survey, 15 subjects (8 women, 7 men) were enrolled in the study. All subjects were between the ages of 18-35 years, healthy, recreationally active, and without dependency (i.e. nicotine, alcohol, or illicit drugs) or comorbidity that could limit normal thermoregulatory function (Table 1). The study was approved by the Colorado State

University Institutional Review Board (#20-10406H) and in accordance with the Declaration of Helsinki.

Screening Protocol

Subjects arrived to Colorado State University's Human Performance and Clinical Research Laboratory for the consent and screening visit as well as 2 passive heat stress study visits. The consent and screening visit was used to brief all participants on the timeline of the project as well as subject expectation. Finally, subjects completed a 30 minute cognitive battery to assess baseline values of both simple and complex cognitive function (Vista LifeSciences, Parker, CO, USA).

Experimental Protocol – Long-Duration Passive Heat Stress

For the 2 passive heat stress visits, the subjects arrived in a fasted state (4 hours) and ingested a core temperature (T^C) sensing pill (HQ Inc., Palmetto, Fl, USA) with 300 mL water approximately 4 hours prior to the start time of the visit. The pill allowed for T_C to be noninvasively measured throughout the bout of passive heat stress as well as validate that the subject's T_C did not reach dangerous levels (>39.5ºC). To standardize clothing, the subjects wore an outfit consisting of liquid perfused garments (LCGs) (CoolShirt Systems, PeachTree City, GA, USA). The dry weight of the subject was measured nude using a digital platform scale (MedWeigh MS-2510). Per a previous protocol used in our lab, subjects voided both bowel and bladder before nude weight measurement (Deming et al., 2020). Once the subject had donned the LCGs, they then laid supine to allow a 10 mL draw of venous blood from an antecubital vein. This blood

was used to assess baseline measurements of blood glucose, blood lactate, calcium (Ca^{2+}), chloride (Cl^{-}), potassium (K^{+}), sodium (Na^{+}), and blood-urea-nitrogen (BUN) via Piccolo Xpress Chemistry Analyzer (Abaxis, Inc., Union City, CA, USA). The metrics were also used to measure pre- and post-heat stress plasma osmolality concentration, which was calculated from the well-established equation by Purssell and colleagues (Purssell et al., 2001):

$$2\ Na^{+}\ (mEq/L) + [Urea\ (mg/dL)/2.8] + [Glucose\ (mg/dL)/18]$$

Once these blood measurements were completed, the subject donned 2 plastic waste bags with pre-cut holes for the subject's arms, legs, and head. These bags were required to limit heat dissipation through evaporation during the bout of heat stress. The subject then laid in a semirecumbent chair (45º from vertical) as a way to simulate occupations such as aviators, navigators, and auto racers. Subjects were covered with emergency thermal blankets to limit the escape of radiant heat. The LCGs were then connected to a cooler equipped with a flow control meter (CoolShirt Systems, PeachTree, GA, USA). The cooler was filled with tap water and heated to 50ºC via heating coil. Once the subject was in this position, baseline measurements were taken for heart rate (HR; pulse oximeter), blood pressure (BP), T_C, respiration rate (RR), end-tidal carbon dioxide ($etCO_2$), blood glucose, blood lactate, internal carotid artery (ICA) velocity, and ICA diameter. HR, BP, RR, and $etCO_2$ were monitored using a Cardiocap/5 (Datex-Ohmeda, Louisville, CO, USA) (Hearon et al., 2016). T_C was measured via remote (CoreTemp Data Recorder, HQ Inc., Palmetto, FL, USA). Glucose

and lactate were measured through finger prick and Contour® Next EZ Meter analysis (Ascensia Diabetes Care, Parsippany, NJ, USA). A 12 MHz linear-array ultrasound probe (Vivid 7; General Electric, Milwaukee, WI, USA) was used to assess resting ICA mean blood velocity and ICA diameter immediately caudal to the carotid bulb and subsequent bifurcation of the internal and external carotid arteries. Diameter measurements of the ICA were made in triplicate in duplex mode at end diastole and blood flow was calculated as described previously in our lab (Crecelius et al. 2010). Once baseleine measurements were taken, the cooler pump was turned on and the 50ºC water began flowing through the LCGs. Each subject performed the above protocol 2 separate times separated by at least 7 days to limit temperature acclimation (Cheung et al., 2000). The 2 fluid conditions were water supplemented with electrolytes (Gatorade Zero; E), and water supplemented with electrolytes and carbohydrates (Gatorade; E+C). Similar to previous protocols used in our lab (and others), Gatorade Zero was chosen to observe whether electrolyte supplementation alone (without carbohydrate) would alter fluid consumption amount as well as maintain pOsm concentrations during a long-duration bout of passive heat stress (Cuddy et al., 2008; Deming et al., 2020, 2021).

The order of fluid condition was randomized and counter-balanced. Subjects were not informed of which fluid they were assigned. Gatorade Zero contained 0.46 mg/mL Na^+ and 0.14 mg/mL K^+ while Gatorade contained 0.46 mg/mL Na^+, 0.13 mg/mL K^+, and 60.9 mg/mL of carbohydrates (mixture of glucose and dextrose). After the 120 minute bout of passive heat stress concluded, the subjects voided their bowel and bladder prior to dry weight measurement in the nude. Total fluid consumption was

measured prior to the start of heat stress. The ingested fluid amount matched a previous study performed in our lab focusing on long-duration exercise heat stress so we could accurately compare outcome variables (hydration status, glucose ingestion, and cognitive function) in both exercise and passive heat stress settings (Deming et al., 2021). Post-exercise pOsm was assessed identical to the pre-exercise technique through comprehensive metabolic panel analysis. The sum of 1) fluid consumed through drinking, 2) fluid lost through sweating, and 3) change of pOsm concentrations, allowed the assessment of hydration status. Finally, cardiovascular and thermal strain were measured via the Physiological Strain Index (PSI) equation established by Moran and colleagues (1998):

$$PSI = 5(T_{ret} - T_{re0}) * (39.5 - T_{re0})^{-1} + 5(HR_t - HR_0) * (180 - HR_0)^{-1}$$

where T_{ret} and HR_t are measurements for core body temperature and HR taken at predetermined points throughout an exercise stimulus, while T_{re0} and HR_0 are the initial measurements for core body temperature and HR immediately prior to the initiation of exercise (Moran et al., 1998). For the present study protocol, T_{ret} and HR_t were assessed at 15 minute intervals throughout the passive heat stress stimulus. All measurements (HR, BP, RR, $etCO_2$, T_C, glucose, lactate, and PSI) were measured at each 15 minute interval throughout the 120 minute passive heat stress stimulus. All study day visits were performed in a temperature-controlled laboratory room of 21ºC.

Cognitive Function Battery

A series of cognitive tests (Automated Neurophysiological Assessment Metric, Vista LifeSciences, Parker, CO, USA) were utilized to measure response inhibition, selective attention, reaction time, visuo-motor response timing, spatial processing, concentration, working memory, interference inhibition, and executive functioning. Baseline values of these tests were measured at the consent and screening visit.

Response inhibition was evaluated via the Go/No-Go Test. This test presented the subject with an “x” or an “o”. The subject was instructed to respond with a click on their computer mouse as quickly as possible when an “x” was seen. When the “o” appeared, the subject was instructed to do nothing (i.e. response inhibition). Attention, reaction time, and visuo-motor response timing was assessed through the Simple Reaction Time Test. This test presented the “*” symbol on the computer screen and asked the subject to respond to the symbol as quickly as they could by clicking the computer mouse button. Spatial processing and visuo-spatial working memory were evaluated via the Spatial Processing Test, which displayed 2 four-bar histograms to the subject. The subject would have to quickly ascertain if the 2 histograms were identical or different. One of the histograms would be rotated either clockwise or counterclockwise by 90º requiring the subject to process the spatial orientation of the figures (i.e. spatial processing). Sustained attention, concentration, and working memory were evaluated through the Standard Continuous Performance Test. This test required the subject to view letters presented on the computer screen one-by-one. The subject was given a target letter at the beginning of the test (ex: the letter “B”) and when that letter was displayed, the subject would click the computer mouse button. The

subject would not click the button for all other letters presented on the screen. Lastly, processing speed, selective attention, interference inhibition, and executive function (collective known as the Stroop Effect) were assessed through the Stoop Color and Word Test (SCWT) as described previously (Deming et al., 2021).

The Go/No-Go Test, Simple Reaction Time Test, and Standard Continuous Performance Test are considered to be “simple” cognitive tasks while the Spatial Processing test and SCWT are known to be “complex” or “higher-order” cognitive tasks (Taylor et al., 2016). On each of the 2 passive heat stress study day visits, these 5 cognitive tests were repeated following the 120 minute bout of heat stress. In addition to the 120 minute evaluation of these tests, the SCWT was also assessed at 60 minutes into the heat stress stimulus. This was selected because (1) the SCWT was the quickest cognitive test of the 5 tests chosen and (2) it allowed the comparison of the effects of short-duration passive heat stress (60 minutes) to a study previously published study in our lab (Deming et al., 2021). All tests have been validated to have no “training” or “learning” effect (Roebuck-Spencer et al., 2007).

Data Acquisition and Statistical Analysis

Data were collected and stored on computer at 250 Hz and analyzed with signal-processing software (WinDaq; DATAQ Instruments, Akron, OH, USA). Baseline ICA blood flow represent an average of the last 30 seconds of the measuring period. Data for the 2 fluid conditions were group averaged and statistically compared using a 2-way, repeated measures ANOVA and multiple comparisons. Individual fluid conditions were also compared to baseline values via 2-tailed T-tests. The presence of main and

interaction effects between conditions were determined with repeated measures ANOVA at each of the exercise time points (condition versus time). Statistical significance was set at $P < 0.05$ (*a priori*).

