THE INFLUENCE OF OSMORECEPTORS AND BARORECEPTORS ON HEAT LOSS RESPONSES DURING A WHOLE-BODY PASSIVE HEAT STRESS

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ABSTRACT

Exercise and/or heat-induced dehydration is associated with decreases in plasma volume (hypovolemia) and increases in plasma osmolality (hyperosmolality), which are thought to stimulate peripheral baroreceptors and central osmoreceptors respectively. Independently, plasma hyperosmolality and baroreceptor unloading have been shown to attenuate sweating and cutaneous vasodilation during heat stress, and therefore, negatively impact body temperature regulation. However, to date little is known regarding the combined influence of plasma hyperosmolality and baroreceptor unloading on thermoefferent activity.

Therefore, we evaluated the separate and combined effects of baroreceptor unloading (via lower body negative pressure, LBNP) and plasma hyperosmolality (via infusion of 3% NaCl saline) on heat loss responses of sweating and cutaneous vascular conductance (CVC) during progressive whole-body heating.

We show that the combined nonthermal influences of plasma hyperosmolality and baroreceptor unloading additively delay the onset threshold for CVC, relative to their independent effects. In contrast, baroreceptor unloading has no influence on the sweating response regardless of osmotic state. These divergent roles of plasma hyperosmolality and the baroreflex on heat loss responses might serve to enhance blood pressure and body core temperature regulation during dehydration and heat stress.

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EMPIRICAL AND THEORETICAL CONSIDERATIONS

CHAPTER 1

INTRODUCTION

1.0 Introduction

As homeotherms, humans maintain a relatively constant 37°C core body temperature in varying environments. In conditions of heat stress, core body temperature change is primarily influenced by the rate of heat loss from the surface of the body. Increases in skin blood flow (SkBF) and sweating allow for adequate heat removal and attenuate the rise in core body temperature, making these two variables critical in the maintenance of thermal homeostasis. Typically, afferent information from skin and core thermoreceptors (thermal factors) determines the temporal pattern, magnitude and rate of change in the heat loss responses of sweating and SkBF (Shibasaki *et al.*, 2006). While thermal factors are considered the main drivers of SkBF and sweating responses, other nonthermal factors are believed to modulate these responses independent of thermal control (Crandall *et al.*, 1996; Kondo *et al.*, 2003; Shibasaki *et al.*, 2003a; Kenny *et al.*, 2007; Kenny & Journeay, 2010)

Blood flow to the skin is increased during heat stress for heat dissipation and can require as much as 60% of the total cardiac output (Charkoudian *et al.*, 2003). When heat stress is combined with exercise, strain on the cardiovascular system is extended as blood flow to active muscles increases to ensure adequate oxygen and nutrient delivery. Furthermore, under conditions of prolonged exercise and/or heat stress, the large volumes of water loss associated with sweating can quickly lead to dehydration despite water replacement (Shibasaki *et al.*, 2003a). Significant body water deficits will lead to reductions in total blood volume, resulting in a progressive reduction in SkBF. For example, 2 hours of exercise in the heat (35°C) resulted in a 23% reduction in SkBF from

the 20 min baseline value when participants become 5% dehydrated. The same protocol resulted in no change in SkBF from the 20 min baseline value when participants were kept hydrated (Gonzalez-Alonso *et al.*, 1995). Thus, the reduction in SkBF limited heat loss from the body and was partly responsible for the 1.2°C higher core temperature at the end of exercise compared to when dehydration was prevented. Such reductions in SkBF result from competition between the cardiovascular and thermoregulatory systems during exercise in the heat for a limited blood supply that is exacerbated when individuals become dehydrated (Gonzalez-Alonso *et al.*, 1997; Sawka *et al.*, 2001).

Changes in blood osmolality and plasma volume associated with exercise and/or heat-induced dehydration have been shown to have significant negative impacts on body temperature regulation. Studies show that dehydration is associated with a greater increase in core temperature during exercise and the magnitude of increase is proportional to the level of dehydration (Nadel *et al.*, 1980; Sawka *et al.*, 1985; Montain & Coyle, 1992). This graded increase in core temperature is attributed to a decrease in the heat loss responses, usually observed as a delay in the core temperature onset for sweating or increases in SkBF or a reduction in the sensitivity of these responses to changes in core temperature (Gonzalez-Alonso *et al.*, 1997; Sawka *et al.*, 2001). Specifically, dehydration leads to a decrease in plasma volume (hypovolemia) and an increase in plasma osmolality (hyperosmolality), both of which have been shown to independently alter SkBF and sweating responses during exercise and/or passive heat stress (Nadel *et al.*, 1980; Fortney *et al.*, 1981b; Fortney *et al.*, 1984; Takamata *et al.*, 1997). For example, Nadel *et al*. (1980) induced plasma volume changes without significant alterations in osmolality (i.e., hypovolemic isosmotic condition) by administrating diuretics to the study participants 4 days before exercise. During cycling

exercise, the isosmotic hypovolemic condition resulted in a 0.42°C increase in the core temperature onset threshold for cutaneous vasodilation and a 50% reduction in the maximal forearm blood flow, but no changes in the forearm blood flow-core temperature relationship (thermal sensitivity). In a subsequent study, Fortney *et al.* (1984) induced thermal dehydration by 24 hour fluid restriction, followed by mild intensity exercise (<30% VO_{2max}) in 40-45°C, 40-100% relative humidity conditions until body weight was reduced by 3%. A 3% NaCl saline solution was subsequently infused to restore plasma volume while maintaining a hyperosmotic condition. After 1 hour of rest in a normothermic environment, subjects then exercised for 30 min in 30°C and 40% relative humidity. Compared to when participants were not thermally dehydrated before the exercise test (control), hyperosmolality elevated esophageal temperature thresholds for both SkBF and sweating, while the sensitivity of the response was unchanged. Similar results were also obtained by Takamata *et al.* (1995) and Shibisaki *et al.* (2009) who used a passive heat stress to mitigate the possible confounding effects exercise can have on the control of heat loss responses (Taylor *et al.*, 1988; Kondo *et al.*, 2003; Shibasaki *et al.*, 2005).

It has been well document that heat loss responses can be modulated by changes in baroreceptor loading status. While the effects of baroreceptor unloading on changes in SkBF have been well documented (Zoller *et al.*, 1972; Johnson & Park, 1981; Kellogg *et al.*, 1990; Crandall *et al.*, 1996; Peters *et al.*, 2000; Mack *et al.*, 2001; Keller *et al.*, 2006), there remains disagreement as to whether baroreceptors can modulate sweating (Sawka, 1992; Crandall *et al.*, 1996; Sawka *et al.*, 2001; Shibasaki *et al.*, 2006). Changes in central blood volume and plasma osmolality which occur with progressive dehydration are known to activate both peripheral baroreceptors and central osmoreceptors. However,

it remains unclear how the separate and combined influence of baroreceptor and osmoreceptor activation influences heat loss responses. Studies suggest that the independent effect of plasma osmolality on SkBF and sweating is mediated centrally in the hypothalamus, where neurons that are both thermosensitive and osmosensitive have been identified. This belief is based on research performed on animals where preoptic-anterior and medial hypothalamic neurons have been found to respond to changes in temperature and plasma tonicity (Silva & Boulant, 1984; Owen *et al.*, 1989). However, it is also possible that plasma osmolality changes could exert a peripheral effect whereby high interstitial osmotic pressure could inhibit fluid availability for the eccrine sweat gland (Greenleaf & Castle, 1971; Sawka, 1992).

To the best of our knowledge, Ito *et al.* (2005) is the only study to examine the separate and combined effects of baroreceptor unloading and plasma hyperosmolality on the cutaneous vascular response. In this study, subjects were either infused with isotonic (0.9% NaCl) or hypertonic (3% NaCl) saline solution to maintain or increase plasma osmolality by ~12 mosmol•kgH_2O^{-1} from baseline, respectively. Subjects were then passively heated for 60 min with a water perfusion suit circulated with 42°C water, after which a negative pressure was progressively applied to the lower limbs (i.e., lower body negative pressure, LBNP) in 3 min intervals (between -10 to -40 mmHg). The percent reduction in forearm vascular conductance was greater in the hyperosmotic condition as compared to the isosmotic conditions (-58.8 ± 4.1% vs. -44.7 ± 8.1%) during the application of -40 mmHg LBNP. This response is consistent with an interaction between the nonthermal influences on heat loss responses associated with hydration induced changes in baroreceptor loading status and plasma osmolality. However, the combined influence of plasma osmolality and baroreceptor loading status on sweat rate was not

reported. Hence, it is unclear how these nonthermal factors may modulate sweating, a key avenue of heat loss during a heat stress, and therefore core temperature response.

Additional studies are therefore required to advance our understanding of the separate and combined effects of changes in plasma hyperosmolality and baroreceptor unloading associated with heat-induced dehydration. A study of these nonthermal modulators of heat loss responses will provide information critical to advancing our knowledge of the potential mitigating influences nonthermal factors can have on heat loss responses during body fluid regulation challenges.

1.1 Rationale

Both plasma hyperosmolality and baroreceptor unloading have been shown to independently influence thermoefferent activity. However, thermal dehydration results in simultaneous plasma hyperosmolality and baroreceptor unloading and at present, there is limited research on the simultaneous examination of these mechanisms during heat stress. Using established experimental techniques to modify plasma osmolality (3% and 0.9% NaCl infusion) and baroreceptor loading status (application of negative pressure to the lower limbs), we can observe the separate and combined effects of these nonthermal factors on thermoregulatory control during passive heating by evaluating the mean body temperature onset threshold and thermal sensitivity for increases in sweating and SkBF.

1.2 Purpose

The following study was conducted to evaluate the separate and combined effects of changes in plasma osmolality and baroreceptor loading status on heat loss responses of SkBF and sweating during a passive heat stress. Through this examination, we are able to

provide greater insight into the mechanisms responsible for the observed attenuations in heat loss responses during exercise and/or heat-induced dehydration.

1.3 Study Objectives

1. To compare the separate and combined effects of plasma hyperosmolality and baroreceptor unloading on SkBF thresholds and sensitivities during a passive heat stress.
2. To compare the separate and combined effects of plasma hyperosmolality and baroreceptor unloading on sweating thresholds and sensitivities during a passive heat stress.

1.4 Hypothesis

1. The separate effects of plasma hyperosmolality and baroreceptor unloading will cause an upward shift in the onset threshold for SkBF, but no change in the slope of the SkBF to mean body temperature relationship (sensitivity) will be observed. Furthermore, the combined effects of plasma hyperosmolality and baroreceptor unloading will cause a greater delay in the onset threshold for SkBF, relative to their independent effects.
2. The separate effects of plasma hyperosmolality and baroreceptor unloading will cause an increase in the onset threshold for sweating with no observable change in thermal sensitivity. Finally, the combined effects of plasma hyperosmolality and baroreceptor unloading will cause a greater delay in the onset threshold of sweating, relative to their independent effects.

1.5 Relevance

Exercise and/or heat exposure results in both plasma hyperosmolality and hypovolemia, a prime example of when nonthermal factors can influence heat loss responses. Plasma hyperosmolality and hypovolemia are thought to stimulate central osmoreceptors and peripheral baroreceptors, respectively and although they have been shown to independently attenuate SkBF and sweating responses to heat stress, their combined effect remains unknown. Therefore, this research project will expand our knowledge surrounding the mechanisms involved in the attenuation of heat loss responses associated with dehydration and provide a greater understanding of thermoregulatory control during challenges to maintain body water balance.

1.6 Delimitations and Limitations

Recruited subjects will be healthy males, between the ages of 18 and 30 years, physically active and lean individuals. Therefore, the results of this study cannot be applied to female, children, older adult, elderly, obese, sedentary or diseased populations. Also, SkBF and sweating will only be measured locally on the forearm, which will not allow for regional differences in the pattern of response to be evaluated.

1.7 Definitions and abbreviations

Osmoreceptors – sensory receptors located in the hypothalamus that are sensitive to changes in osmotic pressure and therefore help maintain water balance in the body.

Baroreceptors – pressure sensitive receptors in the body responsible for detecting changes in blood pressure and blood volume.

Esophageal temperature – an index measure of core temperature that reflects the changes in the temperature of the blood leaving the heart.

Skin Blood Flow (SkBF) – refers to blood flow to the skin vasculature.

Cutaneous vascular conductance (CVC) – reflects changes in cutaneous circulation that are independent of pressure changes and therefore, reflect either changes in vasoconstrictor tone or active vasodilation.

Vasodilation - relaxation of smooth muscle within a blood vessel causing the widening of the blood vessel.

Vasoconstriction - contraction of smooth muscle within a blood vessel causing the narrowing of the blood vessel.

Hyperosmolality – refers to a relatively high concentration of solutes in the blood. Values can increase from 283 mosmo·kgH_2O^{-1} when hydrated to values exceeding 300 mosmo·kgH_2O^{-1} when dehydrated.

Hypovolemia – refers to a significant decrease in the total amount of circulating blood, lost as blood plasma.

Dehydration – a process of losing body water because water output is greater than water input.

Total peripheral resistance- blood flow out of the arteries, influenced primarily by the resistance of the arterioles. When total peripheral resistance is increased, an increase in vasoconstriction is present which serves to increase blood pressure.

Hypotension- blood pressure falls too low because the driving force for bringing blood back to the heart cannot overcome the opposition by gravity.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Thermoregulation

2.1.1. Heat Exchange

Humans maintain a relatively constant internal temperature between 36.5°C and 37.5°C, despite different environmental conditions and/or rates of metabolic heat production. Thermal balance is achieved through the adjustment of physiological mechanisms and behavioural responses. This relationship may be viewed as a dynamic balance between heat transfer with the external environment and heat generation from the body (i.e. during exercise) (Parsons, 2003). Conceptually, this relationship is best described by the heat balance equation (Parsons, 2003):

$$M - W = (K + C + R + E_{SK}) + (C_{RES} + E_{RES}) + S$$

where:
M = rate of metabolic heat production
W = rate of mechanical work (effectively = 0)
K = rate of conductive heat loss
C = rate of convective heat loss from the skin
R = rate of radiative heat loss from the skin
E_{SK} = rate of evaporative heat loss from the skin
C_{RES} = rate of convective heat loss from pulmonary ventilation
E_{RES} = rate of evaporative heat loss from pulmonary ventilation
S = rate of body heat storage (all units $W \cdot m^{-2}$)

Inefficient substrate metabolism leads to metabolic heat production (M-W) and physiological responses that dissipate this heat, which will affect rates of dry heat exchange (R, C, & K) and evaporative heat loss (E). Mainly, these physiological responses include SkBF, sweating and shivering and will be discussed later. Within a thermoneutral or hot environment, increases in heat production not initially offset by heat output lead to positive body heat storage (S) and therefore an increase in core

temperature. Conversely, if dry heat exchange and evaporative heat loss exceed metabolic heat production, body heat storage becomes negative and core temperature decreases, which will occur during cold exposure and the post-exercise period.

2.1.2. Control

Many system models have been developed in order to understand how the body specifically regulates its temperature in response to differing environments and physical activity levels. For example, the set-point theory of temperature regulation proposes that the controlled system (body temperature) is tightly regulated around a reference or set-point temperature (Hardy, 1961; Hammel, 1968). Any disturbances in the controlled system will result in an error signal that will evoke the activation of effector responses (i.e., sweating, SkBF and shivering). Therefore, the goal of this closed-loop feedback regulator is to minimize the error signal. It has been suggested by some experts that core temperature is maintained within a prescribed zone (i.e., the null zone or interthreshold zone) whereby regulation within this zone is first achieved by vasomotor tone (Mekjavic & Eiken, 2006). Once vasomotor tone is no longer able to maintain a core temperature within this zone, effector responses of sweating and increases in SkBF or shivering will be initiated to minimize the displacement of core temperature from the null zone and maintain it within an acceptable range (Mekjavic & Eiken, 2006; Kenny *et al.*, 2007). For example, if the "null" zone is between ~36.7°C and ~37.2°C, then core temperatures above 37.2°C will trigger an increase in SkBF and sweating. In contrast, a decrease in core temperature below ~36.7°C will initiate an increase in vasoconstriction and depending on the rate of core temperature decay, an increase in metabolic heat production (i.e. shivering) (Mekjavic & Eiken, 2006; Kenny *et al.*, 2007). In contrast to these two

control systems where body core temperature is the regulated variable, it has also been suggested that thermoeffector activity is regulated by changes in body heat content and therefore, changes in body heat content is the controlled variable (Webb, 1995). In this way, the active system doesn't defend a set core temperature, but rather adjusts the outflow of heat according to the inflow of heat into the system.

Central to each of these models is the recognition that the preoptic/anterior hypothalamus is the primary control center of these systems, which integrates afferent information from the body, processes it, and activates the appropriate effector response (Parsons, 2003; Mack, 2004). In this way, the hypothalamus is considered the body's thermostat. The system itself is made up of four subsystems in a closed-control loop (Werner, 1981). These four subsystems include 1) receptors that detect and transmit changes in the controlled variable through afferent pathways to 2) the control system (hypothalamus), which sends efferent signals to the 3) effector mechanisms in the active system (SkBF, sweating, and shivering), which thereby 4) control the passive system. In the context of this system, temperature sensitive receptors called thermosensors are responsible for afferent signals to the hypothalamus. Peripherally, warm and cold sensitive receptors have been identified in the skin and other areas (described as 'core' receptors) such as blood vessels, the abdominal cavity and spinal cord (Hensel, 1981). Centrally, there are also thermosensitive neurons located in the hypothalamus (Parsons, 2003; Mack, 2004). When increases in skin and/or core temperatures (controlled variables) are detected by peripheral and/or central thermosensors, activation of sudomotor (sweating) and vasomotor (cutaneous blood vessels) effector mechanisms via the hypothalamus occurs to enhance heat dissipation (Parsons, 2003). This response

serves to equilibrate the heat balance equation after it has been disturbed by either endogenous heat production (i.e. exercise) or exogenous heat sources.

2.2. Mechanisms of Heat Dissipation

There are two mechanisms, SkBF and sweating, which mainly alter heat transfer from the body to the environment. The onset and rate of increase in heat loss responses are defined by the level of hyperthermia or core temperature. After the onset has occurred, the sensitivity of the SkBF and sweating responses are described by the slope of the SkBF/sweating-core temperature relationship (Johnson & Park, 1981). Therefore, changes in either the onset threshold or sensitivity of the SkBF/sweating-core temperature relationship have been used to identify altered thermal control (see figure 1). Delays in the onset threshold or decreases in the thermal sensitivity of heat loss responses will result in greater heat storage, and therefore larger increases in core temperature for a given level of heat stress.

2.2.1. Skin Blood Flow

Vasomotor activity associated with thermoregulation in the heat refers to the thermoeffector reflex of increasing SkBF in response to elevations in core and skin temperature (Johnson, 1986). Increasing SkBF helps to transfer heat from the core to the skin surface where it can be transferred to the environment and assist in the evaporation of sweat or minimize heat gain from the environment when ambient temperature exceed

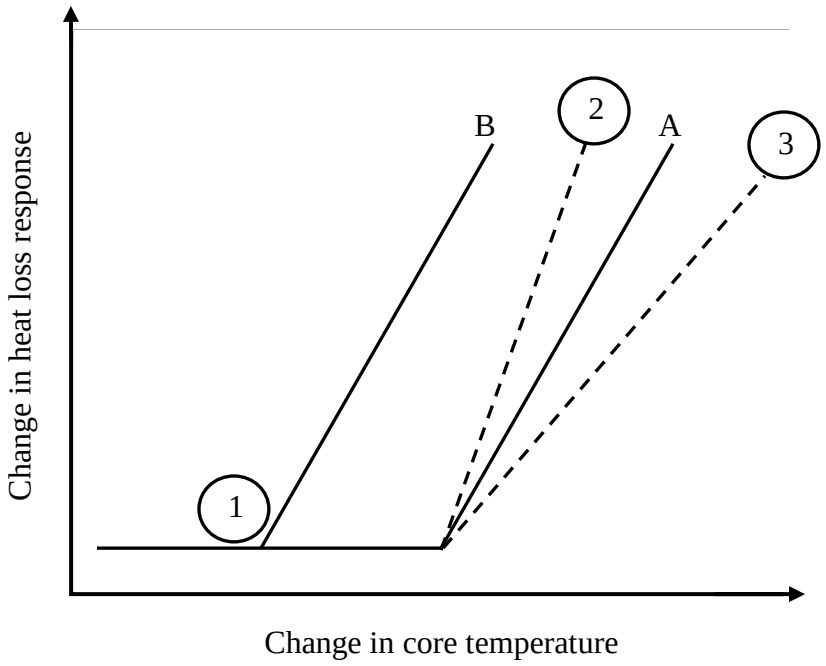

Figure 1. Schematic illustrating how the relationship of changes in the heat loss responses of SkBF and sweating to changes in core temperature can be used to identify altered thermal control. [1] The onset threshold is represented here in condition B by a significant increase in SkBF/sweating, which occurs at an earlier core temperature than condition A. [2] An increase in the sensitivity of the heat loss response to changes in core temperature is illustrated here compared to A, while [3] represents a decrease in sensitivity. Although core temperature (thermal factor) is a main driver for changes in SkBF and sweating, other nonthermal factors can modulate these changes.

skin temperature (Hammel, 1968; Charkoudian *et al.*, 2003). Similarly, the evaporation of sweat helps cool the blood in the skin before it returns to the core.

In addition to local factors, the cutaneous circulation responsible for this convective heat transfer from non-glabrous skin (head, limbs and trunk) is controlled by separate vasoconstrictor and vasodilator sympathetic neural pathways (Gisolfi *et al.*,

1993). The vasomotor tone responsible for maintenance of normal body temperature at rest in a normothermic environment is controlled by the vasoconstrictor system. Under these conditions SkBF is ~250 ml/min, which allows for heat dissipation (80-90 kcal/h) to equal that of metabolic heat production (Johnson & Proppe, 1996). This system is able to maintain core temperature at rest in thermoneutral environments because small changes in SkBF can result in large changes in heat dissipation (Charkoudian *et al.*, 2003). In contrast, large increases in SkBF observed during hyperthermia (6 – 8 L/min or 60% of cardiac output) are mostly (80-90%) due to the sympathetic active vasodilator system, while inhibition of the vasoconstrictor system plays a minor role (Charkoudian *et al.*, 2003).

The neural mechanism responsible for initiating increases in SkBF is that of the anterior preoptic hypothalamus (Gisolfi *et al.*, 1993; Parsons, 2003; Mack, 2004). This closed-loop feedback control center receives afferent information from core and skin temperatures, integrates this information, and adjusts the level of skin perfusion accordingly. During heat stress, an initial rise in SkBF is attributed to a release of vasoconstrictor tone that is closely correlated with an increase in skin temperature. Further heating causes active vasodilation that is responsible for large increases in SkBF and is closely correlated with increases in core temperature. Overall, research indicates that core temperature is ~20 times more influential in the control of SkBF above a threshold core temperature than changes in skin temperature (Wyss *et al.*, 1974). Consequently, increases in SkBF during exercise or passive heating are proportional to increases in core temperature until a steady state is reached as defined by an elevated plateau in core temperature, or until maximal increases in SkBF are reached.

2.2.2. Sweating

Similar to the control of SkBF, sweating is also a reflex response to an elevation in core and skin temperatures controlled centrally by the hypothalamus that elicits an increase in sweat secretion via sympathetic cholinergic activity. Although SkBF can provide adequate heat dissipation during heat stress, sweating becomes increasingly important when SkBF is limited, such as during high intensity exercise and high ambient temperatures. The high latent heat of vaporization (2426 J · g sweat^{-1}) provides a large potential for heat to be lost through evaporation (Wenger, 1972). For these reasons, the evaporation of sweat from the human body is the most important heat loss mechanism, especially during exercise performed in the heat.

Of the two types of sweat glands found on the body, eccrine glands located in non-glabrous regions are those responsible for thermoregulatory function (Parsons, 2003; Shibasaki *et al.*, 2003a). These glands secrete sweat when stimulated by nerve impulses which release acetylcholine that binds to muscarinic receptors on the sweat gland. These efferent nerve impulses originate from the preoptic hypothalamus, where local heating and afferent stimuli from peripheral core thermosensors activates sweating and skin vasodilation (Hammel, 1968; Hensel, 1981; Gisolfi *et al.*, 1993). This is why the elevation of the hypothalamic temperature that occurs with the increase in body temperature is the strongest stimulus for thermoregulatory sweating responses.

The amount of heat lost through sweating will depend both on the amount of sweat secreted and the conditions of the ambient environment. Kondo *et al*. (2001) demonstrated that sweat rate increased abruptly in the first 8 min of exercise and passive heating. This initial increase in sweating was found to be a result of both increased

density of activated sweat glands and increases in sweat output per gland. Further increases in sweating after 8 min were a result of increases in sweat output per gland. Although sweating increases abruptly at the start of passive heating and exercise, it must evaporate to serve as a functional heat loss mechanism. Although it has been shown that sweating can occur immediately at the start of exercise, it has been suggested that delays in the evaporation of sweat can be 2-5 min for a range of ambient conditions (Saltin *et al.*, 1970). In addition, direct calorimetry measurements of evaporative heat loss indicate that the rate of evaporative heat loss lags significantly behind increases in metabolic heat production. Consequently, there is a pronounced increase in body heat storage and rate of core temperature increase at the start of exercise (Kenny *et al.*, 2008; Kenny *et al.*, 2009). Furthermore, evaporation is determined by the water vapour pressure gradient between the skin and the environment. If humidity is high in a hot environment, then the water vapour pressure gradient between the moist skin and surrounding environment is greatly reduced, along with evaporation.

2.3 Hydration

The effects of hydration on cardiovascular function and thermoregulation has been investigated extensively (Greenleaf & Castle, 1971; Nadel *et al.*, 1980; Fortney *et al.*, 1981b; Fortney *et al.*, 1984; Sawka *et al.*, 1985; Montain & Coyle, 1992; Gonzalez-Alonso *et al.*, 1995; Gonzalez-Alonso *et al.*, 1997). Despite the fact that normal body water content remains relatively constant, exercise in the heat and/or prolonged passive heat stress will lead to significant body water deficits due to sweating (Shibasaki *et al.*, 2003a). Although sweat rates of 1 L/h are common, sweat rate losses of >3 L/h have been reported with exercise in the heat (Armstrong *et al.*, 1986), while typical daily fluid

requirements range from 8-16 l/day in hot climates depending on the level of activity (Latzka & Montain, 1999). Considering that the upper limit for fluid replacement is 1-1.5 L/h, this makes people exposed to exercise and/or heat stress extremely susceptible to dehydration (Sawka, 1992). Such high rates of sweat loss without adequate fluid replacement can impose significant strain on the body's ability to maintain water balance, while increasing an individual's risk of heat related injury and decreasing work performance (Gonzalez-Alonso *et al.*, 1997).

2.3.1 Effects of hydration on cardiovascular control

The maintenance of cardiac output is critical during heat stress because it is partly responsible for maintaining adequate SkBF. During heat stress, cardiac output increases and blood is redirected to the skin for heat dissipation, while blood flow to the renal, inactive muscle and splanchnic regions is reduced (Rowell, 1974). This increase in SkBF can reach 8 L/min and require as much as 60% of the total cardiac output, placing significant strain on the cardiovascular system to maintain cardiac output (Charkoudian *et al.*, 2003). The strain placed on the cardiovascular system is exacerbated when heat stress is combined with exercise, as blood flow to active muscles must now increase to ensure adequate delivery of oxygen and nutrients. However, although competition between the thermoregulatory and cardiovascular systems for blood circulation is augmented during exercise in the heat, research indicates that adequate cardiac output can be maintained if the combination of hyperthermia and dehydration is avoided. For example, increases in SkBF that occur in response to exercise-induced hyperthermia (end-exercise esophageal temperature of 39.3°C) leads to decreases in venous pressure and total peripheral resistance. This in turn decreases blood return to the heart, resulting in a decreased stroke

volume and a compensatory increased heart rate that maintains cardiac output and blood pressure (Gonzalez-Alonso *et al.*, 1997). Similarly, the commencement of exercise in a dehydrated state (-4% of total body water) and preventing hyperthermia (exercise in a cold environment at 2°C) invoked comparable cardiovascular responses so that cardiac output was not compromised (Gonzalez-Alonso *et al.*, 1997).

Despite these similar responses, Gonzalez-Alonso et al. (1997) showed that when a 4% dehydrated condition was combined with intense exercise (71% VO_{2max}) in the heat to an end exercise esophageal temperature of 39.3°C, increases in heart rate could not compensate for decreases in stroke volume and therefore cardiac output declined by 13%. Using saline infusion to restore blood volume, it was demonstrated that blood volume reductions only account for part of the reduced stroke volume and cardiac output during exercise in the heat when subjects become hyperthermic, suggesting additional mechanisms that have yet to be identified (Montain & Coyle, 1992; Gonzalez-Alonso *et al.*, 1997). Essentially, the strain placed on the cardiovascular system during hyperthermia cause by heat stress and/or exercise is exacerbated when sweat loss is not replaced and individuals become dehydrated.

2.3.2 Effects of hydration on thermoregulation

In addition to the cardiovascular strain associated with dehydration and exercise in the heat, dehydration has also been shown to adversely affect heat loss mechanisms. For example, Sawka (1985) subjected men to heat stress tests (49°C, 20% relative humidity) beginning at increased levels of dehydration (3, 5, and 7% respectively). Regression analysis revealed that rectal temperature linearly increased with the level of dehydration with a slope of 0.15°C for each percent decrease in body weight. However,

they did not observe a significant difference in rectal temperature between trials when subjects were either euhydrated or moderately dehydrated, failing to report that small levels of dehydration augment hyperthermia during exercise. In contrast, Montain & Coyle (1992) had participants exercise for 2 hours in the heat (33°C, 50% relative humidity) with and without fluid replacement to graded levels of dehydration equivalent to 1, 2, 3, and 4% of total body water loss. Increases in esophageal temperature were highly correlated with the level of dehydration incurred, while the end-exercise esophageal temperatures of 37.9°C ± 0.1 and 38.2°C ± 0.1 for levels of 1 and 2% dehydration respectively, were significantly different. Therefore, it was shown that even small levels of dehydration can augment heat storage and core temperature during exercise in the heat. In summary, dehydration is associated with a greater increase in core temperature during exercise in the heat that is graded in proportion to the level of dehydration (Nadel *et al.*, 1980; Montain & Coyle, 1992).