Results

Body Mass and Fluid Consumption

Body mass was not significantly different from baseline in the 2 fluid conditions following the passive heat stress protocol (Table 2 and Figure 1). During the protocol, fluid consumption was matched to equal the amount a previous cohort consumed in a 2021 study performed in our lab (Deming et al., 2021). As such, all subjects drank enough fluid to consume approximately 1.8 L of fluid (both Gatorade Zero;E and Gatorade;E+C) or approximately 109.6 g of carbohydrate (mixture of glucose and sucrose). Because the amount of fluid was matched, there were no significant differences in total fluid consumed during the 120 minute bout of passive heat stress between the 2 fluid conditions.

Cardiovascular and Thermal Strain

As anticipated, there was a main effect of time for T_C and PSI during the 120 minute bout of passive heat stress ($P < 0.05$) (Figures 2 and 3, respectively). However, there were no significant differences between the 2 fluid conditions in cardiovascular or thermal strain at any 15 minute measurement interval.

Ventilatory Measures

Similar to PSI, both etCO2 and respiration rate demonstrated a main effect of time over the 120 minute bout of passive heat stress ($P < 0.05$) (Figures 4 and 5, respectively). There were no significant differences between the 2 fluid conditions for either ventilatory measure for pre- to post-heat stress or at any 15 minute time point.

Haematological Measures

At baseline (pre-heat stress), there were no significant differences in pOsm or electrolyte concentration between the 2 fluid conditions. Additionally, there were no significant changes in pOsm or electrolyte concentration after the 120 minute bout of passive heat stress (Table 3). Baseline glucose ranged from ~90-95 mg/dL and was similar for the first 30 minutes of passive heat stress. Compared the E condition, blood glucose concentration was significantly higher in the E+C condition at the 45, 60, 90 105, and 120 minute time points ($P < 0.05$) (Figure 6). Additionally, blood glucose continued to be significantly higher in the E+C condition at the 150 minute mark, when venous blood samples were analyzed again via comprehensive metabolic panel ($P < 0.05$). Compared to baseline, glucose significantly decreased over time in the E condition from the 60 minute mark through end-heat stress ($P < 0.05$).

Cognitive Battery

Compared to baseline, there were no significant differences between the Go/No-Go, Simple Reaction Time, and Standard Continuous Performance Tests (i.e. the simple cognitive tasks). Contrary to these tests, compared to baseline, the Spatial

Processing Test and the SCWT (i.e. the complex cognitive tasks) were both significantly impaired (increased reaction times and Stroop Effect, respectively) following the 120 minute bout of passive heat stress in the E condition (Table 5 and Figures 8 and 9, respectively). Specifically for the different components of reaction time measured within the Spatial Processing Test, compared to baseline, mean reaction time, mean reaction time for responding to histograms that were the same, and mean reaction time for responding to histograms that were different, were all significantly increased in the E condition ($P < 0.05$) (Figure 8). Similarly, in regards to the Stroop Effect, compared to baseline, at the 60 minute SCWT assessment, there were no significant differences between the 2 fluid conditions. However, at the 120 minute SCWT assessment, compared to baseline, there was a significant increase in Stroop Effect in the E condition only ($P< 0.05$). Notably, this effect of increased reaction times and Stroop Effect scores were almost completely erased (~99%) in the E+C condition ($P < 0.05$).

Haemodynamics and Vascular Measures

Systemic haemodynamics are presented in Table 4. A main effect of time was observed for HR, ICA blood velocity, and ICA blood flow during the 120 minute bout of passive heat stress ($P < 0.05$). There were no significant differences in ICA diameter between the 2 fluid conditions. Compared to the E condition, ICA blood flow was significantly increased within the E+C condition at the 90, 105, and 120 minute marks ($P < 0.05$) (Figure 7).

Discussion

The primary aim of this study was to determine the effects of 2 different fluid conditions (electrolytes alone and electrolytes+carbohydrates) on cognitive function following a bout of long-duration passive heat stress. Our hypotheses were that, compared to the electrolytes alone, the decline in cognitive function following a bout of prolonged passive heat stress would be significantly attenuated when subjects consumed fluids supplemented with glucose, similar to a previous study performed in out lab focusing on long-duration exercise heat stress (Deming et al., 2021). The principle findings of this study are that compared to the electrolyte only condition, cognitive function was less impaired in the electrolytes+carbohydrates condition during complex cognitive tasks. Interestingly, when compared to the electrolyte only condition, we also observed a significant increase in global cerebral blood flow through the ICA in the electrolyte+carbohydrate condition.

Body Mass and Fluid Consumption

Changes in body mass throughout the 120 minute bout of passive heat stress was not significantly different between the 2 fluid conditions. Independent of supplementation variety (electrolytes vs. carbohydrates), subjects lost ~0.5% of their body mass from pre- to post-heat stress (Table 2). This reduction in body mass does not reach the clinical threshold value of >2% for a diagnosis of dehydration (Nybo et al., 2001; Cheuvront et al., 2003). As we made the attempt to equal fluid consumption from a recently published study in our lab as a way to match glucose ingestion as well as

compare the results of this study to long-duration exercise heat stress, the negligible amount of body mass lost during the stimulus was not of concern (Deming et al., 2021).

Similar to body mass, there were no significant differences in fluid consumption among the 2 fluid conditions. As previously mentioned, we intentionally had subjects consume identical amounts of fluid that a cohort of subjects consumed in a previously published study in our lab (Deming et al., 2021). This was selected as a way to validate that the subjects in the present study were ingesting roughly the same amount of glucose (~109.6 g) as the previous cohort (see methods), allowing us to ascertain and compare the differences between long-duration passive and exercise heat stimuli on hydration status and cognitive function. Furthermore, this data is consistent with previous studies, which suggest that when fluid consumption was not significantly different (water vs. electrolytes vs. carbohydrates), there were no significant differences in body mass lost during long-duration hyperthermic exercise (Burstein et al., 1994; Byrne et al., 2005; Deming et al., 2020). To our knowledge, this is the first study to investigate the effects of supplementation variety (electrolytes vs. carbohydrates) on fluid consumption, body mass loss, and plasma osmolality changes (i.e. changes in hydration status) in a long-duration passive heat stress stimulus.

Cardiovascular and Thermal Strain – Physiological Strain Index

Secondary to the chosen passive heat stress stimulus (50ºC water infusion for 120 mintues) and a core temperature termination of 39.5ºC, we anticipated for there to be an increase in both metrics used to calculate Physiological Strain Index (HR and T_C). Both cardiovascular and thermal strain were not significantly different between the 2

fluid conditions. Similar to previous studies performed in a controlled laboratory, PSI increased in a time-dependent manner during the 120 minute bout of passive heat stress. Peak-PSI for the electrolyte and electrolyte+carbohydrate conditions were 6.0 and 5.9, respectively. These PSI values are similar to previously published long-duration passive heat stress studies (Nunneley et al., 2002; Yokota et al., 2002). The negligible differences in PSI for both the electrolyte and electrolyte+carbohydrate conditions suggests that supplementation variety (electrolytes vs. carbohydrates) does not significantly affect PSI during long-duration passive heat stress, which is similarly observed during long-duration exercise heat stress (Deming et al. 2020, 2021)

Ventilatory and Haematological Measures

No significant differences were observed from baseline for $etCO_2$ or respiration rate between the 2 fluid conditions. Our data is similar to other studies where during long-duration passive heat stress, there is a rise in respiration rate and a subsequent drop in $etCO_2$ secondary to hyperventilation-induced hypocapnia(Nybo et al., 2001; Nielsen & Nybo, 2003; Ainslie et al., 2009).

Comparable to our lab's most recent study focusing on the differences between long-duration hyperthermic and normothermic exercise, there were no significant changes in response to the long-duration passive heat stress stimulus to either pOsm or plasma electrolyte concentrations (Deming et al., 2021). This is not surprising secondary to both the electrolyte and electrolyte+carbohydrate conditions not meeting a clinically dehydrated state (>2% body mass loss). This is consistent with other studies indicating that 120 minutes of passive heat stress may not be sufficient to stimulate

significant changes in pOsm or electrolyte concentration particularly when subjects are allowed to consume fluids during the heat stress stimulus (Nunneley et al., 2002; Ainslie et al., 2009). Blood glucose increased during the bout of passive heat stress in the in electrolyte only condition while the opposite was noted in the electrolyte+carbohydrate condition (Figure 4). The electrolyte+carbohydrate condition was the only fluid that offered exogenous glucose and as such, the observed increase in blood glucose was expected within this cohort. Interestingly, this increase in plasma glucose may in fact offer a protective effect to cognitive decline secondary to long-duration passive heat stress (see below).

Cognitive Battery

Simple Cognitive Tests

Following the 120 minute bout of passive heat stress, there were no significant differences between the 2 fluid conditions for the battery of simple task cognitive tests (i.e. the Go/No-Go, Simple Reaction Time Test, and Standard Continuous Performance Test). These data relate well to other studies focusing on the effect of long-duration exercise heat stress on simple tasks of cognition (Bandelow et al., 2010). Although this may be the first study to monitor changes in these tasks following a bout of long-duration passive heat stress, our data suggests that these cognitive domains remain well preserved during this form of heat stress challenge.