These graded increases in core temperature are attributable to changes in the heat loss responses of SkBF and sweating (Gonzalez-Alonso *et al.*, 1997; Sawka *et al.*, 2001). That is, dehydration has been shown to increase the core temperature at which the onset threshold for skin vasodilation occurs, as well as decrease sweat rate for a given core temperature (Nadel *et al.*, 1980; Sawka *et al.*, 1985). Specifically, dehydration leads to a decrease in plasma volume (hypovolemia) paralleled by increases in plasma osmolality (hyperosmolality), both of which have been shown to independently alter sweating and SkBF responses during heat stress (Nadel *et al.*, 1980; Fortney *et al.*, 1981b; Fortney *et al.*, 1984; Takamata *et al.*, 1997). The effect of hypovolemia on sweating and SkBF are thought to be manifested via a baroreflex-mediated response (i.e., baroreceptor unloading associated with a reduction in central blood volume), while plasma hyperosmolality is

believed to have either a central effect on osmoreceptors located in the hypothalamus and/or peripheral effect on the eccrine sweat gland due to high interstitial osmotic pressure changes (Sawka, 1992; Mack *et al.*, 1995; Ito *et al.*, 2005). Although both plasma hyperosmolality and baroreceptor unloading have been shown to inhibit thermoregulatory responses separately, their combined effects on sweating and SkBF remain unresolved.

2.3.3 Hydration, Heat stress and Exercise

The sweat losses that lead to dehydration can result from either exercise or passive heating, or a combination of passive heating and exercise. For example, 2 hours of moderate intensity cycling (~60% VO_{2max}) in the heat (35°C, 50% relative humidity) will result in dehydration of 4-5% (Montain & Coyle, 1992; Gonzalez-Alonso *et al.*, 1999; Gonzalez-Alonso *et al.*, 2000), while 5-6 hours of passive heating in 46°C, 23% relative humidity can result in dehydration of 4.3% of body weight when water is restricted (Craig & Cummings, 1966). However, understanding thermal control when exercise has been introduced creates a more complex situation and is different when compared to a thermal stress at rest.

Although both passive heating and exercise will lead to increases in SkBF and sweating, exercise creates additional challenges by increasing competition between the thermoregulatory and cardiovascular systems for blood flow (see figure 2). Unlike during a passive heat stress, blood flow to active muscles increases during exercise (Rowell, 1974). This increase in muscle blood flow is associated with decreases in SkBF at the start of exercise (Jonhson & Park, 1982). As exercise continues, thermoregulatory reflexes will begin to compete with exercise demands to increase SkBF. This increased

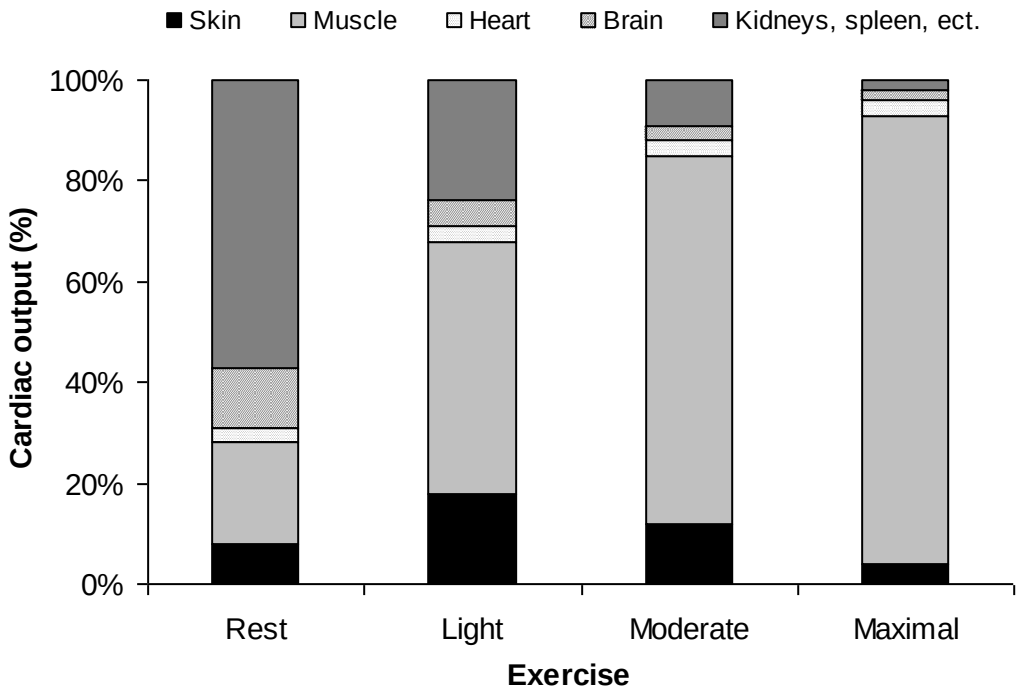

Figure 2. Schematic illustrating the distribution of cardiac output during rest and at increasing intensities of exercise. Source: Wilmore & Costill (2004)

competition during exercise has been shown to increase the onset threshold for SkBF and leave the sweating threshold unchanged when compared to the same thermal stress at rest (Kellogg *et al.*, 1991). This threshold change associated with exercise is a result of a delayed increase in active vasodilation (Kellogg *et al.*, 1991). It has also been reported that the SkBF threshold increases in a curvilinear fashion with exercise intensities that exceed 125W (Taylor *et al.*, 1988).

In addition to these blood flow regulation changes that occur with exercise, other nonthermal factors are also present during exercise that have been shown to have a significant influence on the control of SkBF and sweating. These factors include muscle mechanoreceptors, metaboreceptors, central command and their overall general effect on SkBF and sweating is summarized in table 1 (Kondo *et al.*, 2000; Kondo *et al.*, 2003;

Shibasaki *et al.*, 2003b). Studies that isolate these nonthermal factors to determine their influence on the control of SkBF and sweating utilize an isometric hand grip (IHG) exercise protocol. In general, this protocol requires individuals to isometrically contract their forearm at a certain percentage of their maximal contraction (30-60%) for 1-2 min. Next, a blood pressure cuff is inflated to ~250 mm Hg to occlude blood flow for 2 min (muscle ischemia). Changes in SkBF or sweating during the IHG exercise occur without any changes in skin or core temperature and are considered to be under the control of mechanisms that are nonthermal in nature (i.e., central command, mechanoreceptors, metaboreceptors, and baroreceptors). The period of muscle ischemia stimulates metaboreceptor afferents, while eliminating central command and stimulation of mechanoreceptor afferents. Furthermore, administration of a partial neuromuscular blockade augments the effects of central command during IHG exercise by eliminating force production.

Therefore, inducing a thermal stress at rest attenuates the competition between the thermoregulatory and cardiovascular systems and negates the confounding influence muscle mechanoreceptors, metaboreceptors and central command can have on thermoregulation during exercise.

Table 1. Effect of central command and muscle metaboreceptor stimulation on heat loss responses of sweating and skin blood flow.

Nonthermal Factor	Study	Heat stress Condition	Δ Core temperature	Sweating	SkBF
Metaboreceptors & Central Command	(Crandall *et al.*, 1995)	normothermic	↔	↔	↔
		mild	↑ 0.5°C	↑	↓
Metaboreceptors	(Kondo *et al.*, 2002)	mild	↑ 0.5°C	↑↑	↑
		moderate	↑ 0.9°C	↑	↓
	(Crandall *et al.*, 1998)	normothermic	↔	↔	↔
		mild	↑ 0.5°C	↑	↓
	(Kondo *et al.*, 1999)	normothermic	↔ (↑$\bar{T}_{sk}$)	↑ and ↔	↔
	(Kondo *et al.*, 2003)	normothermic	↔ (↑$\bar{T}_{sk}$)	↑	↔
Central Command	(Shibasaki *et al.*, 2001)	normothermic	↔	↑	-
		mild	↑ 0.5°C	↑	-
	(Shibasaki *et al.*, 2003b)	normothermic	↔	↑	-
		mild	↑ 0.5°C	↑↑	-
		moderate	↑ 1.0°C	↑	-
	(Shibasaki *et al.*, 2005)	normothermic	↔	-	↔
		moderate	↑ 1.0°C	-	↓

2.4 Thermoregulation and nonthermal influences

Nonthermal factor is a general term in thermoregulation literature that refers to variables other than changes in skin and core temperature (thermal factors) that can influence thermoregulatory control. Although it is generally thought that activation of autonomic thermoeffector activity is primarily influenced by thermal sensors (peripheral and central), evidence suggests that nonthermal stimuli can also modulate these responses (Crandall *et al.*, 1996; Shibasaki *et al.*, 2003a; Journeay *et al.*, 2006; Kenny *et al.*, 2007; Kenny & Journeay, 2010). Consequently, various nonthermal factors have been studied, such as metaboreceptors, mechanoreceptors, baroreceptors and central command in order to further understand thermoregulatory control (Kenny & Journeay, 2010). Given that the observed changes in heat loss responses that occur with dehydration are believed to be

related to baroreceptors and osmoreceptors, a review of studies and their findings on these nonthermal factors will be presented below.

2.4.1 Baroreceptors

Baroreceptors have been shown to have a significant influence on thermoeffector activity (Zoller *et al.*, 1972; Kellogg *et al.*, 1990; Crandall *et al.*, 1996; Peters *et al.*, 2000; Mack *et al.*, 2001; Carter III *et al.*, 2002; Kenny *et al.*, 2003; Journeay *et al.*, 2004; Journeay *et al.*, 2006; Gagnon *et al.*, 2008). Baroreceptors are stretch-sensitive receptors that primarily detect changes in arterial blood pressure. Afferent impulses originating from baroreceptors are sent via the autonomic nervous system to the cardioregulatory center located in the medulla oblongata, where the firing rate of their action potentials are received and interpreted (Shibasaki *et al.*, 2003a). If blood pressure is high (increase in rate of firing) then baroreceptors become "loaded". If blood pressure is low (decrease in rate of firing) then baroreceptors become "unloaded". In response to this afferent information, efferent signals are sent from the cardioacceleratory or cardioinhibitory center to the heart and blood vessels by means of sympathetic or parasympathetic divisions (Van Wynsberghe *et al.*, 1995). For example, when baroreceptors become unloaded, sympathetic activity increases and parasympathetic activity decreases to cause an increase in heart rate, stroke volume, cardiac output, cutaneous vasoconstriction and total peripheral resistance (Van Wynsberghe *et al.*, 1995).

It has been proposed that there are two main groups of baroreceptors (high and low pressure). The first group are located in the carotid sinus and aortic arch and are referred to as high-pressure arterial baroreceptors. These baroreceptors monitor both brain blood supply and blood vessel pressure, respectively. The second group of

baroreceptors are the cardiopulmonary receptors located in large systemic veins and right atrium. These baroreceptors monitor blood volume levels through both circulatory and renal pathways. Different lab techniques have been employed to study the effects of baroreceptors on heat loss responses, which unload different populations of baroreceptors. Application of lower body negative pressure (LBNP) at low levels and using a 30°C head-up tilt manoeuvre likely causes greater unloading of the cardiopulmonary baroreceptors, while pharmacological induced reductions in blood pressure cause a greater unloading of arterial baroreceptors (Mack *et al.*, 2001; Wilson *et al.*, 2001; Wilson *et al.*, 2005). Yet, higher levels of LBNP (>20 mm Hg) may engage both populations of baroreceptors (Mack *et al.*, 1995; Mack *et al.*, 2001). However, recent work by Fu *et al.* (2008) demonstrated unloading of arterial baroreceptors even at low levels of LBNP, making it difficult to distinguish between baroreceptor populations.

The baroreceptor reflex has been studied extensively in thermoregulation and is considered to have a profound influence on the control of SkBF (Solack *et al.*, 1985; Kellogg *et al.*, 1990; Crandall *et al.*, 1996; Mack *et al.*, 2001; Wilson *et al.*, 2001; Keller *et al.*, 2006; Kenny *et al.*, 2010). For example, during moderate exercise (118 ± 8 W) in normothermic conditions, Mack *et al*. (2001) used lower body negative pressure (LBNP at -40 mm Hg) to unload baroreceptors. The onset core temperature for SkBF was greater during LBNP (0.50 ± 0.10°C) compared to a control condition (0.25 ± 0.08°C), resulting in a greater esophageal temperature at the end of 15 minutes of exercise (37.44 ± 0.09 vs. 37.30 ± 0.06°C). Similarly, Tripathi & Nadel (1986) applied various levels (-10 to -50 mmHg) of LBNP for 3 min intervals under normothermic conditions at rest to study changes in forearm blood flow. Forearm blood flow decreased in a graded fashion to decreasing levels of LBNP. However, they determined that a LBNP of -20 mmHg was

required to initiate decreases in SkBF (and increases in skin vasoconstriction) and that changes in muscle blood flow are responsible for the changes in forearm blood flow at low levels of LBNP (<20 mm Hg). The importance of a baroreceptor control of SkBF is critical during times of heat stress to ensure adequate regulation of blood pressure. In general, adequate blood pressure is maintained in the face of cardiovascular strain and heat stress. However, as with all physiological systems there is an upper limit whereby cardiovascular regulation cannot maintain sufficient control during severe heat stress, and syncope is likely to occur.

In contrast to the well established influence of baroreceptors on the control of SkBF, Kenny & Journeay (2010) have recently concluded that "there remains significant controversy regarding the role of baroreceptors in modulating thermal sweating". Mack *et al.* (2001) demonstrated that LBNP (-40 mm Hg) delayed the onset of sweating during moderate exercise in a normothermic environment (25°C). The increase in esophageal temperature required to elicit sweating averaged 0.39 ± 0.10°C and 0.20 ± 0.10°C with and without LBNP, respectively. In this study, the sensitivity of the sweating-esophageal temperature relationship was not changed. This was in contrast to an earlier study by Mack *et al*. (1995) that used a similar protocol. They found that the sensitivity of the sweating-esophageal temperature relationship was decreased with the application of LBNP (-40 mm Hg) during exercise, while there was no difference in the onset core temperature threshold for increases in sweating. However, upon reanalysis of core temperature thresholds using a change from baseline resting required to elicit a response instead of an absolute value, they found results consistent with their later study. Also, they attributed changes in the sweating sensitivity to 3 individuals who showed little or no sweating in the original study. Consequently, the work by Mack *et al.* (1995; 2001)

demonstrated considerable evidence that baroreceptor unloading can influence thermal sweating during exercise. Conversely, Solack *et al.* (1985) passively heated subjects while measuring forearm sweating. LBNP was then applied (-20 to -60 cmH_2O), which elicited no decrease in sweating but did cause a 28% decrease in the sensitivity of sweating-core temperature relationship. However, since the sensitivity of this slope did not recover with the cessation of the LBNP, they concluded that sweating is not an effector of low pressure baroreceptors. In addition, Wilson *et al.* (2001) used pharmacological agents to manipulate blood pressure during normothermic and heat-stressed humans, therefore altering baroreceptor loading status without the confounding influence of skin cooling that may occur with the application of LBNP (Vissing *et al.*, 1994). Neither sweating nor skin sympathetic nerve activity (an index of sweating) was altered by the pharmacological manipulation of blood pressure in either condition.

It has been suggested that the level of heat stress may alter the baroreceptors influence on thermal sweating. Consequently, Wilson *et al*. (2005) performed 30°C head-up tilts from a supine position (baroreceptor unloading) during a progressive passive heat stress (36.8 to 38.0°C) while measuring skin sympathetic nerve activity and local sweating. They observed no differences in local sweating or skin sympathetic nerve activity at any point throughout the heat stress. Although there have been proposed explanations for the contradictory findings on baroreceptor modulation of sweating, such as changes in skin temperature during LBNP, manipulation of different population of baroreceptors and the confounding influence of exercise, stress and hyperventilation, it is clear further investigation is needed to understand the impact baroreceptors can have on eccrine sweating.