Complex Cognitive Tests

Compared to the electrolyte condition, the electrolyte+carbohydrate condition demonstrated a significate reduction in reaction time for the battery of complex cognitive tests (Spatial Processing Test and The Stroop Color Word and Word Test). The Stroop Effect is the final score of the SCWT and it assesses the frontal lobe's ability to provide selective attention and some executive control processes as well as reaction time and cognitive processing speed (Zomeren, 1992). Interestingly, this area of the brain's blood flow is fed through the ICA and its subsequent bifurcations to the middle cerebral artery (MCA) and anterior cerebral artery (ACA). Indeed, there may be other physiological mechanisms as to why glucose attenuated the decline in cognitive function secondary to long-duration passive heat stress in the E condition, however, it is noteworthy to consider that this attenuation may have occurred from a novel mechanism relating glucose-sensitive chemoreceptors in the carotid bulb modulating sympathetic tone of the ICA (see below).

Haemodynamics and Vascular Measures

There were no significant differences in baseline ICA diameter or at any of the 15 minute time points between the 2 fluid conditions. This is comparable to other studies concentrating on changes to global and regional cerebral blood flow with and without a passive heat stress stimulus (Nybo et al., 2001; Nielsen & Nybo, 2003; Ainslie et al., 2009). However, these studies concluded that the decreases in cerebral blood flow were secondary to hyperventilation-induced hypocapnia. Conversely, compared to the electrolyte condition, the electrolyte+carbohydrate condition had a significant increase in

ICA mean blood velocity and blood flow at the 90, 105, and 120 minute time points suggesting that exogenous glucose supplementation may in fact increase global or regional cerebral blood flow secondary to long-duration passive heat stress.

Mechanistically speaking, the effect of glucose on cerebral blood flow is unknown but could possibly work through chemoreceptors in the carotid bulb, potentially modulating the threshold for sympathetic nervous system activation. This has been noted in animal models and humans in vivo during bouts of hypoglycemia (Koyama et al., 2000, 2001; Holmes et al., 2012; Ward et al., 2007; Joyner et al., 2018). The sympathetic nervous system's role in lipolysis, glycolysis and hepatic glucose output has been well documented in the literature (Cannon, 1929; Weiss & Maickel, 1968; Hayes et al., 2009, 2010; Fliers et al., 2010). In moments of "fight or flight", the sympathetic nervous system increases the above-mentioned metabolic pathways to increase glucose availability for the likelihood of increased energy demands on the body (Hayes et al., 2009, 2010). Additionally, with activation of the sympathetic nervous system, the subsequent secretion of catecholamines (epinephrine and norepinephrine) occurs, which can cause systemic vasoconstriction peripherally and centrally, to include the cerebral vasculature (Dinenno et al., 2003; Richards et al., 2017, MacKenzie et al., 1976). Increased sympathetic tone on the cerebral vasculature, globally or regionally, will cause decreased blood flow and consequently, decreases in nutrient delivery for cerebral metabolism (Ide et al., 2000; Ogoh et al., 2009; Sato et al., 2009). During times of long-duration passive heat stress, other labs have observed that blood flow through the ICA decreases while other arteries that feed the more autonomic portions of the brain, such as the vertebral, posterior, and middle cerebral arteries, do not demonstrate

significant changes in flow (Nybo et al., 2001; Ainslie et al., 2009). For example, the external cerebral artery increases flow during both exercise and passive heat stress as a way to increase cutaneous flow to dissipate heat. Additionally, the vertebral and posterior cerebral arteries do not demonstrate significant changes in blood flow secondary to short- or long-duration heat stress. This has been suggested to be secondary to the areas of the brain that these arteries feed are for autonomic functions and hence, more important during life or death situations (Nybo et al., 2001, Ainslie et al., 2009). The ICA on the other hand, may not be as important during these types of situations. The ICA bifurcates to become and anterior and middle cerebral arteries (Mchedlishvili et al, 1973; Gilbert & Burgess, 2008). The ACA supplies blood to the frontal and parietal lobes while the MCA supplies blood to the lateral lobes and areas of cerebral hemispheres that are charged with more voluntary and executive functions, such as the memory, problem solving, attention, and impulse control (Gilbert & Burgess, 2008). In periods of “fight or flight”, these portions of executive function may not be as vital for survival as our motor output areas or higher order centers of the brain (i.e. the cardiovascular and ventilatory control centers) (Nybo et al., 2001, Nielsen & Nybo, 2003, Ainslie et al., 2009).

In this study, compared to the electrolyte condition, we observed a significant decrease in both HR and BP in the electrolyte+carbohydrate condition, suggesting that exogenous glucose administration may modulate the sympathetic nervous system secondary to increased glucose concentration for cerebral metabolism. If this is indeed possible, the question remains: through what physiological mechanism(s) does exogenous glucose modulate the sympathetic nervous system to acutely affect blood

flow through the ICA? Several studies have pointed to the carotid bulb as an area with a high concentration of chemoreceptors that can be very sensitive to glucose (Koyama et al., 2000, 2001; Holmes et al., 2012; Ward et al., 2007; Shin et al., 2014; Joyner et al., 2018). It is possible that these specialized chemoreceptors are charged with modulating the sympathetic nervous system in the presence of increased concentrations of glucose as a way to maintain flow to the areas of the brain that the ICA, ACA, and MCA feed in an effort to preserve blood flow for carrying out higher order/thinking cognitive tasks during situations like “fight or flight”. Ribeiro and colleagues (2013) as well as Limberg and colleagues (2014) have shown that highly specialized chemoreceptors do indeed exist, however that they are very sensitive to insulin concentrations (Ribeiro et al. 2013; Limberg et al. 2014). Unfortunately, because we did not measure insulin, nor did we measure catecholamine or cortisol concentrations during the 120 minute bout of passive heat stress, framing definitive conclusions regarding glucose and sympathetic modulation is difficult. However, the literature supports the presence of specialized chemoreceptors in the carotid bulb making the concept of glucose-sensitive chemoreceptors not too unimaginable. Uncovering (1) whether or not glucose has this effect on carotid chemoreceptors and (2) the presence of glucose-sensitive chemoreceptors are areas of future research.

Limitations

There are some limitations for this study that merit discussion. First, this experiment was highly controlled in a laboratory setting. Consequently, our discussion points and conclusions are specific to the experimental conditions employed, and we

acknowledge that any inferences to real-world occupations are somewhat limited. A second limitation is that we were unable to measure cortisol or catecholamine concentrations to gain insight to whether reductions in cerebral perfusion is related to impaired cognitive function during long-duration passive heat stress secondary to sympathetic nervous system activation. Adding cortisol and catecholamine measurements would have allowed for an enhanced understanding of changes in cerebral blood flow specific to glucose delivery as well as the possible effect of exogenous glucose ingestion on sympathetic nervous system modulation through the specialized chemoreceptors within the carotid bulb. Ultimately, this would have permitted for a greater comprehension of mechanisms directly involved in cognitive control under long-duration passive heat stress.

Conclusions

It is our understanding that these data are the first to demonstrate that (1) glucose supplementation attenuates the decline in cognitive function secondary to long-duration (≥120 minutes) passive heat stress and (2) glucose may be working through an unknown mechanisms to modulate the sympathetic nervous system to acutely change global cerebral blood flow through the internal carotid artery. In fact, compared to ingesting electrolytes alone, the attenuation in cognitive decline was nearly 100%. Maintaining normal cognitive function and processing speeds during a bout of long-duration passive heat stress is a fundamental trait for many military (aviators, navigators, aircrew, astronauts, and decontamination teams) and athletic (auto racing

sports) personnel. As such consuming beverages that may indeed attenuate cognitive decline is of the utmost importance and must be considered for future scientific inquiry.

Table 4.1: Subject characteristics (mean ± SD)

	n = 15
Age (years)	26.2 ± 2
Body Mass (kg)	68.9 ± 2.6
BMI (kg/m^2)	23.2 ± 0.6
HR_{max} (BPM)	183 ± 8

Table 4.2: Body mass change as a marker of hydration status over 2 hours of passive heat stress (mean ± SD)

	E	***E+C***
Pre-Heat Stress Body Mass (kg)	69.5 ± 9.8	68.8 ± 9.3
Post-Heat Stress Body Mass (kg)	69.1 ± 9.6	68.6 ± 9.3
Body Mass Reduction (%)	0.6 ± 0.8	0.3 ± 0.9

E = Gatorade Zero; E+C = Gatorade (n = 15)

Table 4.3: Plasma Osmolality and electrolyte concentrations pre- and post-passive heat stress stimulus (mean ± SD)

		E	***E+C***
Pre-Heat Stress	**pOsm (mmol/L)**	288.2 ± 2.1	287.5 ± 1.8
	[Na^+] (mmol/L)	138.9 ± 1.0	138.5 ± 0.9
	[Ca^{2+}] (mmol/L)	9.9 ± 0.1	9.9 ± 0.1
	[Cl^-] (mmol/L)	108.1 ± 0.3	108.9 ± 0.4
	[K^+] (mmol/L)	4.4 ± 0.1	4.2 ± 0.1
		E	***E+C***
Post-Heat Stress	**pOsm (mmol/L)**	291.5 ± 1.0	293.4 ± 1.5
	[Na^+] (mmol/L)	140.4 ± 0.6	140.9 ± 0.7
	[Ca^{2+}] (mmol/L)	10.2 ± 0.2	9.8 ± 0.1
	[Cl^-] (mmol/L)	107.3 ± 0.9	108.1 ± 0.8
	[K^+] (mmol/L)	4.5 ± 0.2	4.2 ± 0.7

pOsm = plasma osmolality
E = Gatorade Zero; E+C = Gatorade (n = 15)

Table 4.3: Internal Carotid Artery Haemodynamics at baseline and at 2 hours of passive heat stress (mean ± SD)