2.4.2 Osmoreceptors

In addition to thermosensors, osmosensitive neurons have been identified in the preoptic-anterior hypothalamus (Silva & Boulant, 1984; Owen *et al.*, 1989). Animal studies indicate that targeting these osmosensitive neurons with hypertonic solutions can elevate core temperature during rest and exercise in the heat (Owen *et al.*, 1989; Sawka, 1992). Therefore, it has been proposed that the precisely regulated plasma osmolality might compete centrally with thermoregulation in the control of thermal effector mechanisms (sweating and SkBF).

During dehydration, intracellular and extracellular volumes decrease leading to plasma hyperosmolality. Studies show that increases in plasma osmolality are associated with reductions in sweating and SkBF during heat stress and/or exercise (Fortney *et al.*, 1984; Takamata *et al.*, 1997; Ito *et al.*, 2005; Shibasaki *et al.*, 2009). For example, Fortney *et al.* (1984) demonstrated that hyperosmolality increases the onset threshold for sweating and SkBF during exercise in the heat. In this study, participants exercised in the heat prior to the experimental protocol to become dehydrated. Participants were then infused with 3% saline to return plasma volume levels to normal, while maintaining an average 11 mosmo•kgH_2O^{-1} increase in plasma hyperosmolality from a pre-exercise level of 282 mosmo•kgH_2O^{-1}. This increase in plasma osmolality is similar to those measured when individuals are 5% dehydrated (Sawka *et al.*, 1985). After resting in a cool environment for 1 hour, participants exercised in the heat (30ºC, 40% relative humidity) for 30 minutes. The hyperosmotic condition resulted in a 0.22°C increase in the esophageal temperature threshold for skin vasodilation and sweating compared to control (isosmotic). However, no changes in the thermal sensitivity for sweating and vasodilation were observed. Similar results were reported by Shibasaki et al. (2009) during passive

heat stress (lower leg water emersion in 42°C). Subjects were infused with either hypertonic (3% NaCl, increase plasma osmolality by 14 mosmo•kgH_2O^{-1}) or isotonic (0.9% NaCl, to maintain plasma osmolality) saline after which they were passively heated for 60 min. Compared to the isosmotic condition, the onset threshold for skin vasodilation and sweating increased by 0.88°C and 0.71°C respectively. Again, the sensitivity of the sweating/SkBF-esophageal temperature response was unchanged between conditions.

To address a different question, Fortney *et al.* (1981a; 1981b) studied the effects of changes in blood volume (hypovolemic and hypervolemia) on sweating and SkBF responses during moderate intensity exercise (30 min at 60% VO2max in 30-35°C, 40% relative humidity), while keeping osmolality constant. Their findings suggest that isosmotic hypovolemia reduces the sensitivity of the sweating response without changes in the onset threshold. Isosmotic hypovolemia also reduced the sensitivity of SkBF-esophageal temperature relationship and increased the esophageal temperature onset threshold. Interestingly, increasing blood volume (isosmotic hypervolemia) had no significant effects on the control of sweating or SkBF when compared to a control. A similar study by Nadel *et al.* (1980) also reported changes in thermoeffector activity during isosmotic hypovolemia. However, these changes were reported as an increase in the core temperature onset threshold for cutaneous vasodilation and a 50% reduction in the maximal forearm blood flow, but no changes in the forearm blood flow-core temperature relationship (thermal sensitivity).

A study by Ito *et al.* (2005) sought to identify the interactive effects of central hypovolemia and hyperosmolality on regulation of the peripheral vascular response. Again, subjects were first infused with either hypertonic (3% NaCl) or isotonic (0.9%

NaCl) saline, passively heated for 60 min with a water perfusion suit (42°C), and then subjected to progressive LBNP (-10 to -40 mmHg) after core temperature had increased by 0.3°C and 0.9°C in the isosmotic and hyperosmotic conditions, respectively. The reduction in forearm vascular conductance was greater in the hyperosmotic condition than isosmotic (-9.99 ± 0.96 units vs. -6.02 ± 1.23 units, respectively). Their findings suggest there is an interactive effect between plasma hyperosmolality and central hypovolemia on the peripheral vascular response during heat stress. However, it is possible the greater level of hyperthermia reached in the hyperosmotic condition contributed to the observed reductions in forearm vascular conductance, since reductions in skin blood flow are more readily evidenced as the level of heat stress increases (Peters *et al.*, 2000). Additionally, the study by Ito *et al.* (2005) fails to determine the interactive effect of hyperosmolarity and central hypovolemia during a passive heat stress on the sweating response, since sweat rate was not recorded.

2.5 Summary

In summary, prolonged sweating without adequate water replacement will lead to dehydration, which can easily occur during exercise and/or heat stress. In such an event, there is increased competition between the cardiovascular and thermoregulatory system for a limited blood supply. This creates a situation where afferent information from nonthermal factors may more easily modify sweating and SkBF responses, which are primarily thermally driven. Specifically, dehydration leads to changes in blood volume and plasma osmolality, which stimulate baroreceptors and central osmoreceptors respectively. While the independent effects of blood volume alterations and plasma hyperosmolality have been well documented during exercise and passive heating, their

combined effects on sweating and SkBF are not well studied. Although it is possible that the roles of plasma hyperosmolality and baroreceptor unloading on heat loss responses are different during exercise (i.e. influence of mechanoreceptors, metaboreceptors and central command), the following thesis examined these influences during a passive heat stress in an attempt to provide a more comprehensive understanding of human thermoregulatory control during challenges to maintain body water balance.

PART TWO: METHODS & RESULTS

Divergent roles of plasma osmolality and the baroreflex on sweating and skin blood flow

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ABSTRACT

Plasma hyperosmolality and baroreceptor unloading have been shown to independently influence the heat loss responses of sweating and cutaneous vasodilation. However, their combined effects remain unresolved. On four separate occasions, eight males were passively heated with a liquid conditioned suit to 1.0ºC above baseline core temperature during a resting isosmotic state (infusion of 0.9% NaCl saline) (i) with (**LBNP)** and (ii) without (**CON**) application of lower body negative pressure (-40 cm H_2O) and during a hyperosmotic state (infusion with 3.0% NaCl saline) (iii) with (**LBNP+HYP**) and (iv) without (**HYP**) application of lower body negative pressure. Forearm sweat rate (ventilated capsule) and skin blood flow (laser-doppler), as well as core (esophageal) and mean skin temperatures were measured continuously. Plasma osmolality increased by ~10 mosmol•kgH_2O^{-1} during **HYP** and **LBNP+HYP** conditions, while it remained unchanged during **CON** and **LBNP** ($p \leq 0.05$). The change in mean body temperature (0.8•core temperature + 0.2•mean skin temperature) at the onset threshold for increases in cutaneous vascular conductance (CVC) was significantly greater during **LBNP** (0.56±0.24°C) and **HYP** (0.69±0.36°C) conditions, compared to **CON** (0.28±0.23°C, $p \leq 0.05$). Additionally, the onset threshold for CVC during **LBNP+HYP** (0.88±0.33°C) was significantly greater than **CON** and **LBNP** conditions ($p \leq 0.05$). In contrast, onset thresholds for sweating were not different during **LBNP** (0.50±0.18°C) compared to **CON** (0.46±0.26°C, $p=0.950$), but were elevated ($p \leq 0.05$) similarly during **HYP** (0.91±0.37°C) and **LBNP+HYP** (0.94±0.40°C). Our findings show an additive effect of hyperosmolality and baroreceptor unloading on the onset threshold for increases in CVC during whole-body heat stress. In contrast, the onset threshold for sweating during heat stress was only elevated by hyperosmolality with no effect of the baroreflex.

INTRODUCTION

Dehydration is associated with decreases in plasma volume (hypovolemia) and increases in plasma osmolality (hyperosmolality), which are thought to stimulate peripheral baroreceptors and central osmoreceptors respectively (9, 27, 38, 40). Independently, hypovolemia or baroreceptor unloading and plasma hyperosmolality have both been shown to have a significant negative influence on thermoefferent activity and therefore, body core temperature regulation (8, 9, 24, 27, 33). Plasma hyperosmolality has consistently been shown to increase the core temperature at which onset threshold of sweating and increases in cutaneous vascular conductance (CVC) occur without any changes in the thermal sensitivity (10, 33, 40). Similarly, baroreceptor unloading has been shown to cause a delay in the core temperature onset threshold for cutaneous vasodilation without any changes in the thermal sensitivity of the response (20, 24, 27). These responses result in a greater increase in core temperature for a given level of heat stress. However, modulation of the sweating response by the baroreflex is less consistent, with some studies reporting an attenuated sweating response (8, 24, 36) and others showing no effect (6, 42, 44, 45) during baroreceptor unloading.

Although numerous studies have examined the effects of baroreceptor unloading and hyperosmolality on the heat loss responses of cutaneous blood flow and sweating, they have generally examined these conditions independently. However, in the setting of real-time heat stress, dehydration results in the convergent signals by plasma hyperosmolality and hypovolemia (4). Therefore, it remains to be determined if one of these stimuli has a predominant effect, or if both act together (i.e. synergistically or additively) in causing the observed disturbances in the heat loss mechanisms of sweating and skin blood flow while dehydrated. Furthermore, it is unknown if plasma

hyperosmolality and baroreceptor unloading affect sweating and skin blood flow to a similar extent, or if one stimulus preferentially modulates either one of these heat loss responses.

To the best of our knowledge, Ito et al. (16) is the only study that has addressed a potential interaction between plasma hyperosmolality and baroreceptor unloading, focusing solely on the skin blood flow response. By applying lower body negative pressure (LBNP) after 60-min of passive heating under either an isosmotic or hyperosmotic state, they examined the capacity of the baroreflex to cause reductions in forearm vascular conductance as a function of plasma osmolality. Interestingly, greater reductions in forearm vascular conductance were observed in the hyperosmotic state than the isosmotic state, but only at higher levels of LBNP (-40 mmHg). Although these findings suggest a potential interaction between plasma hyperosmolality and the ability of the baroreflex to reduce forearm vascular conductance, they do not provide any information as to how this interaction may affect the initiation of active vasodilation since they applied LBNP once forearm vascular conductance had attained elevated levels. Furthermore, the interactive response on sweating, the main avenue of heat loss during heat stress, was not reported. As such, it remains to be determined how the combination of baroreceptor unloading and plasma hyperosmolality influences the thermal control of sweating and cutaneous vasodilation.

Therefore, we examined the separate and combined effects of baroreceptor unloading and plasma hyperosmolality on human temperature regulation as measured by the mean body temperature at which the onset of sweating and increases in CVC occur, as well as the thermal sensitivity of these responses during whole-body heating. We hypothesised that combined LBNP and plasma hyperosmolality would result in a greater

delay in the mean body temperature at which sweating and increases in CVC occur, relative to the independent effects of each condition. A second hypothesis was that the thermal sensitivity of each response would be unaffected by the baroreflex and plasma hyperosmolality.

METHODS

Ethical approval

The current experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to their participation in the study.

Participants

A sample size of 6 participants was determined using a power calculation (α = 0.05, β = 0.95) estimated from the changes in core temperature required to cause cutaneous vasodilation and sweating in a hyperosmotic condition relative to an isosmotic condition during passive heating (33). A total of eight healthy (no history of respiratory, metabolic or cardiovascular disease, non-smoking and normotensive) and physically active (exercised 3-5 times per week for a minimum of 30 min) males volunteered for the study. Participants were recruited by advertisement within the university community and their mean (±SD) physical characteristics are as follows: age: 24 ± 4 yrs, height: 178 ± 9 cm, weight: 80.4 ± 8.8 kg, and body surface area: 1.98 ± 0.15 m^2.

Experimental protocol

All subjects volunteered for one screening visit and four experimental sessions that were separated by a minimum of 48 hours. During the screening visit, participants were informed of the study protocol before written consent was obtained.

On the day prior to each experimental session, subjects were instructed to abstain from salty foods and drink water throughout the day. Subjects then reported to the laboratory between 0730 and 1000 after drinking 500 mL of water, eating a light breakfast and avoiding caffeine, alcohol and physical activity for 24 hours prior to their trial. For each subject, trials were performed at the same time of day to avoid circadian variations in core temperature (37). Upon their arrival, subjects dressed in shorts and were asked to void their bladders and provide a urine sample before weighing themselves nude. Next, an 18-gauge catheter was inserted into an antecubital vein of the right arm. The subjects then entered an environmental chamber regulated at a thermoneutral ambient condition (semi-nude in 30°C and 20 % relative humidity) whereby they sat resting for a minimum of 30 min before a baseline blood sample was drawn.

Subjects were then infused with either 0.9 or 3.0% NaCl solution for 90 min to either maintain (isosmotic) or increase (hyperosmotic) blood osmolality. The infusion rates were 0.2 and 0.125 $mL \cdot min^{-1} \cdot kg^{-1}$ of body weight for the 0.9% and 3.0% NaCl solutions, respectively. These infusion rates were based on previous studies and were intended to provide similar changes in plasma volume between conditions (33, 40).

After the infusion period, subjects were dressed in a high density tube-lined water perfusion suit (Delta Temax Inc., Pembroke, ON, CAN) and placed upright seated in a pressure box sealed at the participant's waist with a neoprene skirt. The subjects also donned neoprene pants to help minimize air movement across the lower limbs during the

application of LBNP. After a 15 min baseline data collection period, either -40 cmH_2O LBNP (-29 mmHg) or sham pressure (0 mmHg) was applied for an additional 15 min before whole-body heating began. A sham pressure condition was performed by allowing air to escape near the top of the pressure box but remote to the sealed waist of the participant to create similar air movement patterns to that observed during the LBNP conditions, but without any change in pressure. The four experimental conditions, performed in random order were as follows: 1) isosmotic and sham pressure (**CON**) 2) isosmotic and LBNP (**LBNP**), 3) hyperosmotic and sham pressure (**HYP**), and 4) hyperosmotic and LBNP (**LBNP+HYP**). At the start of whole-body heating, ambient air temperature was increased to 40°C and warm water maintained at 48°C was perfused through the suit. Whole-body heating continued until a 1°C increase in core temperature relative to baseline 1 (B1) was achieved. Transition time after infusion to the start of heating was ~50 min for all trials.

Measurements

Core temperature was estimated by esophageal temperature, measured by placing a paediatric thermocouple probe of approximately 2 mm in diameter (Mon-a-therm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St-Louis, MO, USA) through the participant's nose to a depth of 40 cm past the opening of the nose. Skin temperature was measured at 9 points over the body's surface using 0.3 mm diameter T-type (copper/constantan) thermocouples (Concept Engineering, Old Saybrook, CT, USA). Thermocouples were attached using surgical tape (Blenderm, 3M, St. Paul, MN, USA). Mean skin temperature was calculated using the 9 skin temperatures weighted to the regional proportions as determined by Hardy and DuBois (15): head 7%, upper back

9.5%, chest 9.5%, lower back 9.5%, abdomen 9.5%, bicep 10%, quadriceps 19%, hamstring 10%, front calf 16%. All temperature data were collected using an HP Agilent data acquisition module (model 3497A) at a sampling rate of 15 s. Data was simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0, National Instruments, TX, USA).

Heart rate was monitored using a Polar coded transmitter, recorded continuously and stored with a Polar Advantage interface and Polar Precision Performance software (Polar Electro Oy, Finland).

Mean arterial pressure was estimated using a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) from the beat-to-beat recording of the left middle finger arterial pressure waveform with the volume-clamp method (29) and physiocal criteria that is used to calibrate the finger arterial size at which the finger cuff air pressure equals finger arterial blood pressure (43). Prior to the start of measurement recording, brachial artery pressure reconstruction (13, 14) was calibrated with an upper arm return-to-flow systolic pressure detection (2). Furthermore, the left arm was supported at heart level, and a level calibration was performed.

Cardiac output was measured noninvasely using an Innocor^{TM} inert gas rebreathing unit with breath-by-breath ergospirometry option and arterial oxygen saturation sensor (Innovisions, Odense, Denmark) that has been previously validated against the direct oxygen Fick method and thermodilution (31). For rebreathing gases, we used 5% nitrous oxide and 1% sulphur hexafluoride, diluted with atmospheric air (1, 23). The closed rebreathing system consisted of a 3-way respiratory valve connecting a facemask, an anti-static rubber bag, and an infrared photoacoustic gas analyzer. Before each rebreathing manoeuvre, the gas mixture was filled into the bag with a previously

determined volume (~2 L) that the participant could fully and easily empty into their lungs during each inhalation. Stroke volume was calculated as cardiac output divided by heart rate.

Forearm skin blood flow was estimated using laser-Doppler velocimetry maintained locally at 35°C (PeriFlux System 5000, Main control unit; PF5010 LDPM) on the left mid-anterior forearm. After the skin was shaved and cleaned with alcohol, the probe (Perimed integrating probe 413, Järfälla, Sweden) was placed on an area that, superficially, appeared to be minimally vascularized. The probe was not moved from its location throughout the experimental trial. To determine maximum skin blood flow, a local heating period to 42°C for 20 min and then to 44°C for an additional 25 min was performed at the end of each experimental trial (3). CVC was subsequently calculated as laser-Doppler velocimetry output in arbitrary perfusion units divided by mean arterial pressure and expressed as a percentage of maximum.

Forearm sweat rate was estimated from a 3.8 cm^2 ventilated capsule placed on the left mid-anterior forearm. Anhydrous compressed air was passed through the capsule over the skin surface at a rate of 1 $L \cdot min^{-1}$. Water content of the effluent air was measured at known barometric pressure using dew point hygrometry (RH Systems model 473, Albuquerque, NM, USA). Sweat rate was defined as the product of the difference in water content between effluent and influent air and the flow rate and was adjusted for skin surface area under the capsule (expressed in $mg \cdot min^{-1} \cdot cm^{-2}$).

Measurements of nude body weight to the nearest 0.01 kg were obtained prior to each experimental session using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, CAN). Urine specific gravity was

measured using refractometry (TS 400 Reichert, Inc., Depew, NY, USA) before each experimental session.

Venous Blood samples were collected without stasis through an indwelling plastic catheter in a superficial vein. Blood samples (approximately 10 mL) were drawn and transferred into K2 EDTA and SST^{TM} vacutainers (BD Vacutainer, Franklin lakes, NJ, USA) for the determination of plasma volume and osmolality respectively. Four samples were taken throughout each experimental trial at the following time points: 1) at rest following 30 min in the seated position, 2) prior to the start of whole-body heating, 3) following a 0.5°C increase in core temperature and 4) at the end of heating, equal to a 1°C increase in core temperature. Hematocrit and hemoglobin concentrations were determined using the Coulter method (Coulter® A^{c}•T diff 2^{TM} analyzer, Beckman Coulter, Miami, Florida, USA). Plasma osmolality was determined by centrifuging each blood sample and storing the separated plasma frozen at -20°C until measurement. All samples of plasma osmolality were analyzed ~48 hours after collection by freezing point depression (Osmometer, Advanced Instruments Inc., MA, USA). Changes in plasma volume from baseline resting were estimated from changes in hemoglobin and hematocrit using the formula proposed by Dill & Costill (7).

Statistical analysis

To determine the effect of LBNP prior to the heating period, the dependent variables were analyzed at baseline (baseline 1, **B1**) and during the application of LBNP or sham pressure (baseline 2, **B2**) using a one-way repeated measure ANOVA with the factor of treatment condition. Furthermore, to determine the effect of heating and LBNP on the dependent variables, a two-way repeated measure ANOVA was employed with the

repeated factors of increase in core temperature (levels: 0°C, 0.25°C, 0.5°C, 0.75°C, 1°C) and treatment condition. Relative core temperature increases were determined from the 15 min of baseline data collection (**B1**), prior to the application of LBNP. Subsequently, dependent variable values were determined at core temperature increases for analysis by taking a 3 min average of data that included 1 min before and 1 min after each core temperature increase was reached.

The onset thresholds for sweating and increases in CVC are expressed as mean body temperature (0.8 • core temperature + 0.2 • mean skin temperature) to account for the relative influences of changes in skin and core temperatures on thermoefferent activity during whole-body heating (12). Onset thresholds were defined as the mean body temperature at which a rapid increase in CVC and sweating occurred over three consecutive measurements (24, 39). Thermal sensitivity was defined by the slope of the linear portion of the CVC/sweating to mean body temperature relationship, fitted with a least squares linear regression line. The independent and combined effects of baroreceptor unloading and hyperosmolality on changes in onset threshold and thermal sensitivity were analyzed by comparing each condition (**HYP, LBNP** and **LBNP+HYP**) relative to CON using paired sample T-tests. To determine which treatment condition had the greatest effect on changes in onset threshold and thermal sensitivity, each condition (**HYP, LBNP** and **LBNP+HYP**) was subsequently compared using a one-way repeated measures ANOVA. For all analyses, when a significant main effect of condition was observed, post-hoc comparisons were carried out with paired sample T-tests corrected for multiple comparisons using the Holm-Bonferroni procedure. The level of significance was set to an alpha level of $p \leq 0.05$. All analyses were performed using the statistical

software package SPSS 18.0 for Windows (SPSS Inc. Chicago, IL, USA). All results are presented as mean ± standard deviation, unless otherwise indicated.

RESULTS

No differences in hydration status as determined by total body weight (**CON**, 80.20 ± 8.37 kg; **LBNP**, 80.25 ± 8.49 kg; **HYP**, 80.25 ± 8.15 kg; and **LBNP+HYP**, 80.04 ± 8.21 kg, p=0.889) and urine specific gravity (**CON**, 1.015 ± 0.008; **LBNP**, 1.017 ± 0.010; **HYP**, 1.018 ± 0.008; and **LBNP+HYP**, 1.017 ± 0.008, p=0.670) were measured between conditions prior to the start of the experimental protocol.

Plasma osmolality and plasma volume

Mean changes in plasma osmolality and plasma volume after infusion and during heat stress are presented in Figure 1. Pre-infusion osmolality did not differ between conditions (p=0.714). Isotonic saline infusion did not change plasma osmolality in either the **CON** or **LBNP** conditions, whereas hypertonic saline infusion significantly increased plasma osmolality to a similar extent in the **HYP** and **LBNP+HYP** conditions ($p \leq 0.05$). During whole-body heating, plasma osmolality remained similar to pre-infusion levels in the **CON** and **LBNP** conditions ($p > 0.05$). In contrast, plasma osmolality remained significantly elevated above pre-infusion levels during whole-body heating in the **HYP** and **LBNP+HYP** conditions ($p \leq 0.05$).

There was no statistical effect of condition on plasma volume increases after infusion and before whole-body heating (p=0.241). Whole-body heating caused a decrease in plasma volume from post-infusion values in all conditions ($p \leq 0.05$), with a

greater decrease observed during LBNP conditions (**LBNP** and **LBNP+HYP**) compared to sham pressure conditions (**CON** and **HYP**) ($p \leq 0.05$).

Cardiovascular responses

Baseline period. During baseline rest (**B1**), there were no significant differences in cardiac output ($p=0.426$), heart rate ($p=0.641$), stroke volume ($p=0.508$), mean arterial pressure ($p=0.694$), and total peripheral resistance ($p=0.253$) among conditions. During the baseline period with application of LBNP or sham pressure (**B2**), cardiac output and stroke volume were significantly lower in the **LBNP** and **LBNP+HYP** conditions as compared to the **HYP** condition (Fig. 2, $p \leq 0.05$). Similarly, there was a main effect of condition ($p<0.001$) for differences in total peripheral resistance (Fig. 3) and heart rate (Fig. 2) during **B2**, demonstrated by generally greater heart rate and total peripheral resistance values in the **LBNP** and **LBNP+HYP** conditions compared to the **HYP** and **CON** conditions ($p \leq 0.05$). There was no main effect of LBNP or sham pressure on mean arterial pressure prior to whole-body heating (Fig. 3, $p=0.865$). The observed differences in cardiovascular responses during the application of LBNP compared to the sham pressure conditions demonstrate that a similar level of baroreceptor unloading had been successfully induced in the **LBNP** and **LBNP+HYP** conditions prior to beginning the whole-body heat stress period.

Whole-body heating. During whole-body heating, stroke volume and total peripheral resistance decreased as a function of increases in core temperature for all conditions ($p \leq 0.05$), but to a greater extent in the LBNP conditions compared to the sham pressure conditions ($p \leq 0.05$). Cardiac output significantly increased in the **CON**, **LBNP** and **HYP** conditions ($p \leq 0.05$), but no significant change in the **LBNP+HYP** condition

was observed (p=0.134). Throughout whole-body heating, cardiac output was significantly greater in the **CON** and **HYP** conditions compared to **LBNP** and **LBNP+HYP** conditions ($p \leq 0.05$). Similarly, heart rate increased during whole-body heating in all conditions ($p < 0.001$), with significantly greater increases observed in the **LBNP** and **LBNP+HYP** conditions compared to **CON** and **HYP** conditions at 0.5°C, 0.75°C and 1.0°C core temperature increases ($p \leq 0.05$). In contrast, there was no significant effect of condition (p=0.193) or core temperature (p=0.191) on mean arterial pressure during whole-body heating. These different cardiovascular responses indicate that the baroreceptor unloading achieved prior to beginning the heating period was maintained throughout passive heat stress in the **LBNP** and **LBNP+HYP** conditions.

Thermal Responses

Heating time. The time required for esophageal temperature to increase by 1°C was 54 ± 5 min, 48 ± 6 min, 67 ± 17min , and 85 ± 13 min for the **CON, HYP, LBNP** and **LBNP+HYP** conditions, respectively.

Skin and core temperatures. Baseline resting (**B1**) measures of core temperature were 36.89 ± 0.12°C, 36.83 ± 0.20°C, 36.90 ± 0.17°C, and 36.93 ± 0.07°C for the **CON, LBNP, HYP**, and **LBNP+HYP** conditions, respectively (p=0.370). There were no differences in baseline resting (**B1**) measures of mean skin temperatures (Table 1, p=0.387). There was a significant main effect of condition on mean skin temperature during B2 (p=0.039) and whole-body heating (p=0.032). However, after correcting for multiple comparisons, no significant differences were observed between condition.

Cutaneous vascular conductance. Figure 4 illustrates the typical response of a representative subject for the CVC to mean body temperature relationship during each

condition. The mean body temperature required to elicit an increase in CVC was significantly greater in the **LBNP**, **HYP,** and **LBNP+HYP** conditions compared to **CON** (Table 2, $p \leq 0.05$). The mean body temperature required to initiate an increase in CVC in the **LBNP+HYP** condition was also significantly greater compared to **LBNP** ($p \leq 0.05$) but not **HYP** ($p=0.164$). These observations suggest that plasma hyperosmolality and baroreceptor unloading additively contributed to the increase CVC response during whole-body heating in the **LBNP+HYP** condition. In contrast, there was no effect of condition on the thermal sensitivity of CVC ($p=0.478$).

Sweating. Figure 5 illustrates the typical response of a representative subject for the sweating to mean body temperature relationship during each condition. The mean body temperature threshold at which the onset of sweating occurred was significantly greater in both the **HYP** and **LBNP+HYP** conditions compared to **CON** and **LBNP** conditions (Table 2, $p \leq 0.05$). The mean body temperature onset thresholds for sweating were not statistically different between isosmotic conditions (**CON** and **LBNP**, $p=0.950$) nor did they differ between hyperosmotic conditions (**HYP** and **LBNP+HYP**, $p=0.459$), indicating that threshold changes observed in the **LBNP+HYP** condition were solely the result of increases in plasma osmolality. Furthermore, there was no significant effect of condition on the thermal sensitivity of sweating ($p=0.172$).

DISCUSSION

The present study is the first to examine the separate and combined effects of baroreceptor unloading and plasma hyperosmolality on the thermal control of sweating and CVC during progressive whole-body heat stress. The current results support our hypothesis that combined baroreceptor unloading and plasma hyperosmolality cause a

greater attenuation in the heat loss response of cutaneous vasodilation relative to their independent effects. This is evidenced by the observation that plasma hyperosmolality and baroreceptor unloading caused the greatest delay in the onset threshold for CVC during whole-body heating. However, contrary to our hypothesis, baroreceptor unloading had no effect on the onset threshold for eccrine sweating under both isosmotic and hyperosmotic conditions. In addition, once sweating and increases in CVC occurred, there were no differences in the thermal sensitivity of each response between conditions. Our results suggest that alterations in skin perfusion associated with dehydration during heat stress are mediated by a combination of baroreceptor loading status and plasma hyperosmolality, whereas reductions in sweat rate are primarily mediated by changes in plasma osmolality.

Cutaneous vascular conductance

Previous findings have shown that the baroreflex has the capacity to reduce blood flow to the cutaneous circulation during combined orthostatic challenge and heat stress, which is critical to maintain arterial blood pressure (5, 25, 30, 35). Furthermore, previous reports by Nadel et al. (27) and Mack et al. (24) have shown that isosmotic hypovolemia and baroreceptor unloading delay the onset threshold for increases in skin blood flow during cycling exercise. Consistent with these findings, we show that the application of LBNP can independently delay the onset of CVC during progressive whole-body heating (Table 2 and Fig. 4). Similarly, we show an independent effect of plasma hyperosmolality in elevating the onset threshold for CVC during whole-body heating (Table 2 and Fig. 4), a response consistent with previous findings (10, 33, 38, 40).

The purpose of the present study, however, was to examine the combined effects of baroreceptor unloading and plasma hyperosmolality on thermoefferent activity since dehydration results in simultaneous plasma hyperosmolality and hypovolemia. To our knowledge, only Ito et al. (16) have investigated a potential interaction of the combined effects of baroreceptor unloading and plasma hyperosmolality, focusing solely on the capacity of the baroreflex to cause reductions in CVC in either an isosmotic or hyperosmotic state. They reported greater reductions in forearm vascular conductance during graded levels of LBNP applied under a hyperosmotic state relative to an isosmotic state during heat stress conditions. However, LBNP was only applied after 60 min of heat stress, which occurred at different levels of hyperthermia for the isosmotic and hyperosmotic conditions (i.e., core temperature increase of 0.3°C and 0.9°C, respectively). Although similar levels of forearm vascular conductance were reported prior to the application of LBNP, it is possible that differences in the level of hyperthermia may have contributed to the greater reductions in forearm vascular conductance observed in the hyperosmotic condition, since reductions in skin blood flow are more readily evidenced as the level of heat stress increases (30). In the present study, we continuously applied LBNP before and throughout whole-body heating. As such, this novel approach allowed us to examine the combined effects of baroreceptor unloading and plasma hyperosmolality on the thermal control of cutaneous vasodilation (onset thresholds and thermal sensitivity) at similar levels of thermoafferent stimuli. Relative to **CON**, the average onset threshold for CVC in the **LBNP** and **HYP** conditions were delayed by 0.31 ± 0.13°C and 0.44 ± 0.17°C respectively, the sum of which (0.75°C) approximates the delay observed during the **LBNP+HYP** condition (Table 2). Therefore, our observation that combined plasma hyperosmolality and baroreceptor unloading

caused the greatest delay in the onset threshold for CVC confirm the findings of Ito et al. (16) and extend them by showing that the combined influence of both stimuli appears to be additive.