	E				*E+C*			
	***HR* (bpm)**	***ICA*$_V$ (cm/s)**	***ICA*$_{BF}$ (ml/min)**	***ICA D*$_{mean}$ (cm)**	***HR* (bpm)**	***ICA*$_V$ (cm/s)**	***ICA*$_{BF}$ (ml/min)**	***ICA D*$_{mean}$ (cm)**
Pre-Heat Stress	62.6 ± 9.9	30.0 ± 1.7	240.6 ± 39.4	0.41 ± 0.03	59.5 ± 9.6	29.4 ± 1.2	239.4 ± 37.5	0.41 ± 0.03
Post-Heat Stress	123 ± 9.2*	23.0 ± 2.4*	188.4 ± 27.6*	0.42 ± 0.02	107.1 ± 3.9$^{*\#}$	26.4 ± 5.5$^{*\#}$	216.3 ± 21.3$^{*\#}$	0.42 ± 0.02*

* Indicates within condition difference pre- to post-heat stress (P < 0.05)
# Indicates difference between E and E+C conditions (P < 0.05)
ICA_V = Internal Carotid Artery Mean Velocity; ICA D_{mean} = Internal Carotid Artery Mean Diameter; ICA_{BF} = Internal Carotid Artery Blood Flow; E = Gatorade Zero; E+C = Gatorade (all exercise in the heat; n = 15)

Table 4.5: Cognitive function on complex tasks during and immediately following 2 hours of passive heat stress (mean ± SD)

		Baseline	*Post-Heat Stress E*	*Post-Heat Stress E+C*
Stroop Effect (ms)		28.1 ± 4.2	230.3 ± 18.6*	49.5 ± 3.1*#
Spatial Processing Test	**MRT**	1,752.1 ± 105.8	2,280.9 ± 113.8*	1,788.8 ± 129.2#
	$MRTC_{same}$	1,802.0 ± 143.0	2,470.7 ± 157.6*	1,842.6 ± 153.9#
	$MRTC_{diff}$	1,710.1 ± 89.4	2,246.1 ± 89.2*	1,713.8 ± 114.8#

* Indicates difference between baseline within condition ($P < 0.05$)
# Indicates difference between E, and E+C conditions ($P < 0.05$)
MRT = Mean Reaction Time; $MRTC_{same}$ = Mean Reaction Time Correct Same Histograms; $MRTC_{diff}$ = Mean Reaction Time Correct Different Histograms; E = Gatorade Zero; E+C = Gatorade (all exercise in the heat; n = 15)

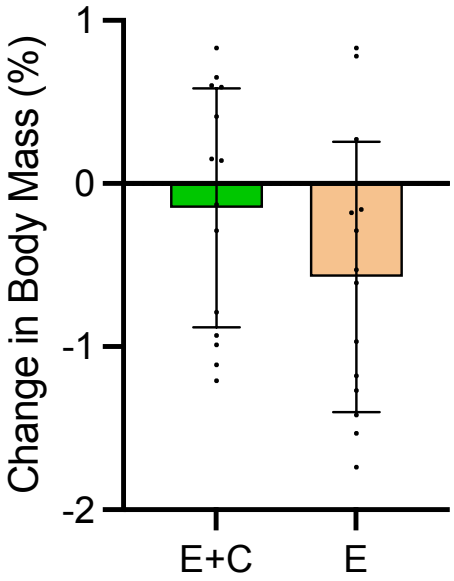

Figure 4.1: Changes in body mass after 120 minutes of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).

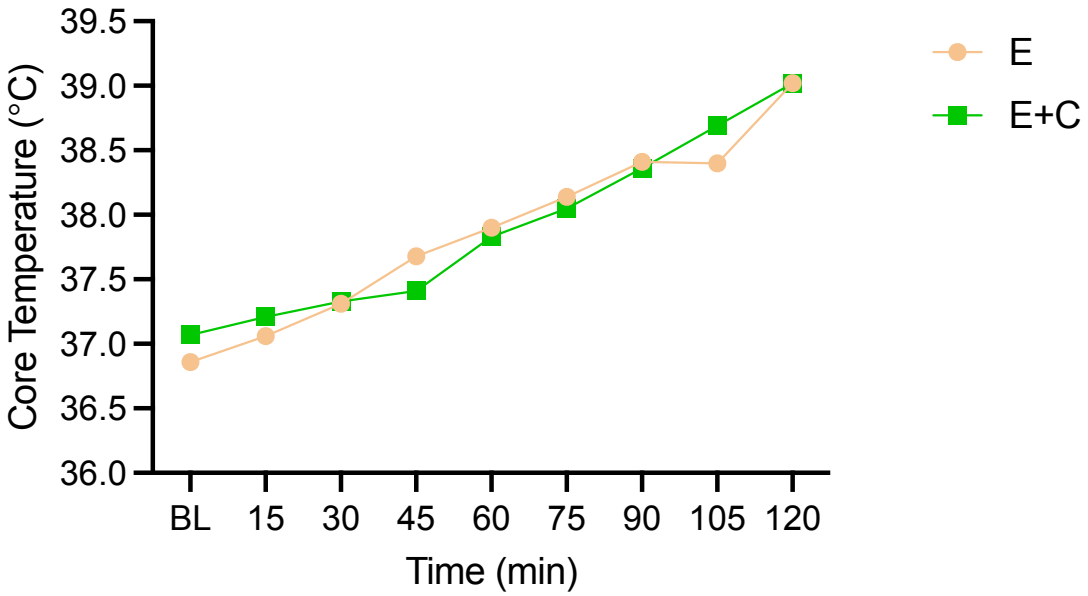

Figure 4.2: Core temperature measured at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).

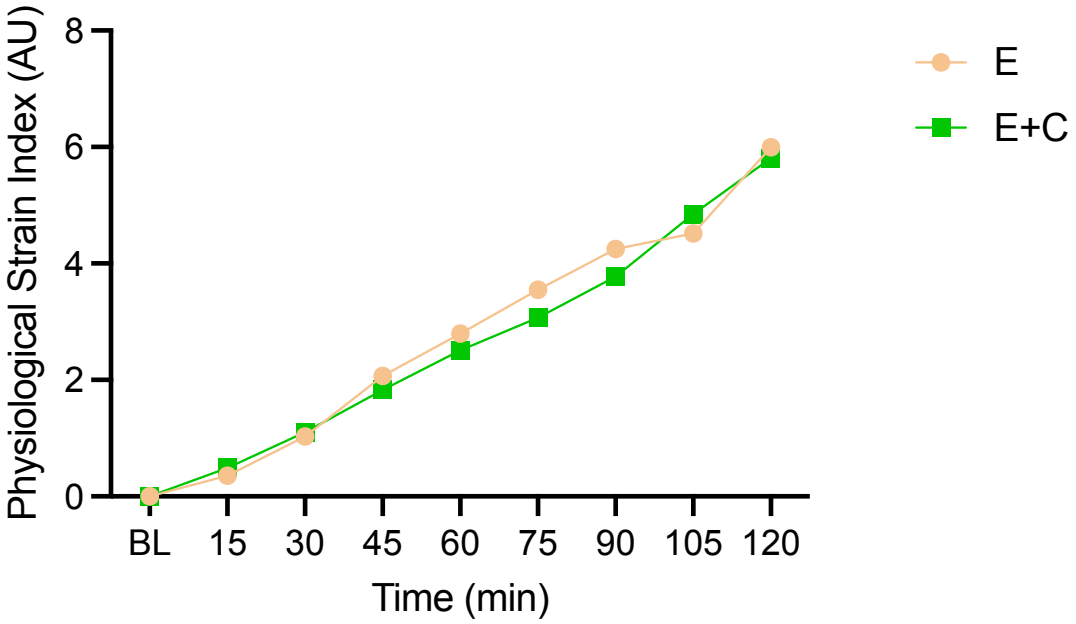

Figure 4.3: Physiological strain index (PSI) measured at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).

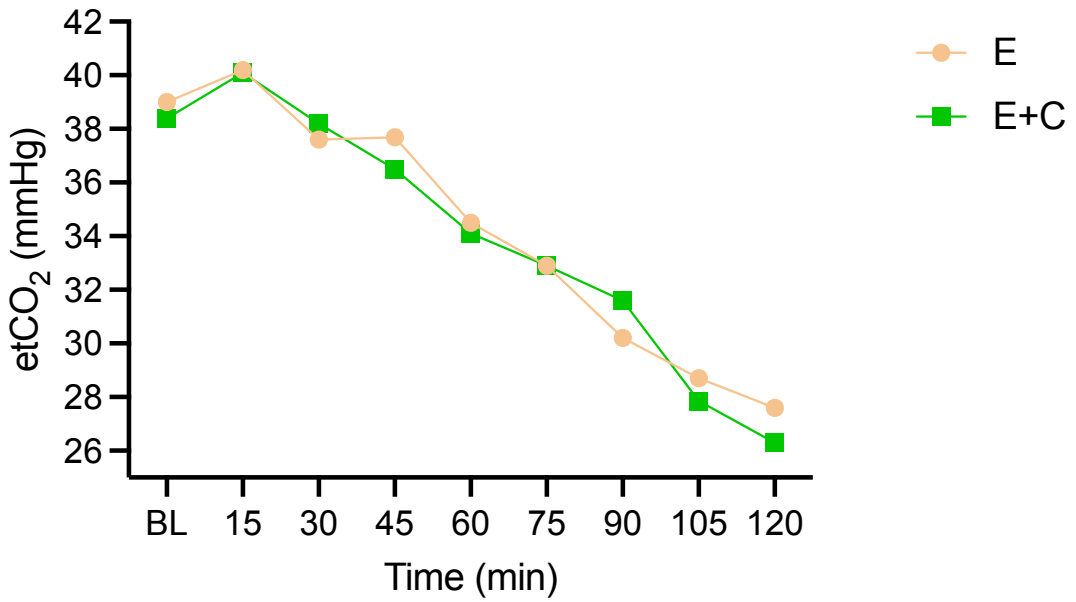

Figure 4.4: End-tidal carbon dioxide measured via nasal cannula at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).
***** Indicates difference compared to BL within condition ($P < 0.05$)