It has been shown that decreases in CVC during moderate to high levels of LBNP are manifested by increases in vasoconstrictor tone without heat stress (19, 41), whereas during heat stress, baroreceptor unloading causes a decrease in the cutaneous vascular response through withdrawal of active vasodilation (5, 19). However, work by Shibasaki et al. (35) provides evidence to suggest that the cutaneous vasoconstrictor system is capable of decreasing CVC during an orthostatic challenge while heat stressed. Therefore, the delayed onset threshold in CVC with combined baroreceptor unloading and plasma hyperosmolality observed in the present study could be the result of a LBNP-induced baroreceptor-mediated increase in vasoconstrictor activity, a delay in active vasodilation or a combination of both. Recently, Shibasaki et al. (33) concluded that the independent effect of hyperosmolality in elevating the internal temperature onset threshold for CVC is due to a delay in cutaneous active vasodilation, and not to increased vasoconstrictor activity. This was evidenced by similar core temperature onset thresholds for cutaneous vasodilation at treated and untreated sites with bretylium tosylate (vasoconstrictor blockade). Therefore, it is likely that the augmented delay in the onset threshold for CVC associated with plasma hyperosmolality and baroreceptor unloading observed in the present study is centrally mediated via a further withdrawal of the active vasodilator system. However, since we did not directly measure the responses of the vasoconstrictor and vasodilator systems, further study is warranted to evaluate this hypothesis.

Sweating response

Consistent with previous studies, we show that the onset threshold for sweating under a hyperosmotic state is delayed as compared to the isosmotic condition (Table 2 and Fig 5) (10, 33, 38, 40). In contrast to previous reports, however, we show that baroreceptor unloading induced by LBNP has no influence on the sweating response during whole-body heating in either the isosmotic or hyperosmotic conditions (Table 2 and Fig 5). A number of studies have demonstrated a role for baroreceptors in modulating sweating responses during heat stress. Mack et al. (24) reported an increase in the esophageal temperature onset threshold for sweating during exercise with combined LBNP. Similarly, Solack et al. (36) reported a reduction in the slope of the sweating to esophageal temperature relationship during simultaneous whole-body heating and LBNP application. In contrast, Johnson and Park (17) found no differences in sweating during exercise and passive heating performed in the supine and upright posture. Additionally, recent work by Wilson et al. (44, 45) reported no changes in skin sympathetic nerve activity and sweat rate during multiple 30° head-up tilts throughout a 1.2°C increase in mean body temperature or during pharmacologically-induced decreases in arterial blood pressure under heat stress (0.6°C increase in sublingual temperature). These later findings propose that the baroreflex does not have an efferent limb in the control of thermal sweating, although it has been shown that pharmacological and postural manipulations of blood pressure perturb baroreceptors differently compared to LBNP (11). There are a number of differences between our study and the study by Mack et al. (24) that might account for the differences in our results. Since they applied LBNP during exercise, the influence of metaboreceptors might have confounded their results (17, 21, 22). More importantly, the LBNP in their study was applied in a room maintained at 28°C which

causes the circulation of cool air around the legs and waist of the participants, thus cooling mean skin temperature. Therefore, it is possible that skin cooling associated with the application of LBNP during the Mack et al. study had a significant influence on the determination of their sweating thresholds, since local skin cooling has been shown to decrease sweating and delay the onset threshold for sweating (28, 44). In the current study, however, a novel approach was to perform LBNP in a temperature controlled chamber regulated at 40°C to minimize any cooling effects through the circulation of warm air. Furthermore, LBNP was continuously applied prior to, and throughout whole-body heating to avoid the inhibitory influence of negative rates of skin temperature change on sweating (46). Finally, any effect of skin cooling on the sweating response would be accounted for in our mean body temperature measurements. Therefore, after minimizing the effects of convective skin cooling associated with applying LBNP and despite a strong baroreceptor drive, a baroreceptor-mediated modulation of sweat rate was still not observed in either an isosmotic or hyperosmotic condition. Our findings, combined with more recent work on the effects of the baroreflex on sweating (6, 44, 45) provide strong evidence that unloading of baroreceptors does not modulate sweating, which is congruent with a number of recent review articles on baroreceptor unloading and the sweating response (20, 35).

Limitations

In the current study, we employed standard infusion rates which have been previously shown to elicit similar changes in plasma volume with marked differences in plasma osmolality (33, 40). However, this procedure ultimately results in different infusion volumes between the hyperosmotic and isosmotic conditions, which may

influence the cardiovascular stress imposed by the application of LBNP. This is particularly important when comparing the **LBNP** and **LBNP+HYP** conditions of the current study. However, plasma volume changes were not different after infusion and remained similar between these conditions throughout the heating period. Furthermore, the cardiovascular responses to the application of LBNP between the **LBNP** and **LBNP+HYP** conditions were similar (Fig 2). Therefore, we are confident that the application of LBNP between these conditions caused similar levels of baroreceptor unloading. Additionally, LBNP in the current study was used to simulate baroreceptor unloading that is thought to occur during dehydration-induced hypovolemia (26, 32). However, it is possible that the mechanism by which hypovolemia during thermal and/or exercise-induced dehydration perturbs baroreceptors differs from baroreceptor unloading during the application of LBNP. Moreover, the differences may be influenced in the current study by the use of saline infusion to manipulate plasma osmolality. While these factors may limit the practical applicability of our results, they do provide important information about the mechanisms mediating altered thermoeffector control of sweating and skin blood flow during thermal dehydration. Finally, it might be expected that the time to reach a 1°C increase in esophageal temperature in the **LBNP** and **LBNP+HYP** conditions (85 ± 13 and 67 ± 17 min) would be shorter compared to the **CON** condition (54 ± 5 min), since we show an effect of LBNP on the control of SkBF. However, the longer time required to reach a 1°C increase in esophageal temperature during the LBNP conditions compared to the CON condition does not reflect the inhibition of thermoregulatory function and is likely due to re-distribution of blood caused by the application of LBNP (18).

Perspectives and Significance

Dehydration associated with prolonged heat exposure has been well documented to result in a progressive attenuation of skin perfusion and sweating, and a concomitant increase in the level of thermal strain. Since heat-induced dehydration leads to both hyperosmolality and hypovolemia (4), central osmoreceptors and peripheral baroreceptors have been implicated as nonthermal modulators of thermoefferent activity during combined dehydration and heat stress. Prior to the current study, however, it remained unknown whether one of these stimuli preferentially caused the observed alterations in temperature regulation during dehydration, or if the combination of both mediated the reductions in heat loss responses. Furthermore, it was unknown if both skin blood flow and sweating were affected to a similar extent by both nonthermal stimuli. Collectively, the results of the current study suggest the activation of osmoreceptors and baroreceptors have divergent effects on the skin blood flow and sweating responses. In addition, most previous studies examining the effects of plasma hyperosmolality or baroreceptor unloading on skin blood flow and sweating responses have done so in the supine posture (5, 6, 24, 30, 33, 34, 36, 40, 42, 44). In the current study, however, we studied these effects in the upright posture and therefore, provide novel insight into the interaction of osmoreceptors and the baroreflex in the control of heat loss responses as it pertains to the upright human. Specifically, we show that, despite being in a relative state of baroreceptor unloading (i.e. the upright posture), the baroreflex maintains the ability to attenuate increases in cutaneous blood flow during further baroreceptor unloading through the application of LBNP. In contrast, no measurable effect was observed in the control of sweat rate with the combination of the upright posture and further baroreceptor unloading with LBNP. Speculatively, an additive effect of hyperosmolality and

baroreceptor unloading on the skin blood flow response could enhance the control of blood pressure during combined dehydration and orthostatic stress (i.e. upright posture), while a preferential effect of plasma hyperosmolality on the sweating response could serve to prevent further reductions in plasma volume, therefore maximising sweat rate and the control of core temperature in such scenarios.

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DISCLOSURES

The authors declare that they have no conflict of interest, financial or otherwise.

GRANTS

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Table 1. Mean ± SD values for mean skin ($\bar{T}_{sk}$) during baseline rest (B1), during the application of lower body negative pressure or sham pressure (B2), and at 0.25°C increments in esophageal temperature during a passive heat stress. Conditions are isosmotic and sham pressure (CON), isosmotic with lower body negative pressure (LBNP), hyperosmotic with sham pressure (HYP), and hyperosmotic with lower body negative pressure (LBNP+HYP).

Measure	Condition	B1	B2	0.25°C	0.5°C	0.75°C	1.0°C
$\bar{T}_{sk}$ (°C)	CON	34.11 ± 0.31	34.10 ± 0.32	36.70 ± 0.60	36.92 ± 0.54	37.14 ± 0.53	37.35 ± 0.53
	LBNP	33.93 ± 0.54	33.82 ± 0.43	36.41 ± 0.65	36.82 ± 0.48	37.00 ± 0.38	37.21 ± 0.51
	HYP	34.12 ± 0.31	34.07 ± 0.34	36.82 ± 0.54	37.07 ± 0.51	37.29 ± 0.51	37.49 ± 0.55
	LBNP+HYP	34.11 ± 0.40	33.80 ± 0.32	36.55 ± 0.63	36.98 ± 0.60	37.21 ± 0.66	37.28 ± 0.68

Table 2. Mean body temperature ($\bar{T}_b$) threshold for cutaneous vascular conductance (CVC) and sweating, the sensitivities of CVC and sweating with respect to $\bar{T}_b$ (slope), and the increase in $\bar{T}_b$ ($\Delta\bar{T}_b$) from preheating to the onset of CVC and sweating in isosmotic and sham pressure (CON), isosmotic with lower body negative pressure (LBNP), hyperosmotic with sham pressure (HYP), and hyperosmotic with lower body negative pressure (LBNP+HYP) conditions.

Condition	Sweating			CVC		
	Threshold	Slope	$\Delta\bar{T}_b$	Threshold	Slope	$\Delta\bar{T}_b$
CON	36.77 ± 0.36	1.29 ± 0.47	0.46 ± 0.26	36.56 ± 0.27	80.93 ± 34.1	0.28 ± 0.23
LBNP	36.77 ± 0.29	1.37 ± 0.54	0.50 ± 0.18	36.83 ± 0.25*	71.24 ± 31.0	0.56 ± 0.24*
HYP	37.19 ± 0.32*†	1.70 ± 0.62	0.91 ± 0.37*†	36.97 ± 0.31*	61.63 ± 24.4	0.69 ± 0.36*
LBNP+HYP	37.25 ± 0.42*†	1.37 ± 0.54	0.94 ± 0.40*†	37.18 ± 0.32*†	73.17 ± 21.1	0.88 ± 0.33*†

Values are °C for threshold and $\Delta\bar{T}_b$ and $\%CVC_{max}$/°C (CVC) or mg•min^{-1}•cm^{-2}/°C (Sweating) for slope. *Indicates significantly different from CON and †indicates significantly different from LBNP conditions, $p \leq 0.05$.

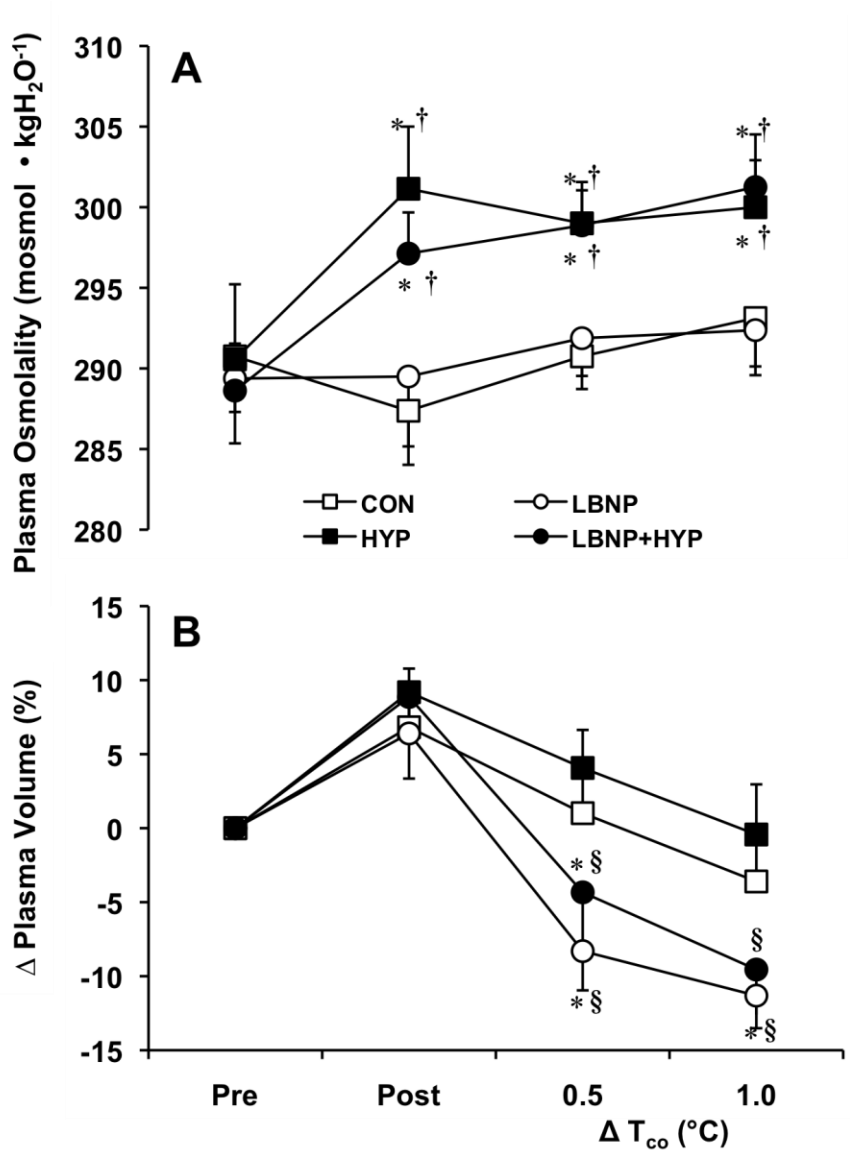


Figure 1. Plasma osmolality (panel A) and percent change in plasma volume (panel B) pre-infusion (Pre), post-infusion (Post), and at 0.5°C and 1.0°C increases in body core temperature (T_{co}) during control (CON), lower body negative pressure (LBNP), hyperosmotic (HYP), and combined HYP and LBNP (LBNP+HYP) conditions. *Indicates significantly different from CON, †indicates significantly different from LBNP, and §indicates significantly different from HYP conditions ($p \leq 0.05$). Error bars represent 95% confidence intervals for 8 subjects.

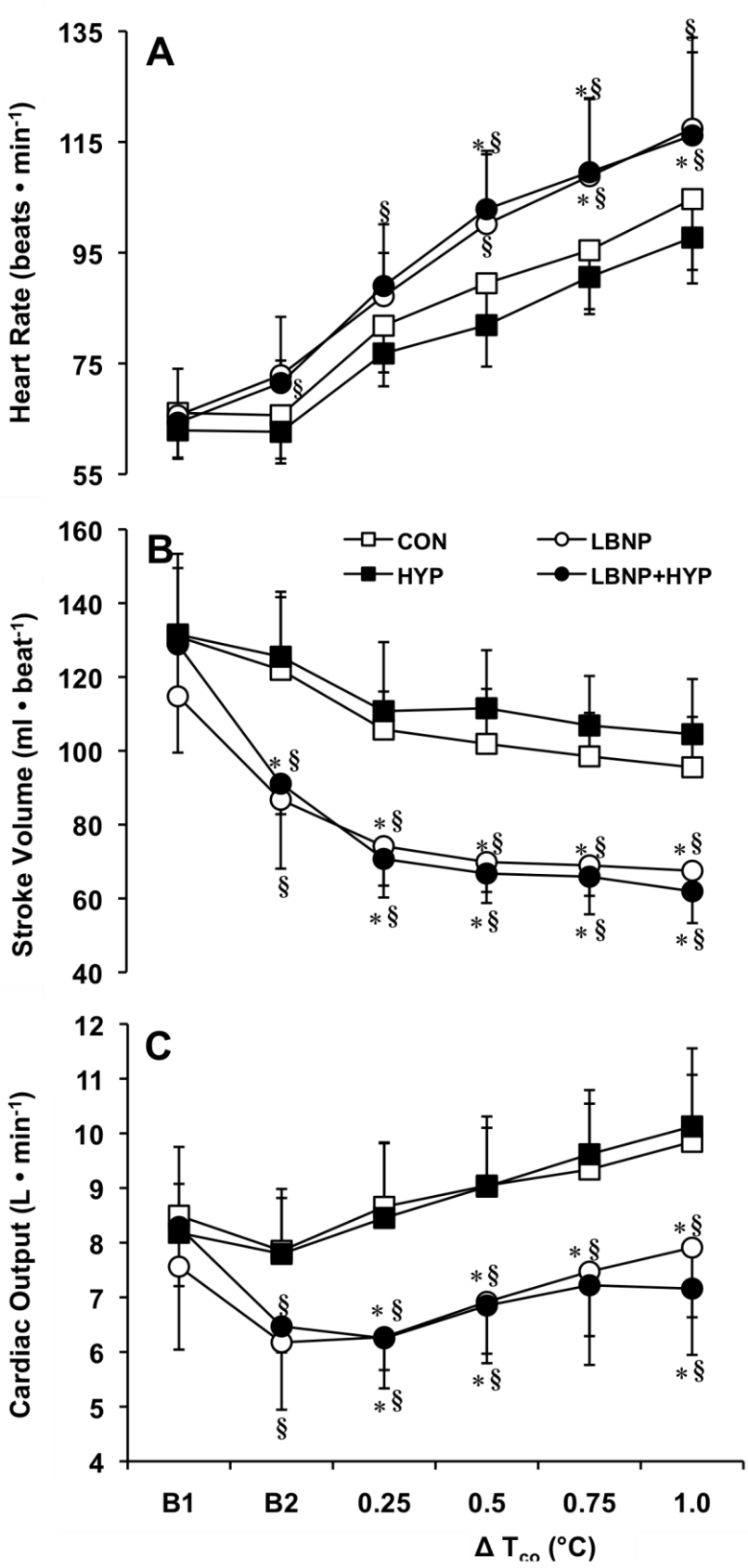


Figure 2. Mean heart rate (panel A), stroke volume (panel B) and cardiac output (panel C) values as a function of increasing body core temperature (T_{co}) during whole-body heating in control (CON), lower body negative pressure (LBNP), hyperosmotic (HYP), and combined HYP and LBNP (LBNP+HYP) conditions. *B1*, baseline rest. *B2*, application of lower body negative pressure or sham pressure. *Indicates significantly different from CON, and §indicates significantly different from HYP conditions ($p \leq 0.05$). Error bars represent 95% confidence intervals for 8 subjects.

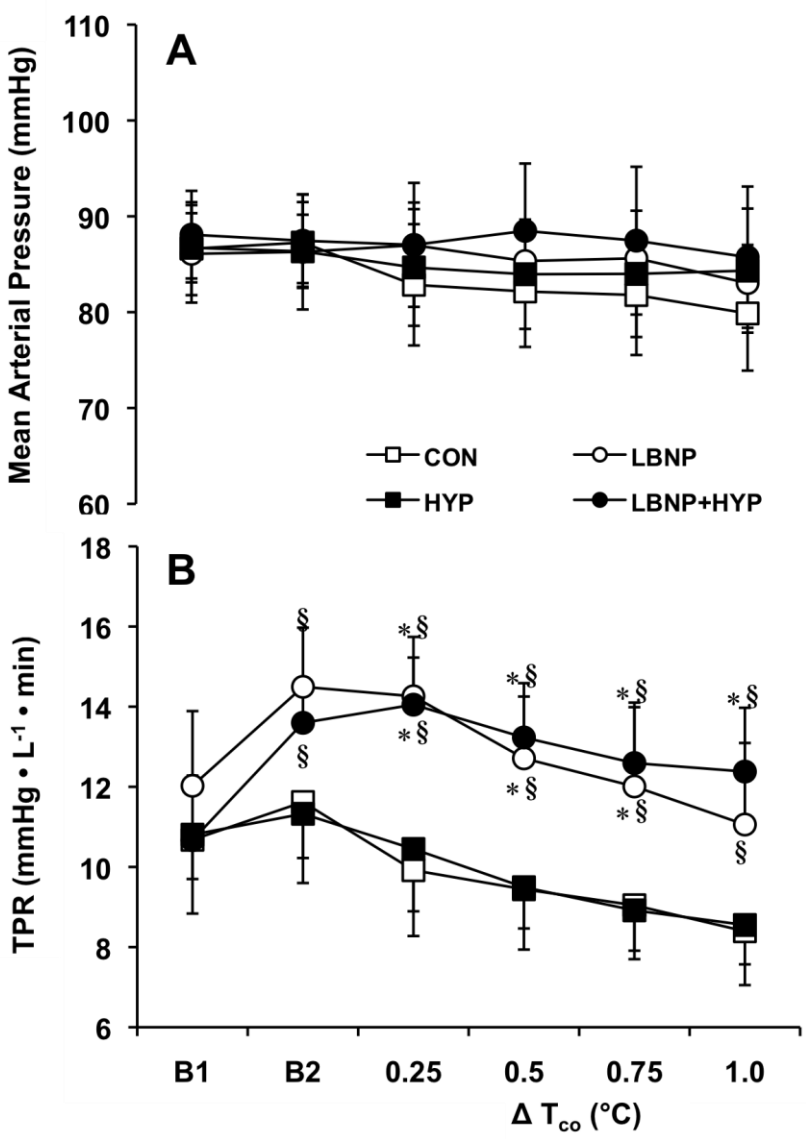


Figure 3. Mean arterial pressure (panel A) and total peripheral resistance (TPR, panel B) as a function of increasing body core temperature (T_{co}) during whole-body heating in control (CON), lower body negative pressure (LBNP), hyperosmotic (HYP), and combined HYP and LBNP (LBNP+HYP) treatment conditions. *B1*, baseline rest. *B2*, application of lower body negative pressure or sham pressure. *Indicates significantly different from CON, and §indicates significantly different from HYP conditions ($p \leq 0.05$). Error bars represent 95% confidence intervals for 8 subjects.

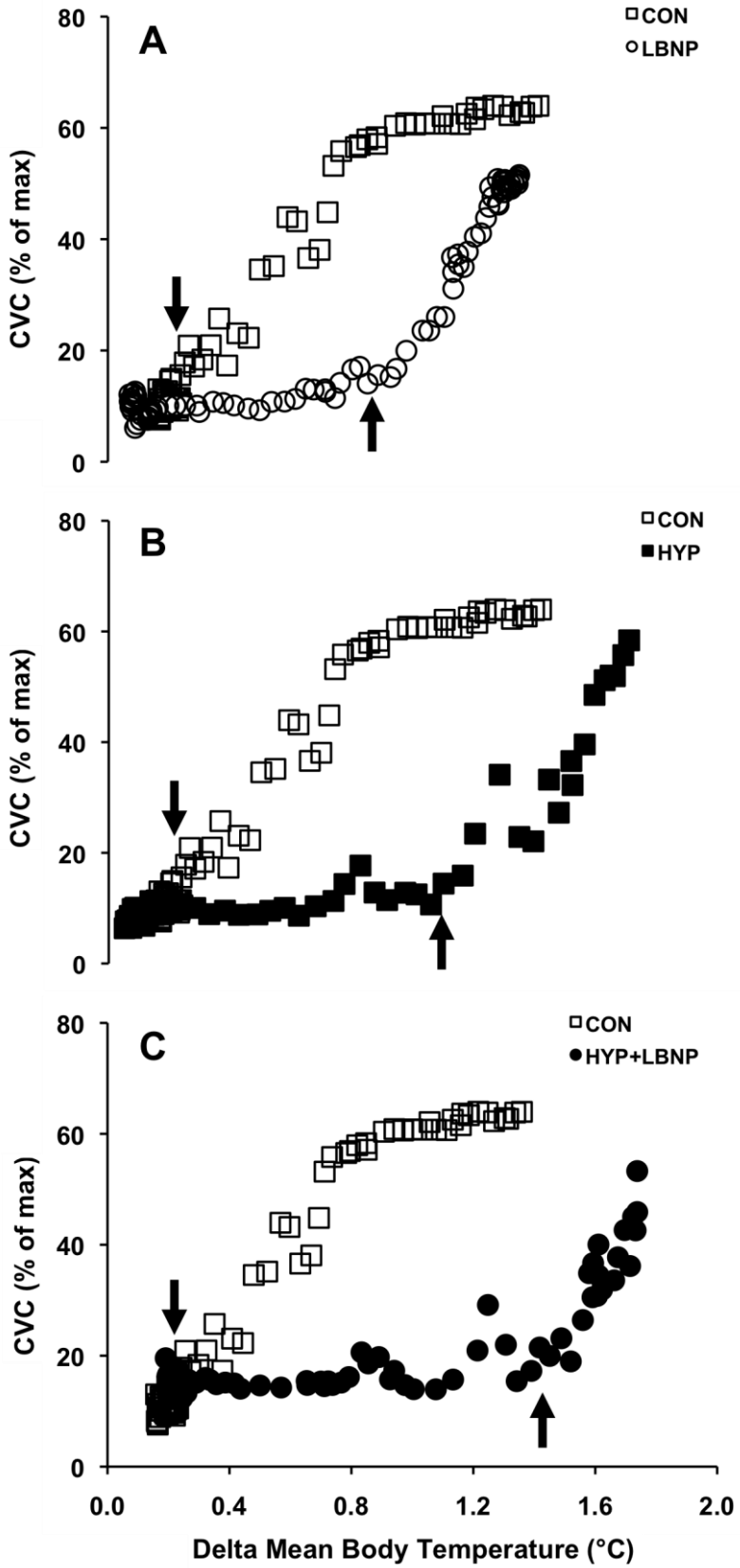
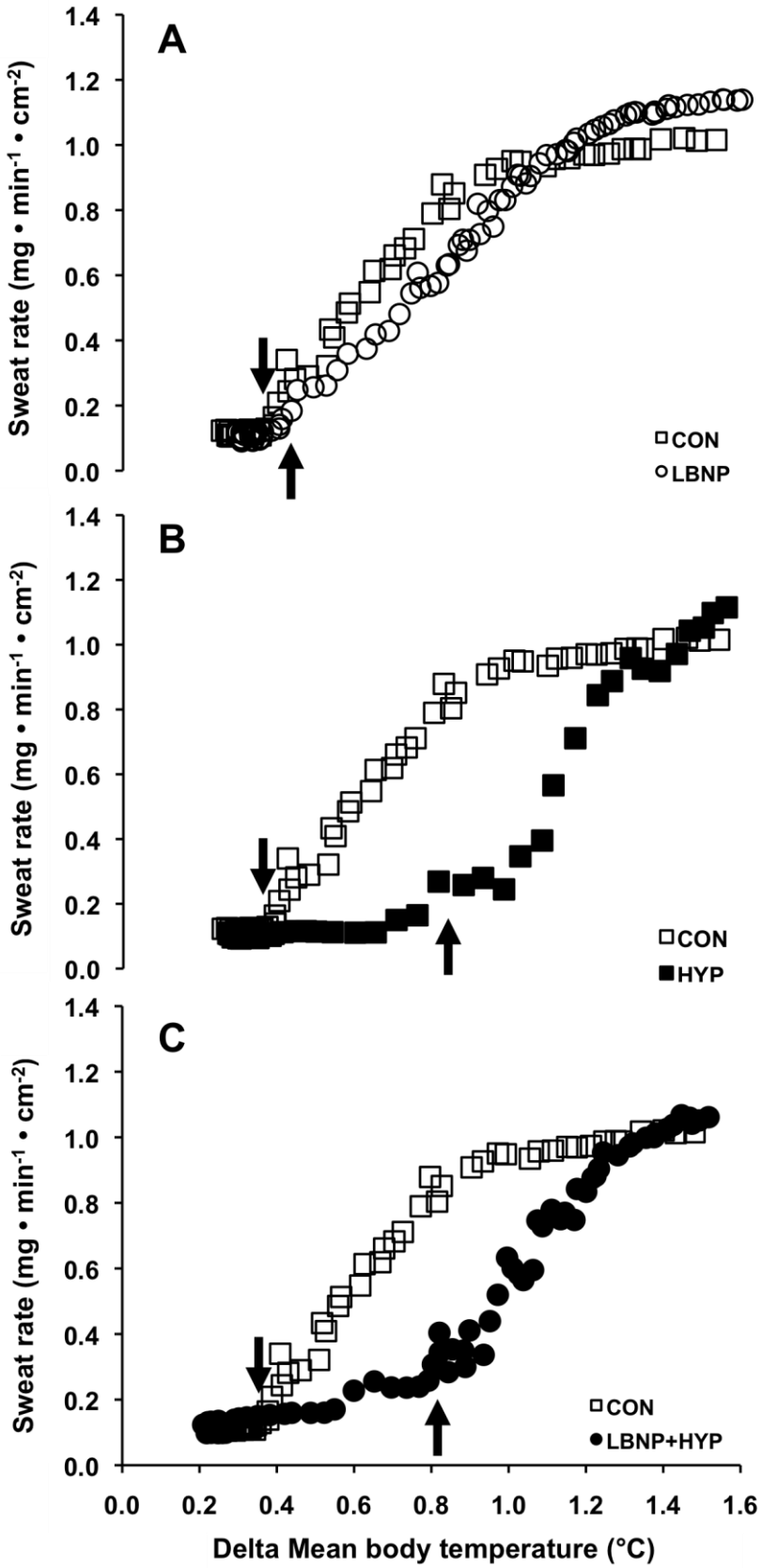


Figure 4. The cutaneous vascular conductance response (CVC) as a function of changes in mean body temperature from B1 during whole-body heating in the lower body negative pressure (LBNP, panel A), hyperosmotic (HYP, panel B), and combined HYP and LBNP (LBNP+HYP, panel C) treatment conditions from a representative subject. Each condition is compared to the control condition (CON). Cutaneous vascular responses are expressed as a percent of CVC_{max}. Arrows indicate the approximate onset threshold for CVC.



F**igure 5.** The local forearm sweating response as a function of increases in mean body temperature from B1 during whole-body heating in the lower body negative pressure (LBNP, panel A), hyperosmotic (HYP, panel B), and combined HYP and LBNP (LBNP+HYP, panel C) treatment conditions from a representative subject. Each condition is compared to the control condition (CON). Arrows indicate the approximate onset threshold for sweating.

PART THREE:

GENERAL CONCLUSIONS OF THE THESIS

This thesis work was directed at examining the mechanisms responsible for the observed attenuations in heat loss responses during exercise and/or heat-induced dehydration. This includes the study of central osmoreceptors and peripheral baroreceptors, which are believed to be the nonthermal factors responsible for these attenuations. More specifically, we studied the effects of plasma hyperosmolality and baroreceptor unloading on the mean body temperature onset threshold and thermal sensitivity during whole-body heat stress. This was achieved by infusing isotonic or hypertonic NaCl solution to maintain or increase plasma osmolality, respectively, and by applying lower body negative pressure to unload baroreceptors.