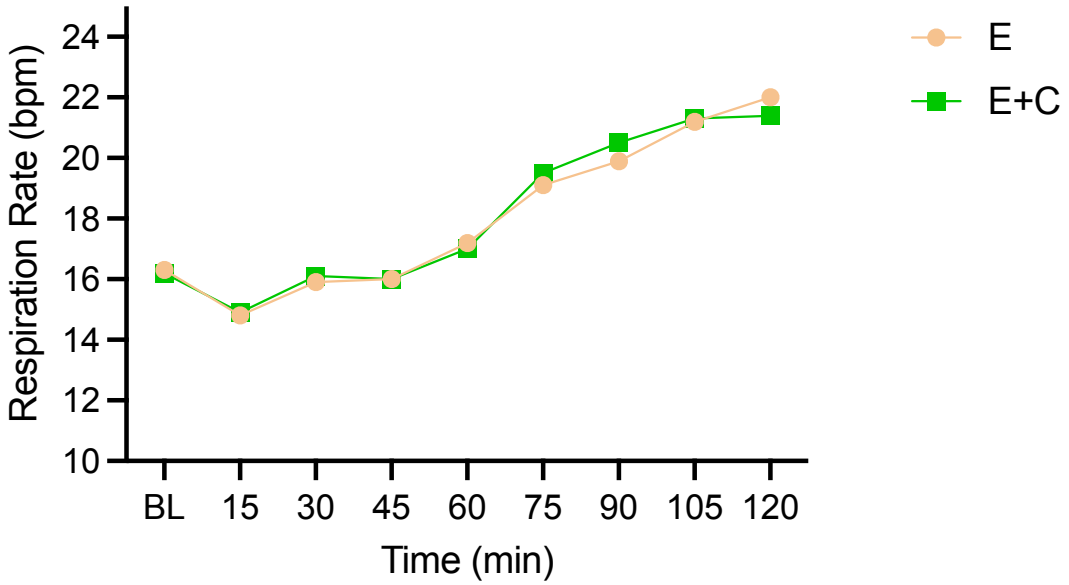

Figure 4.5: Respiration Rate measured at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).

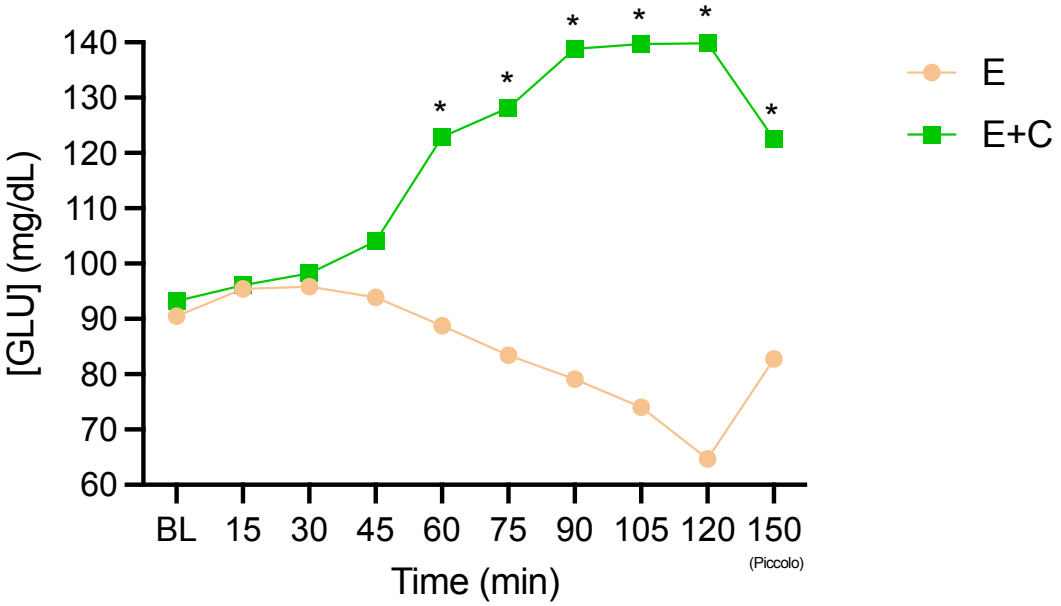

Figure 4.6: Blood glucose concentration (GLU) at baseline (BL) at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).
* Indicates significant compared to E at 60, 75, 90, 105, 120, and 150 minute time points ($P < 0.05$)

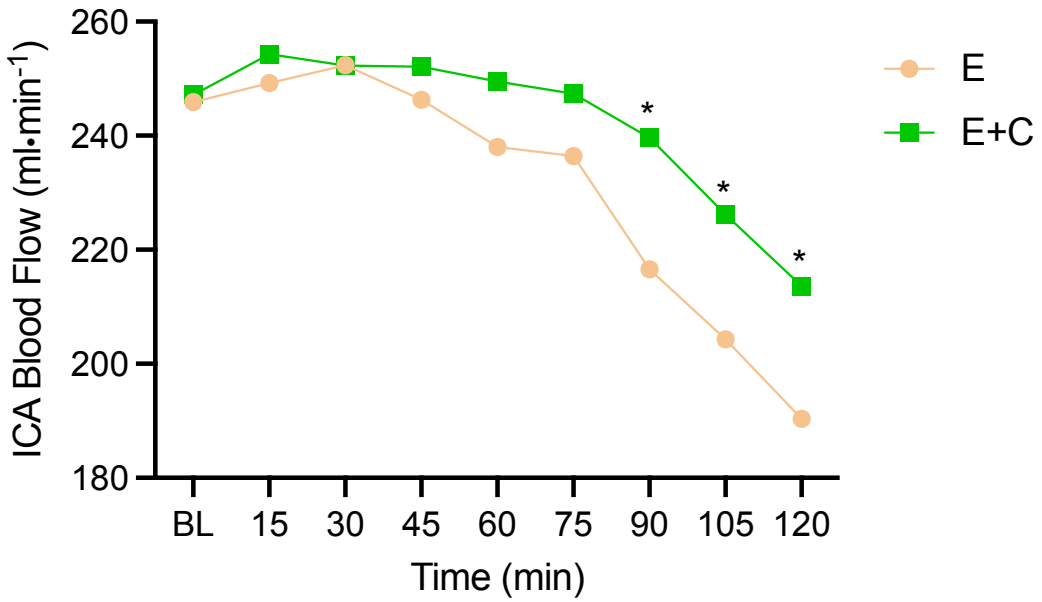

Figure 4.7: Internal Carotid Artery (ICA) blood flow at baseline (BL) and at 15 minute intervals over a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).
***** Indicates significant compared to E at 90, 105, and 120 minute time points ($P < 0.05$)

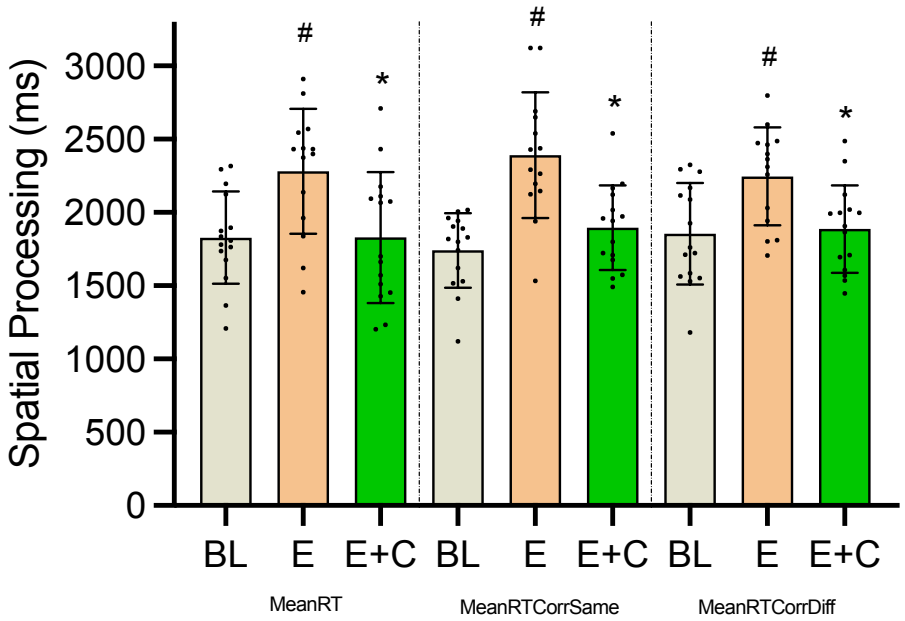

Figure 4.8: The effects of electrolyte and carbohydrate supplementation on spatial processing at baseline (BL) and following a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).
***** Indicates significant compared to E ($P < 0.05$)
# Indicates difference compared to BL ($P < 0.05$)

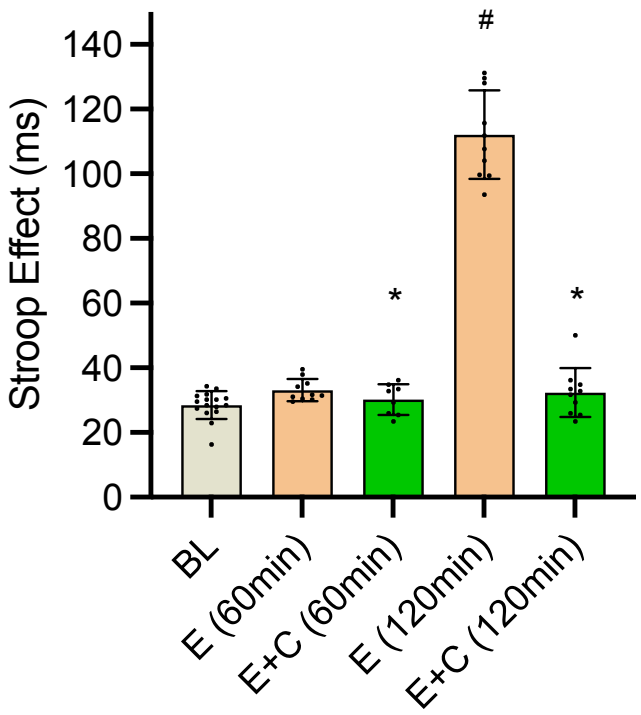

Figure 4.9: The effects of electrolyte and carbohydrate supplementation on Stroop Effect scores at baseline (BL) and following a 120 minute bout of passive heat stress while subjects ingested electrolytes (Gatorade Zero; E) or electrolytes plus carbohydrates (Gatorade; E+C; n = 15).
***** Indicates significant compared to E at 60 and 120 minute cognitive assessments ($P < 0.05$)
# Indicates difference compared to BL ($P < 0.05$)

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CHAPTER V – LIMITATIONS AND CLINICAL IMPLICATIONS

General Experimental Limitations

For this dissertation, the data from both passive and active heat stress models were collected under simulated, laboratory-based conditions. The first and second experiments utilized a steady-state speed and grade as well as an environmental chamber which was chosen to limit fluctuations in work, temperature, and humidity. Performing these experiments in a highly controlled environment restricts the inferences that can be made to populations who have the ability to adjust their work rate based on perceived exertion throughout the duty day, such as military, athletes, and emergency response personnel. As such, the conclusions made throughout this dissertation are only specific to these experimental conditions and will need further interpretation by subject matter experts to be appropriately translated to other patient populations or professions. Although this is not an ideal project design, it did allow our lab to reduce any confounding variables (work rate, exercise intensity, speed, grade, temperature, humidity, and fluid consumption) that may have influenced our primary outcome variables (hydration status and cognitive function)

Additionally, secondary to the organization of the second and third experiments, we were unable to determine the exact mechanism(s) for the observed attenuations in cognitive decline from bouts of long-duration passive or active heat stress. In the final experiment, measuring global cerebral blood flow and arterial tension of carbon dioxide allowed the conceptualization of link(s) between hyperthermia-induced decreases in cerebral blood flow and the subsequent reduction in cognitive function, however, the

physiological mechanism(s) of exogenous glucose ingestion on these processes remains elusive and warrant future investigation.

Finally, closely related to the above limitation is the fact that we did not measure circulating catecholamines or plasma cortisol concentrations as markers of sympathetic nervous system activation resulting from both long-duration passive and active heat stress. This would have allowed an appreciation to the underpinnings of the physiological mechanism(s) behind the effects of exogenous glucose supplementation on carotid chemoreceptors and their potential role in acute modulation of the sympathetic nervous system during long-duration bouts of heat stress.

Clinical Implications

The integrated physiological responses that occur within healthy humans to mitigate dangerous rises in core temperature operate to redistribute blood flow as a means of reducing the possibility of uncompensable heat stress and heat related illness. As noted throughout this dissertation, these physiological responses vary greatly when comparing passive to active heat stress. Indeed, over many decades of research, environmental and exercise physiologists have answered numerous questions to how humans maintain thermoneutrality in all types of austere environmental conditions (heat, cold, and differing altitudes). However, the effects of long-duration passive and active heat stress on cognitive function remain inconclusive at best.

The data in this dissertation provides the first experimental evidence of long-duration heat stress – independent of whether it arose from a passive or active stimulus – and its adverse effects on cognitive function. Furthermore, although the exact

mechanism(s) remain unclear, these findings elucidate the potential protective role of exogenous glucose supplementation on cognition during and immediately following long-duration passive and active heat stress. Accordingly, once translated to real-world populations, this novel therapeutic target for mitigating heat-induced cognitive decline may improve both physical (i.e. cerebral blood flow) and cognitive assessments, providing numerous benefits to occupational-specific tasks requiring high levels of cognitive competence.

APPENDIX A – CONSENT FORMS FOR EXPERIMENTS 1 AND 2

Consent to Participate in a Research Study
Colorado State University

TITLE OF STUDY: EVALUATION OF WILDLAND FIREFIGHTER METABOLIC DEMAND AND HYDRATION STATUS FOLLOWING A SIMULATED HIKE WITH FIREFIGHTING GEAR.

INVESTIGATORS FROM THE DEPARTMENT OF HEALTH AND EXERCISE SCIENCE: Jennifer Richards, Ph.D. (Email: Jennifer.Richards@Colostate.edu)

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH? You are a healthy man or woman aged between 18 and 35 years.

The purpose of our study is to understand the metabolic demand associated with walking on an incline with standard wildland firefighter gear and to examine hydration status prior to and following the hike as well as hydration patterns during the exercise bout.

WHO IS DOING THE STUDY? Dr. Richards and the United States Forest Service are running the study.

WHAT IS THE PURPOSE OF THIS STUDY? Wildland firefighters are exposed to prolonged hot and dry working conditions that often require them to perform moderate intensity exercise for extended periods of time (16 hour shifts) for up to 14 days in a row. It is estimated that the work performed during the hike into a fire, is the most taxing of activities for wildland firefighters, but to date, the amount of work it requires has not been assessed. In addition, during exercise, the human body relies on evaporative cooling, or sweating, as a means to prevent increases in core temperature. The more strenuous the exercise or the hotter the environment, the greater the sweat rate and fluid loss. Wildland firefighters commonly succumb to heat exhaustion due to the nature of their job and working conditions. It is possible that by increasing fluid intake, some wildland firefighters can offset some of the negative effects of heat stress.

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? This whole research project will take place over a period of approximately one year in the Human Performance Clinical Research Laboratory.

WHAT WILL I BE ASKED TO DO? You will visit the laboratory on 3 different days. These visits will include a screening visit and two study day visits. A member of the research team will fully explain each of your visits to our laboratory, including how long each visit will last.

VISIT 1: Screening Visit (2 hours)

Health and Physical Activity Questionnaire: You will be asked to answer some questions about your health and exercise habits to determine if you can participate in the study. (~20 minutes)

Cognitive Function Tests: You will be asked to complete 3 cognitive function tests focusing on your ability to process information, memorize and recall different sets of data, and your baseline mood. (~30 minutes)

Body Composition: Body mass and height will be measured. The fat, muscle, and bone in your body will be measured using an x-ray device (dual-energy x-ray absorptiometry) that will scan you from head to toe while you lie quietly on a special table. The amount of x-ray radiation you will receive is extremely low. (~10 minutes)

Maximal Oxygen Consumption: Exercise testing will be performed on a treadmill; the resistance will be increased until you cannot exercise any more. We will measure your endurance fitness (also known as your VO_{2max}). You will be asked to put your mouth around a scuba-like mouthpiece and wear a nose clip to prevent breathing through your nose. The amount of oxygen your body uses for energy will be determined from the oxygen and carbon dioxide you breathe in and out during the exercise. Your heart rate will be measured using a heart rate monitor. (~30-45 minutes)

Study Day Visit Familiarization: To determine if you are comfortable participating in the study day visits, you will be asked to wear a weighted (50 lb) back-pack and then walk on a treadmill (2-3 mph at 5 % grade) for 10 minutes.

VISITS 2, 3 and 4: Environmental Chamber Study Day Visits (up to 2.5 hours each)

You will be asked to exercise on the treadmill in the environmental chamber. The environmental chamber will be heated to approximately 32˚Celsius (roughly 90˚F) and 10% relative humidity (RH).

For each (2) study day visit you will experience all of the procedures listed below:

Sensor Ingestion: ~4 hours prior to your appointment you will eat a small meal and swallow a temperature sensor that will be used to measure your body (core) temperature throughout the study. The sensor is the size of a multi-vitamin and will leave your body within 24 hours with your normal bowel movement.

Body Weight: Prior to and following exercise, you will be weighed in the same dry clothing to determine how much water you lost (dehydration) during the exercise bout.

Bioelectrical Impedance Spectroscopy: Prior to and following exercise you will lay down on an exam table and have two sticky electrodes placed on your right hand and

foot. A brief and undetectable current will be passed through your body to estimate total body water.

Heart Rate and Blood Pressure: A heart rate monitor strap will be worn around your chest to measure your heart rate throughout the protocol. A blood pressure cuff will be placed over your upper arm and your blood pressure will be measured approximately every 15 minutes.

Weighted Backpack: You will be asked to wear a backpack used by wildland firefighters that contains no more than 8L of fluid that you will be able to drink from as frequently as you wish. The total mass of the pack will be approximately 50 lbs.

Treadmill Exercise: You will be asked to walk on the treadmill for 90-120 minutes. The speed of the treadmill will range between 2-4 mph and the grade will range between 5-10 %. You will be asked to wear the weighted backpack and drink water (plain or flavored) whenever you want. You will also be asked to wear Wildland Firefighter Personal Protective Equipment (PPE) or personal attire of similar weight and length.