The most important findings from this study are that plasma hyperosmolality and baroreceptor unloading additively augmented the attenuation in cutaneous vasodilation during passive heating compared to their independent effects. This was evidenced by an increase in the onset threshold for CVC during combined LBNP+HYP relative to CON that was approximately equal to the sum of the increases in onset threshold for CVC during the independent LBNP and HYP conditions. Additionally, a baroreflex-mediated influence on the local sweat rate response in both an isosmotic and hyperosmotic condition was not observed, suggesting changes in plasma osmolality primarily influence decreases in sweating during dehydration.

Thus, the body's augmented response to plasma hyperosmolality and baroreceptor unloading could serve to help maintain arterial blood pressure through reductions in cutaneous circulation during conditions of dehydration and heat stress. In contrast, a

baroreflex-mediated control of sweating might not lead to greater control of arterial blood pressure. Instead, the preferential effect of plasma osmolality on sweat rate could effectively prevent further reductions in plasma volume, therefore maximizing sweating and core temperature regulation during dehydration.

In summary, our findings provide important new insight regarding the underlying mechanisms responsible for decreases in heat loss responses during exercise and/or heat-induced dehydration. Additionally, these results further knowledge regarding nonthermal modulators in thermoregulatory control by elucidating a combined effect of baroreceptor unloading and plasma hyperosmolality on the cutaneous vascular response and provide strong evidence that sweating is not under control of the baroreflex. Future research should focus on understanding the implications of these divergent roles of plasma hyperosmolality and baroreceptor unloading in the control of skin blood flow and sweating.

PART FOUR:

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PART FIVE:

APPENDIX

5.0 Subject background letter and consent form

The competing influences of baroreceptors and osmoreceptors during passive heat stress

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Background

Exercise in the heat results in progressive dehydration due to loss of body fluids through sweating, which places an individual at further risk of heat related injury. Specifically, dehydration leads to a decrease in plasma volume (hypovolemia) and an increase in plasma osmolality (hyperosmolality), both of which have been shown to independently inhibit thermal responses during heat stress. The mechanisms believed to be responsible for the attenuation of heat loss responses experienced during hypovolemia and hyperosmolality are baroreceptors and osmoreceptors. Baroreceptors are the body's blood pressure regulating receptors, while osmoreceptors are located in the hypothalamus and detect changes in plasma osmolality. Although both hyperosmolarity and baroreceptor unloading have been shown to inhibit thermoregulatory responses separately, their interactive effect on heat loss responses has not been well studied.

Purpose

Therefore, the purpose of this study will be to examine the interactive effects of plasma hyperosmolality and baroreceptor unloading on local control mechanisms of skin blood flow and sweating during a passive heat stress. We hypothesize that baroreceptor unloading and hyperosmolality will attenuate heat loss responses independently, resulting in an elevated core temperature during passive heating compared to a control. However, we also hypothesize that the combined interaction of baroreceptor unloading and plasma hyperosmolality will have the greatest inhibition on heat loss responses, resulting in the greatest increase in core temperature.

Subject profile

To be a participant you must be a healthy (no history of respiratory, metabolic, cardiovascular, blood pressure disease, or of diabetes and not currently on any medication related to these conditions) adult, aged between 18 and 45 years. You must be physically active, that is to say you are physically active at least three to four times a week at a moderate to hard intensity for at least 30 minutes. If you agree to participate in this study, you will be required to participate in one preliminary session and four experimental sessions to be conducted on different days and separated by a minimum of 48 hours.

Preliminary session

Both the preliminary session and the experimental sessions will take place in at the Human and Environmental Physiology Research Unit located on the main campus at the University of Ottawa. The time involvement will be approximately 45 min to 1 hour for the preliminary session. During the preliminary session, we will review all procedures with you. In addition, you will be introduced to all of the equipment and measuring devices that we will be using for the experimental sessions. We will give you the opportunity to read the Background and Informed consent document. If you agree to participate in the study, we will ask you to sign the informed consent below and complete a *Physical Activity Readiness Questionnaire (Par-Q)* and an *American Heart Association/American College of Sports Medicine Health/Fitness Facility Pre-participation Screening Questionnaire*. These questionnaires are standard questionnaires

that have been developed to help us evaluate your readiness for exercise and are also used to assist us in evaluating your general physical health and level of physical activity. Thereafter, we will complete some basic measurements including height and body mass. Following these measures, you will be asked to perform a maximal incremental exercise test on a treadmill. During this exercise test, we will measure the maximum amount of oxygen your body consumes during physical exercise. Typically, this test lasts no longer than 12 minutes. During this test, you will run at a speed of approximately 7.0 miles per hour for males and 6.0 miles per hour for females. The incline of the treadmill will be increased by 2% increments every two minutes until you choose to stop or until you can no longer maintain the required speed. The measurement of oxygen consumption during the test is used to determine your maximal aerobic capacity. We will also assess your body composition by using underwater weighing. You will be asked to wear a bathing suit, enter the tank and situate yourself on the hanging chair. You will be asked to immerse yourself completely under water for 5 seconds. Once the measurement is completed you will be given a few minutes to relax after which you will be asked to perform the same steps again. Five trials will be done in order to obtain accurate results.

Experimental test sessions

The study will consist of 4 experimental test sessions performed in random order. You will be asked to enter a room with an ambient air temperature of 34ºC. You will then be asked to put on a suit which will cover your whole body except your hands, feet and face. We are able to change the temperature of that suit by adjusting the temperature of the water which flows through multiple tubes placed inside the material. You will then be placed standing upright in a lower body pressure box. The experimental session will then proceed with all instrumentation (see below description) and a 15 minute baseline data collection period. After this, saline will be injected at a constant rate for 90 minutes to either increase (3.0% NaCl) or maintain (0.9% NaCl) your plasma osmolality. Next, lower body negative pressure will be applied at -20mmHg for 10 minutes. Finally, whole-body heating will begin by increasing the temperature of the water flowing through your suit to 48ºC for 90 minutes or until your core temperature increases by 1°C from baseline. Therefore, the 4 experimental sessions will include: 1) isoosmotic and no pressure, 2) hyperosmotic and no pressure, 3) isoosmotic and LBNP, and 4) hyperosmotic and LBNP. You should be aware that at any point in the experimental protocol, the test will be terminated should you experience any distress or if the researchers feel that you are in discomfort.

In preparation for the experimental trials, you will be asked to abstain from alcohol, caffeine, high sodium foods and intense or prolonged physical activity for at least 24 hours prior to all sessions. We will ask you to drink at least 100 ml of water for every waking hour prior to the experimental trials to ensure that you are adequately hydrated.

The following instruments will be used to monitor and record your body's physiological responses during the experimental trial:

Esophageal probe: In order to monitor central body temperature, Dr. Kenny will insert a flexible peadiatric esophageal temperature probe (2mm in diameter) (Mon-a-therm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St-Louis, MO, USA) which

is inserted through the patient's nostril to the laryngopharynx. At this point the patient is asked to sip water through a straw which depresses the epiglottis and guards against the tip of the probe from entering the trachea. The tip of the probe, once fully inserted in the esophagus (swallowing tube) is in proximity to the left ventricle and aorta. There can be mild discomfort and a mild gagging reflex during the insertion of the probe. However, the mild irritation and gagging reflex soon passes. The risk of transmission of infectious disease is negligible as each participant has his own sterile probe that is disposed of once all tests have been completed.

Rectal probe: Rectal temperature will be measured using a flexible rectal probe (2mm in diameter) (Mon-a-therm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St-Louis, MO, USA) inserted to a depth of 10-12 cm past the external anal sphincter. The probe is inserted by you with instructions provided by Dr. Kenny. A marker is placed on the rectal probe using sterile surgical tape. The subject inserts the probe until the tape reaches the anal surface. The insertion of the rectal probe may cause some mild discomfort and minor irritation; however, this sensation soon passes. The risk of transmission of infectious disease is negligible as each participant has his own sterile probe that will be disposed of following each testing session.

Skin probes: Twelve skin probes will be taped to the skin surface with hypoallergenic tape. These probes give an indication of skin temperature and heat loss from the skin surface. Some hair may need to be shaved (by the use of disposable razors) in order to secure the probes adequately to the skin surface. Some discomfort may be experienced upon removing the tape.

Sweat capsule: A small plastic capsule will be taped to the back of the shoulder (upper back). This capsule picks up humidity from the skin and provides an indication of sweat rate.

Blood pressure: Blood pressure will be monitored continuously by a Finapress fingertip blood pressure monitor, as well as manually at pre-selected intervals with a sphygmomanometer and a stethoscope. You will feel a slight pressure on your finger while blood pressure will be taken.

Blood flow: A flexible laser probe will measure skin blood flow non-invasively at the mid-forearm. This measuring device does not result in any discomfort or residual medical effects.

Oxygen consumption: An automated metabolic cart (MOXUS system) will be used to assess oxygen consumption. You will be required to wear a breathing valve connected to the metabolic cart and a nose plug for the majority of the study.

Heart rate: Heart rate will be monitored by a strap placed around the chest (Polar Vantage heart rate monitor).

Venous Blood samples: Venous blood samples will be collected through an indwelling plastic catheter in a superficial vein to determine changes in plasma volume and osmolality. Blood samples (approximately 10ml) will be drawn 4 times during each

experimental trial: 1) during initial rest after the subject has been seated for 30 min 2) after 90 min of saline infusion, 3) upon a 0.5°C increase in Teso from baseline during passive heating, and 4) at the end of passive heating or 1°C increase in Teso. The blood samples will be analyzed following the experimental trials at the Gamma-Dynacare laboratory at the University of Ottawa Health Services Clinic on 100 Marie Curie Street, Ottawa, ON, KIN 6N5.

Saline Infusion: Saline will be infused using an indwelling plastic catheter into a superficial vein with either isotonic (0.9% NaCl) or hypertonic (3.0% NaCl) solution for 90 min. The infusion rate will be 0.2 and 0.125 $ml \cdot min^{-1} \cdot kg^{-1}$ body weight for 0.9% and 3.0% saline respectively. These infusion rates were chosen to expand plasma volume by similar amounts in the two conditions and to create a hyperosmotic condition similar to when you are dehydrated.

Risks and discomforts

In the event of a health related emergency, our research staff is trained in CPR and we have emergency phones located in the laboratory for immediate contact with University emergency response (University Protection Office).

Temperature probes: Perforation of the esophagus or oral or nasal cavities, as well as the rectum can occur during insertion of the esophageal and rectal probes (potentially causing inflammation and infection). Perforation of the esophagus or oral or nasal cavities, as well as the rectum is very rare and no such incident has ever occurred in this laboratory. The risk of transmission of infectious disease is negligible as each subject has his own sterile probes that will be disposed of once all tests have been completed.

Exercise testing: The risks of maximal exercise testing are nausea, dizziness, fainting, abnormal blood pressure, chest pain, leg cramps and fatal injury. The incidence of cardiac arrest during maximal exercise tests is 1 in 10 000 tests. To ensure your safety, all tests are conducted under standardized conditions for human exercise experiments as laid out by the Canadian Society for Exercise Physiology and the American College of Sports Medicine. You may stop at any time during the maximal exercise test.

Elevation core body temperature: There are also certain risks that accompany an elevation of 1.5 to 2.0°C in your core temperature. These include: headache, extreme weakness, dizziness, nausea, hyperventilation, hypotension, confusion, diarrhoea, vomiting and loss of consciousness. An increase in your core temperature of this magnitude is unlikely under these experimental conditions. The risk of any ill-effect associated with an increase in your core temperature will be minimized by terminating the session at the first sign of distress. Immediately following the termination of the session, if deemed necessary by the researcher, we will immerse you in a cool water bath to bring your core temperature back down to your normal resting value.

Blood samples and saline infusion: There is a risk of infection during and following a venopuncture for blood sampling and catherization for saline infusion. All venopunctures and/or catherizations will be performed under standard care procedures and drawn by qualified personnel under the approval of the University of Ottawa Risk

Assessment Office. To minimize the occurrence of an infection, the affected region is treated with isopropyl alcohol and/or hydrogen peroxide using sterile gauze pads subsequent to a period of local pressure application. Analysis of the blood samples will be done by the Gamma-Dynacare laboratory at the University of Ottawa Health Services Clinic on 100 Marie Curie Street, Ottawa, ON, KIN 6N5. All blood samples are discarded and properly destroyed by Gamma-Dynacare laboratories immediately upon completion of the analysis.

A first aid kit is readily available if needed in the laboratory for all sessions.

Anonymity and Confidentiality

All raw data will be stored using alphanumeric coding system as such, no one will be able to identify you as your name will not appear on these files. Data will be kept in Dr. Kenny's office in Montpetit Hall in locked file cabinets and only the researchers mentioned above will have access to your data.

No records bearing your name will leave the institution. Only the researchers mentioned above will have access to your data. You are encouraged to request and discuss the results of the experimental trials at any time. The results of the preliminary session (aerobic fitness and body composition) will be available to you upon completion of the study.

The data collected in this study will be published in scientific journals. The data will be kept for a period of 10 years and will subsequently be destroyed by the physical resources service of the University of Ottawa.

For the entire duration of the study, it is fully understood that you may refuse to participate or withdraw from the study at any time.

INFORMED CONSENT OF PARTICIPANT

Research involving human subjects requires written consent of the participants.

I, _____, hereby volunteer to participate as a subject in the study entitled **"The competing influences of baroreceptors and osmoreceptors during passive heat stress"**. I have read the information presented in the above background information and I had the opportunity to ask questions to the investigators. I understand that my participation in this study, or indeed any research, may involve risks that are currently unforeseen.

I recognize that there will be no direct benefit to me from my participation in this study (besides receiving a fitness evaluation).

I have been given a copy of this Background Letter and Consent Form for me to keep.

Signature of participant: _____ Date: _____

Signature of Researcher: _____ Date: _____

5.1 Health Sciences and Sciences REB ethical clearance certificate

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Glen	Kenny	Health Sciences / Human Kinetics	Principal Investigator
Konrad	Binder	Health Sciences / Human Kinetics	Co-investigator
Daniel	Gagnon	Health Sciences / Human Kinetics	Co-investigator
Aaron	Lynn	Health Sciences / Human Kinetics	Co-investigator
Jill	Stapleton	Health Sciences / Human Kinetics	Co-investigator
Heather	Wright	Health Sciences / Human Kinetics	Co-investigator

File Number: H03-10-03

Type of Project: Professor

Title: The Competing Influences of Baroreceptors and Osmoreceptors during Passive Heath Stress

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
07/22/2010	07/21/2011	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

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Ottawa (Ontario) K1N 6N5 Canada Ottawa, Ontario K1N 6N5 Canada
(613) 562-5841 • Téléc./Fax (613) 562-5338
http://www.research.uottawa.ca/ethics/index.html
http://www.recherche.uottawa.ca/deontologie/index.html

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement and other applicable laws and regulations in Ontario, has examined and approved the application for ethical approval for the above named research project as of the Ethics Approval Date indicated for the period above and subject to the conditions listed the section above entitled "Special Conditions / Comments".

During the course of the study the protocol may not be modified without prior written approval from the REB except when necessary to remove subjects from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the study (e.g. change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, information/consent documentation, and/or recruitment documentation, should be submitted to this office for approval using the "Modification to research project" form available at:
http://www.rges.uottawa.ca/ethics/application_dwn.asp

Please submit an annual status report to the Protocol Officer 4 weeks before the above-referenced expiry date to either close the file or request a renewal of ethics approval. This document can be found at:
http://www.rges.uottawa.ca/ethics/application_dwn.asp

Signature:

Germain Zongo
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Sciences and Health Sciences REB

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