***Blood Sample*:** A sample of your blood will be drawn both before and after the treadmill exercise portion of the study. Each sample will consist of about 10 ml (approximately 2 tablespoons) of your blood and will be drawn from a vein on the front of your elbow in a standard fashion using a sterilized hypodermic needle. (~30 minutes)

Cognitive Function Tests: You will be asked to complete the same 3 cognitive function tests that you previous completed during Visit 1 following treadmill exercise of Visit 2, 3, and 4. This will demonstrate any change in your ability to process information, memorize and recall data, and mood from baseline. (~30 minutes)

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?

You will not be allowed to take part in the study for any of the following reasons:

1. You are not physically able to perform this exercise test.
2. You are not 18-40 years of age
3. You are pregnant
4. You are a regular smoker
5. You weigh less than 80 pounds
6. You have a known auto-immune disorders
7. You have alcohol dependence
8. You have the presence of presence of any known or suspected obstructive disease of the stomach and digestive system (gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease)
9. You have a history of disorders or impairment of the gag reflex
10. You have a previous stomach (gastrointestinal) surgery
11. You have been told you have folds or ridges in your throat (also known as felinization or esophageal folds).

12. You have been told you have eosinophilic esophagitis. This is a sickness often linked with heartburn, stomach reflux, and/or regular vomiting
13. You have difficulty making bowel movements (e.g. hypo-motility disorders of the gastrointestinal tract including but not limited to the ileus (obstruction of the intestine)
14. Have been hospitalized for hypo- or hyperthermia (sickness due to extremes in temperature; either too hot or too cold), have an illness that lowers your immune function, or have any diseases that would affect our measurements or significantly increase the risks associated with this study
15. Your blood pressure during visit 1: Systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg
16. You have a BMI > 30 kg/m^2 (we will measure this for you).
17. You use prescription drugs that may impact your body's ability to regulate its temperature.
18. You use illicit substances, such as meth, speed, ecstasy, cocaine, etc.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential (but unknown) risks. The Human Performance Clinical Research Laboratory has emergency supplies including a medicine trolley equipped with heart machines and supplemental oxygen. The research team has a great deal of experience with all of the procedures that will be performed in this study. Some of the procedures for which you are being asked to volunteer have a number of associated risks:

Health and Physical Activity Questionnaire: There are no known risks associated with answering health questions. All information is kept strictly confidential.

Maximal Oxygen Consumption Test: There is a risk of fatigue (temporary muscle tiredness), muscle strain, heart beat abnormalities (arrhythmias), a 0.01% chance of death (in people who have heart problems), a 0.02% risk of cardiac arrhythmias that would require you to go to a hospital (in people who have heart problems), and a risk of an increase or decrease in blood pressure.

Body composition (DEXA) scan: The risks associated with the DEXA are very low. The maximum radiation dose you will receive in this study is less than 1/1000th of the federal and state occupational whole body dose limit allowed to radiation workers (5,000 mrem). Put another way, the maximum dose from any scan we utilize with this DEXA ranges from 1.2 mrem (Whole body scan) to 12.2 mrem (for several of the regional scans, such as lumbar, femur, and forearm scans). The average annual background radiation you already receive is at least 620 mrem/year. The more radiation you receive over the course of your life, the more the risk increases of developing a fatal cancer or inducing changes in genes. The radiation in this scan is not expected to significantly increase these risks, but the exact increase in such risks is not known. There are no discomforts associated with this procedure.

Body (Core) temperature sensor ingestion: There may be some discomfort when swallowing the pill because it is the size of a multi-vitamin pill. You should not ingest a sensor if you:

1. Weigh less than 80 pounds
2. Have the presence of presence of any known or suspected obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease
3. Have been told you have folds or ridges in your throat (also known as felinization or esophageal folds)
4. Have been told you have eosinophilic esophagitis. This is a sickness often linked with heartburn, stomach reflux, and/or regular vomiting
5. Have had previous stomach (gastrointestinal) surgery
6. You might undergo Nuclear Magnetic Resonance (NMR) or MRI scanning during the duration of the study
7. You have hypo motility disorders of the gastrointestinal tract including but not limited to ileus (obstruction of the intestine)
8. Have a cardiac pacemaker or implanted electro medical device

Bioelectrical Impedance and Body Weight: There are no known risks associated with these measures.

Heart Rate and Blood Pressure: There may be some temporary discomfort when the blood pressure cuff is inflated.

Heat exposure at rest and during exercise: There is a risk of dehydration, and heat related illness (heat exhaustion and heat stroke). We will minimize this risk by ending the study in the event your core temperature exceeds 39.0˚C (103.1 °F).

Blood sample: The risks associated with blood drawing include bruising, slight risk of infection, soreness, and fainting. These are minor risks which usually do not last more than one day if they occur.

Cognitive Function Tests: There are no known risks associated with these measures.

WILL I BENEFIT FROM TAKING PART IN THIS STUDY? There are no direct benefits to you for participating in this study beyond receiving information on your body composition and aerobic fitness. Information provided by this study may help to support wildland firefighters.

DO I HAVE TO TAKE PART IN THE STUDY? Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

WHAT WILL IT COST ME TO PARTICIPATE? There is no cost to you for participating except that associated with your transportation to our facilities.

WHO WILL SEE THE INFORMATION THAT I GIVE?
We will keep private all research records that identify you, to the extent allowed by law. For this study, we will assign a code to your data (e.g. 1234ABCD#$) so that the only place your name will appear in our records is on the consent and in our data spreadsheet that links you to your code. Only the research team will have access to the link between you, your code, and your data. The only exceptions to this are if we are asked to share the records of the study for audit purposes with the Food and Drug Administration, Health and Human Services, and/or the CSU Institutional Review Board ethics committee, if necessary. In addition, for funded studies, the CSU financial management team may also request an audit of research expenditures. For financial audits, only the fact that you participated would be shared, not any research data.

When we write about the study to share it with other researchers or the U.S. Forest Service, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private. If you choose to take part in this study your private [information or biospecimen] collected for this study will not be used or distributed for future studies, even if we remove all identifiers linking you to your [information or biospecimen].

CAN MY TAKING PART IN THE STUDY END EARLY? Your participation in the study could end in the rare event of muscle strain, if your core temperature exceeds 39.0 C, if you become pregnant, or if you miss an excessive number of appointments.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY? You may receive up to $125 for taking part in the study. The compensation will be as follows: You will not receive any money for completing the screening visit, you will receive $25 for completing visit 2 and 3, and $75 for completing visit 4. If you are asked to repeat any visits, you will be compensated $25 per any additional visit.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?
We will arrange to get you medical care if you have an injury that is caused by this research.

Should you sustain an injury that is not the direct result of the study, you or your insurance company may have to pay for the required care. The Colorado Governmental Immunity Act determines and may limit Colorado State University legal responsibility if an injury happens because of this study. Claims against the University must be filed with Colorado State University within 180 days of the injury.

WHAT IF I HAVE QUESTIONS? *Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact Jennifer Richards Ph.D., at (970)491-6702, or via email at* Jennifer.Richards@Colostate.edu. *If you would like to ask a medical doctor about your participation in the study, you may contact one of the*

physicians listed below at the corresponding email. If you have any questions about your rights as a volunteer in this research, contact the CSU IRB at: RICRO_IRB@mail.colostate.edu; 970-491-1553. We will give you a copy of this consent form to take with you.

WHAT ELSE DO I NEED TO KNOW?
Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 8 pages.

_____ _____
Signature of person agreeing to take part in the study Date

_____
Printed name of person agreeing to take part in the study

_____ _____
Name of person providing information to participant Date

_____
Signature of Research Staff

**** List of Contact Numbers in Case of Medical Emergency**
Scott Fahrner, M.D. Scott.Fahrner@colostate.edu

Jennifer C. Richards, Ph.D. Jennifer.Richards@Colostate.edu
Work: 970-491-6702
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APPENDIX B – CONSENT FORM FOR EXPERIMENT 3

Consent to Participate in a Research Study
Colorado State University

TITLE OF STUDY: CHANGES IN GLOBAL CEREBRAL BLOOD FLOW AND COGNITIVE PROCESSING SPEED FOLLOWING LONG-DURATION PASSIVE HEAT-STRESS

INVESTIGATORS FROM THE DEPARTMENT OF HEALTH AND EXERCISE SCIENCE: Jennifer Richards, Ph.D. (Email: Jennifer.Richards@Colostate.edu)

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH? You are a healthy man or woman aged between 18 and 35 years.

The purpose of our study is to understand whether long-duration passive heat stress causes changes in cerebral (brain) blood flow and cognitive decline.

WHO IS DOING THE STUDY? Dr. Richards is running the study.

WHAT IS THE PURPOSE OF THIS STUDY? Certain occupations (military, athletes, and emergency response personnel) are required to perform moderate-intensity exercise in austere (hot and humid) climates for long shifts (>16 hours). This study would like to determine whether 1) these individuals have a higher risk of suffering from cognitive decline and 2) if cognitive decline occurs secondary to heat stress alone (i.e. passive heat stress), and not heat stress associated with an exercise stimulus. We will examine global cerebral blood flow (via doppler of the middle cerebral artery and/or internal carotid artery) and cognitive function (via a battery of cognitive tests) to observe whether these variables change during long-duration (~2 hours) passive-heat stress. We will also observe whether solutions supplemented with glucose have a protective affect against this decline (which occurs during long-duration exercise heat stress).

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? The entirety of this research project will take place over a period of approximately one year in the Human Performance Clinical Research Laboratory. Each visit will take approximately 2-3 hours with roughly 5 to 7 days between each study day visit to limit heat acclimation.

WHAT WILL I BE ASKED TO DO? You will visit the laboratory on 3 different days. These visits will include a screening visit and two study day visits. A member of the research team will fully explain each of your visits to our laboratory, including how long each visit will last.

VISIT 1: Screening Visit (2 hours)

Health and Physical Activity Questionnaire: You will be asked to answer some questions about your health and exercise habits to determine if you can participate in the study. (~20 minutes)

Cognitive Battery: You will be asked to complete a battery of cognitive tests through a program called Automated Neurophysiological Assessment Metric (ANAM). This test will challenge different areas of your brain that are charged with response inhibition, visuomotor processing speed, motor speed, attention (sustained and selective), visual spatial skills, concentration, memory formation, interference inhibition, and executive function. (~30 minutes)

Temperature Sensor Pill: You will be given a temperature sensing pill at the conclusion of this first visit. This pill will be taken ~4 hours prior to your first temperature controlled room study day visit (see below). Similarly, at the conclusion of your first temperature controlled room study day visit, you will be given a second temperature sensing pill. This second pill will be used for your second temperature controlled room study day visit.

VISITS 2 and 3: Passive Heat Stress Visits (up to 3 hours each)

You will be asked to lay supine for approximately 2 hours while wearing Water Perfused Suits that will be infused with warm water.

For each (2) study day visits you will experience all of the procedures listed below:

Sensor Ingestion: ~4 hours prior to your appointment you will eat a small meal and swallow a temperature sensor that will be used to measure your body (core) temperature throughout the study. The sensor is the size of a multi-vitamin and will leave your body within 24 hours with your normal bowel movement.

Body Weight: Prior to and following the passive heat stress stimulus, you will be weighed (nude) to determine how much water you lost (dehydration) during the passive heat stress stimulus.

Heart Rate and Blood Pressure: A heart rate monitor strap will be worn around your chest to measure your heart rate throughout the protocol. A blood pressure cuff will be placed over your upper arm and your blood pressure will be measured approximately every 15 minutes.

End-Tidal CO_2 Measurement: You will wear a nasal cannula every 15 minutes to estimate the amount of CO_2 in your arterial blood.

Water Perfused Suits: You will be asked to wear a shirt and pair of pants that have tubes sewed within the fabric. These tubes will allow us to infuse warm water to raise your core body temperature up to approximately 39ºC.

Venous Blood Sample: A sample of your blood will be drawn both before and after the passive heat stress portion of the study. Each sample will consist of about 10 ml (approximately 2 tablespoons) of your blood and will be drawn from a vein on the front of your elbow in a standard fashion using a sterilized hypodermic needle. (~30 minutes)

Global Cerebral Blood Flow Measurement: Throughout the passive heat stress, we will measure the flow of blood to your brain via Doppler Ultrasound through an artery in your neck and head. This form of measurement is non-invasive. (~5 minutes)

Cognitive Battery: You will be asked to complete the same cognitive battery (ANAM) following the passive heat stress that you took during the Consent Visit. This will demonstrate any change in your ability to process information, react to visual stimuli, memorize and recall data, and mood from baseline. (~30 minutes)

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?

You will not be allowed to take part in the study for any of the following reasons:

1. You are not physically able to perform this exercise test.
2. You are not 18-35 years of age
3. You are pregnant
4. You are a regular smoker (i.e. an adult who has smoked **100 cigarettes** in his or her lifetime and who currently smokes cigarettes).
5. You weigh less than 80 pounds
6. You have known auto-immune disorders (any disorder that impairs your body's immune function)
7. You have alcohol dependence (i.e. the inability to control drinking due to both a physical and emotional dependence on alcohol).
8. You have the presence of any known or suspected obstructive disease of the stomach and digestive system (gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease)
9. You have a history of disorders or impairment of the gag reflex
10. You have a previous stomach (gastrointestinal) surgery
11. You have been told you have folds or ridges in your throat (also known as felinization or esophageal folds).
12. You have been told you have eosinophilic esophagitis. This is a sickness often linked with heartburn, stomach reflux, and/or regular vomiting
13. You have difficulty making bowel movements (e.g. hypo-motility disorders of the gastrointestinal tract including but not limited to the ileus (obstruction of the intestine)
14. Have been hospitalized for hypo- or hyperthermia (sickness due to extremes in temperature; either too hot or too cold), have an illness that lowers your immune

function, or have any diseases that would affect our measurements or significantly increase the risks associated with this study
15. Your blood pressure during visit 1: Systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg
16. You have a BMI > 30 kg/m^2 (we will measure this for you).
17. You use prescription drugs that may impact your body's ability to regulate its temperature.
18. You use illicit substances, such as meth, speed, ecstasy, cocaine, etc.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential (but unknown) risks. The Human Performance Clinical Research Laboratory has emergency supplies including a medicine trolley equipped with heart machines and supplemental oxygen. The research team has a great deal of experience with all of the procedures that will be performed in this study. Some of the procedures for which you are being asked to volunteer have a number of associated risks:

Health and Physical Activity Questionnaire: There are no known risks associated with answering health questions. All information is kept confidential with the research team. Following the screening and consent visit, you will be given a subject ID that contains no personal information of any kind. The subject ID will be the only designator for that your information. The PI and co-PI will be the only researcher staff who have access to the electronic data file which links your subject ID with the your actual name, DOB, etc. This electronic file will be password protected and stored on the lab drive, which only faculty/staff/student in the lab have access.

Body (Core) temperature sensor ingestion: There may be some discomfort when swallowing the pill because it is the size of a multi-vitamin pill. You should not ingest a sensor if you:

1. Weigh less than 80 pounds
2. Have the presence of presence of any known or suspected obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease
3. Have been told you have folds or ridges in your throat (also known as felinization or esophageal folds)
4. Have been told you have eosinophilic esophagitis. This is a sickness often linked with heartburn, stomach reflux, and/or regular vomiting
5. Have had previous stomach (gastrointestinal) surgery
6. You might undergo Nuclear Magnetic Resonance (NMR) or MRI scanning during the duration of the study
7. You have hypo motility disorders of the gastrointestinal tract including but not limited to ileus (obstruction of the intestine)
8. Have a cardiac pacemaker or implanted electro medical device

Heart Rate and Blood Pressure: There may be some temporary discomfort when the blood pressure cuff is inflated.

Heat exposure at rest: There is a risk of dehydration, and heat related illness (heat exhaustion and heat stroke). We will minimize this risk by ending the study in the event your core temperature exceeds 39.0ºC (103.1ºF).

Blood sample: The risks associated with blood drawing include bruising, slight risk of infection, soreness, and fainting. These are minor risks which usually do not last more than one day if they occur.

Cerebral Blood Flow Measurement: There are no known risks associated with these measures.

Cognitive Battery: There are no known risks associated with these measures.

WILL I BENEFIT FROM TAKING PART IN THIS STUDY? There are no direct benefits to you for participating in this study beyond receiving information on your body composition and cognitive function. Information provided by this study may help to support occupations which require a high degree of cognitive function during and immediately following long-duration heat stress.

DO I HAVE TO TAKE PART IN THE STUDY? Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.
WHAT WILL IT COST ME TO PARTICIPATE? There is no cost to you for participating except that associated with your transportation to our facilities.

WHO WILL SEE THE INFORMATION THAT I GIVE? We will keep private all research records that identify you, to the extent allowed by law. For this study, we will assign a code to your data (e.g. 1234ABCD#$) so that the only place your name will appear in our records is on the consent and in our data spreadsheet that links you to your code. Only the research team will have access to the link between you, your code, and your data. The only exceptions to this are if we are asked to share the records of the study for audit purposes with the Food and Drug Administration, Health and Human Services, and/or the CSU Institutional Review Board ethics committee, if necessary. In addition, for funded studies, the CSU financial management team may also request an audit of research expenditures. For financial audits, only the fact that you participated would be shared, not any research data. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private. If you choose to take part in this study your private [information or biospecimen] collected for this study will not be used or distributed for future studies, even if we remove all identifiers linking you to your [information or biospecimen].

CAN MY TAKING PART IN THE STUDY END EARLY? Your participation in the study could end if your core temperature exceeds 39.0ºC, if you become pregnant, or if you miss an excessive number of appointments.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY? You may receive up to $100 for taking part in the study. The compensation will be as follows: You will not receive any money for completing the screening visit, you will receive $50 for Visit 2 and $50 for Visit 3 for a total of $100. If you are asked to repeat any visits, you will be compensated $50 per any additional visit.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?
We will arrange to get you medical care if you have an injury that is caused by this research.

Should you sustain an injury that is not the direct result of the study, you or your insurance company may have to pay for the required care. The Colorado Governmental Immunity Act determines and may limit Colorado State University legal responsibility if an injury happens because of this study. Claims against the University must be filed with Colorado State University within 180 days of the injury.

WHAT IF I HAVE QUESTIONS? *Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact Jennifer Richards Ph.D., at (970)491-6702, or via email at* Jennifer.Richards@Colostate.edu. *If you would like to ask a medical doctor about your participation in the study, you may contact one of the physicians listed below at the corresponding email. If you have any questions about your rights as a volunteer in this research, contact the CSU IRB at: RICRO_IRB@mail.colostate.edu; 970-491-1553. We will give you a copy of this consent form to take with you.*

WHAT ELSE DO I NEED TO KNOW?
Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 8 pages.

_____ _____
Signature of person agreeing to take part in the study Date

_____
Printed name of person agreeing to take part in the study

_____ _____
Name of person providing information to participant Date

_____
Signature of Research Staff

**** List of Contact Numbers in Case of Medical Emergency**

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APPENDIX C –DISCLOSURES

Department of the Air Force, Department of Defense, United States Government, and Air Force Institute of Technology Disclosure

The authors declare that there are no conflicts of interests. The views expressed are those of the authors and do not necessarily reflect the official policy or position, either expressed or implied, of the United States Forest Service, Department of the Air Force, Department of Defense, or the U.S. Government.